NIDDK

Recent Advances & Emerging Opportunities

February 2012

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

The cover images illustrate the fascinating relationships between the diets of mammals and the bacteria that inhabit their guts, as described within this compendium. Bacterial residents of the intestinal tract have been found to influence normal health and disease, and have been an important focus for NIDDK-supported research. The mammalian intestine is "host" to an enormous ecosystem of microorganisms. In exchange for a nutrient-rich environment, the bacterial community provides essential metabolic functions that mammals cannot carry out on their own. Recent research has shown that the composition of bacteria that naturally reside in the intestines of human beings and other mammals can influence a range of physiological traits, including metabolism and appetite, as well as diseases and conditions, such as obesity, malnutrition, inflammatory bowel diseases, type 1 and type 2 diabetes, and irritable bowel syndrome.

On the cover, the diagrams depict the relationships between different mammalian diets and species of bacteria in the gut (left) or the types of genes carried by these bacteria (right). In the left diagram, DNA sequences were used to identify the different bacterial species that colonized the guts of 33 animals (mammals) from three different dietary groups: meat eaters (red), plant eaters (green), and those that eat both (blue). Circles represent individual animals. The lines radiating from each circle represent connections to bacterial species found within that animal's intestine, some of which were found in other animals as well. The clusters of green, red, and blue circles show that patterns of certain bacterial species in the gut tend to be similar in mammalian hosts that share the same diet. In contrast, the right diagram shows connections between the animals and bacterial genes. The overlap of all the circles (in the center) revealed that there is a core set of bacterial genes in the guts of all animal hosts, regardless of diet. However, diet did correlate with other aspects of the bacterial genes in different animals, and seemed to match the corresponding metabolic requirements. For example, bacterial genes involved in breaking down proteins were more abundant in the guts of meat eaters, while the plant-eaters' bacteria were enriched for genes involved in synthesizing the building blocks of proteins. Similar analyses in human beings showed that the intestines of people with different diets contained different bacterial species and different sets of bacterial gene functions.

These studies demonstrate that diet can influence the types of bacterial communities that flourish in the guts of their animal hosts, and suggest that altering diet could change the microbial makeup of the human intestine. To fully characterize the complement of microbes and their collective genomes (the microbiome) in different parts of the body, the NIH has established the Human Microbiome Project, for which NIDDK Director Dr. Griffin Rodgers serves as Co-chair. Through continued support for research in this important area, the NIDDK aims to define the role of the microbiome in disease progression, with the hope of developing new therapeutic approaches to improve health.

Images provided by Dr. Jeffrey I. Gordon, and from Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, and Gordon JI: Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970-974, 2011. Reprinted with permission from AAAS.

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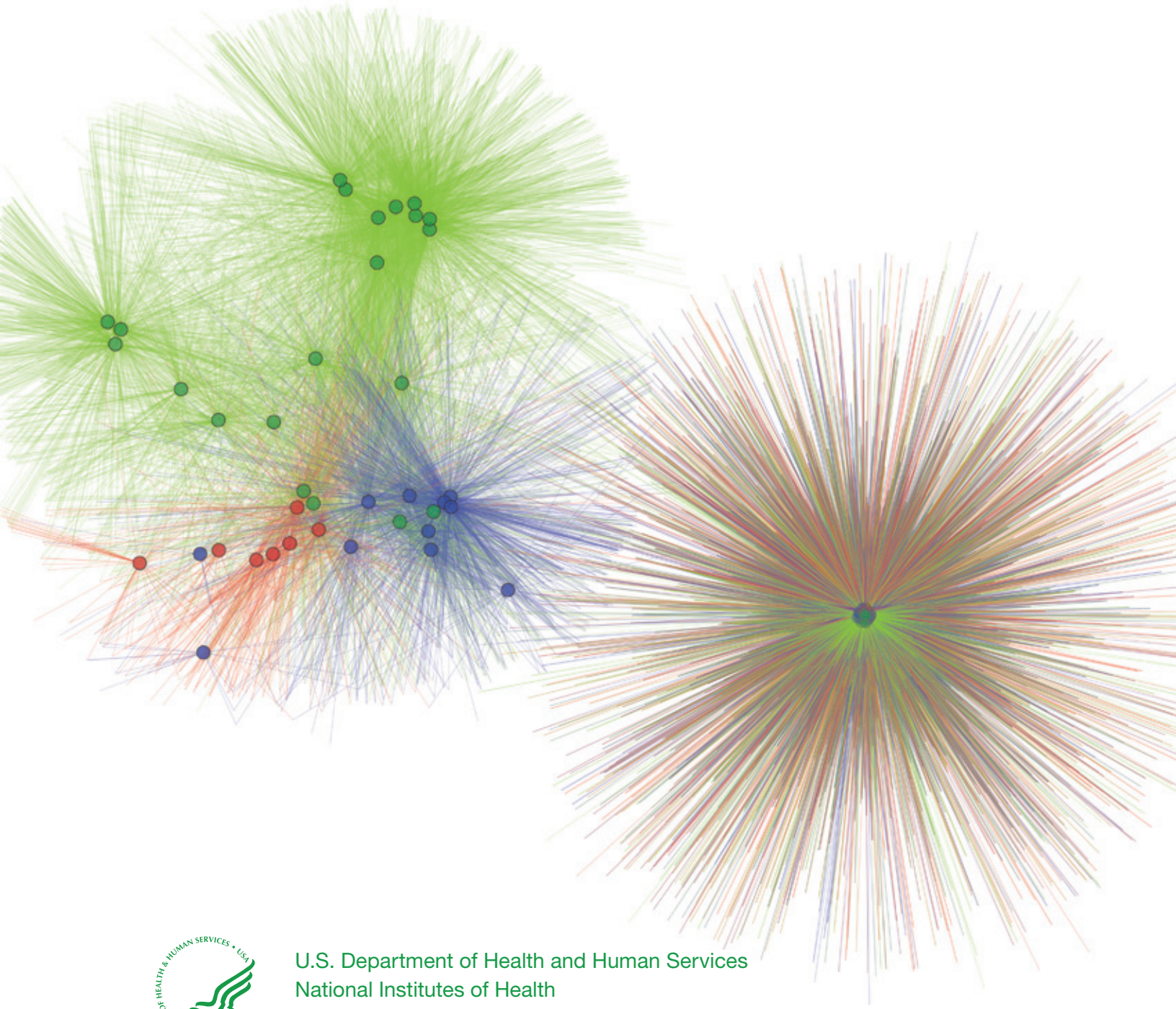

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Message from the Director

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual compendium highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility, which 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, such as inflammatory bowel diseases; nutritional disorders and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases and conditions, such as interstitial cystitis/painful bladder syndrome; and hematologic diseases, such as Cooley's anemia.

The 12th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Identification of genetic variants associated with body fat percentage, body fat distribution, and body mass index
- Successful replacement of defective urethral tissue in children with new, functional urethras engineered using biodegradable scaffolds and cells from patients
- Discovery that a naturally produced compound can improve metabolic defects in mouse models of insulin resistance
- Demonstration that the combination of two drugs, pegylated interferon and ribavirin, more effectively treats hepatitis C infection in children
- Demonstration that the hormone hepcidin reduces the toxic buildup of iron in tissues and organs and improves anemia in rodent models of beta-thalassemia
- Discovery that vitamin E helps diminish severe nonalcoholic fatty liver disease in some children
- Discovery of genetic variants associated with increased risk for inflammatory bowel diseases
- Finding that the drug abatacept can slow disease progression in people with new-onset type 1 diabetes
- Identification of a compound that protects cells from the abnormal antibody responsible for Graves' disease

This report also includes personal stories of patients. A woman with chronic pancreatitis describes the challenges of managing her painful disease while maintaining an active life as a mother and radio show host. A youth explains the daily effort required to manage type 1 diabetes, and how he raises awareness for continued research toward a cure for this disease. A man shares his experience participating in an observational study to understand the long-term health consequences of acute kidney injury.

The NIDDK is continuing efforts to ensure that knowledge gained from its research 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 in printed format and on the NIDDK web-site so that they are readily available for patients, health care providers, and the public. I invite you to visit the web-site at *www.niddk.nih.gov*

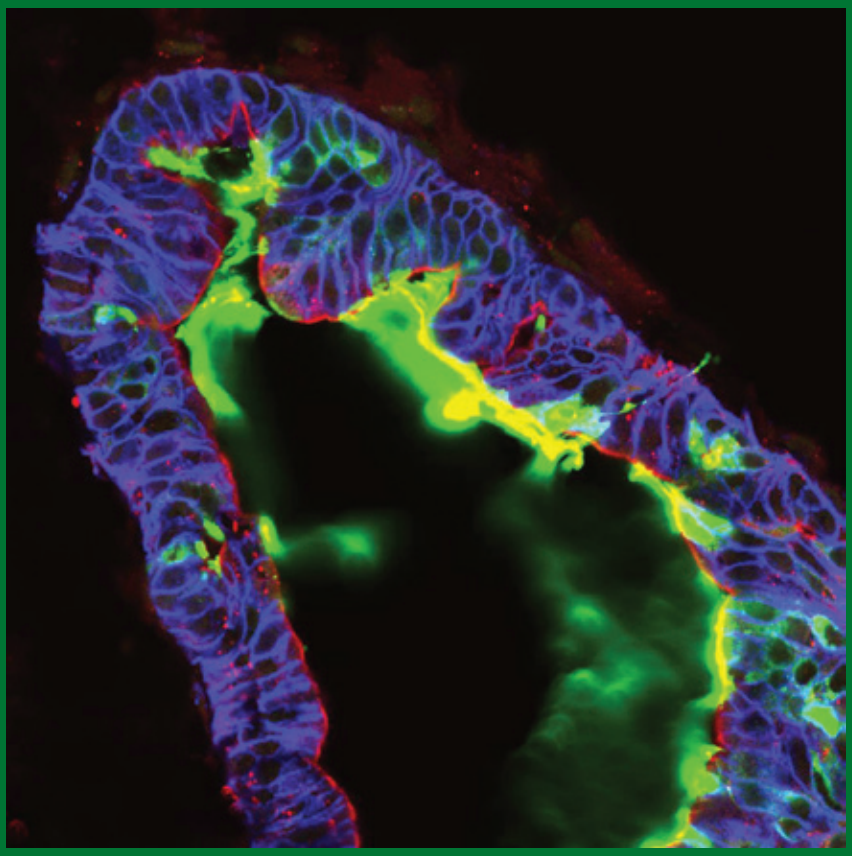
This report reflects only a fraction of the immense body of research performed by basic scientists, clinical investigators, and patient volunteers. We remain committed to translating their efforts into improvements in the health and quality of life of all people.

Griffin P. Rodgers, M.D., M.A.C.P.
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
U.S. Department of Health and Human Services

The 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

Scan the QR code with your mobile device to link to the NIDDK web-site at *www.niddk.nih.gov*



Studies described in this chapter are uncovering the signals that turn stem cells into unique cell types capable of maintaining their identity over multiple cell divisions and even developing into whole tissues in the lab. For example, this image shows a three-dimensional intestinal "organoid," a tissue grown in the lab from stem cells to mimic the structure, cellular complexity, and function of the human intestine. The organoid includes the column-shaped "epithelial" cells found in human intestine (outlined in blue by the presence of a protein called "E-cadherin"). The location of a protein called "villin"—marked in red—on one side of the epithelial cells demonstrates that the organoid epithelial cells are arranged in a manner similar to their organization in the human intestine. Finally, the presence of a protein called "mucin"—shown in green in this image—indicates that the organoid also contains a specialized cell type, which functions as it does in the human intestine by secreting mucin, the major component of intestinal mucus. This pioneering work can help facilitate multiple research directions related to intestinal health and disease, including identifying disease processes, testing new drugs, and regenerating the intestine or generating tissue for transplantation. Other research findings highlighted in this chapter show the promise of related studies on kidney, pancreatic, and blood cells for similar benefits in these tissues.

Image provided by Dr. James M. Wells, and reprinted by permission from Macmillan Publishers Ltd: Nature Methods 8: 111, copyright 2011.

Cross-Cutting Science

Advances in medicine are largely dependent on the accumulation of new knowledge about biologic processes, often at the smallest levels of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other. Such basic research discoveries can have broad and far-reaching implications. Major strides in fighting disease can be traced to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Opportunities to make exciting discoveries are arising ever more rapidly with the development of innovative technologies, novel approaches, and even new scientific disciplines as teams of talented, creative researchers join together to pursue increasingly complex challenges. Described in this chapter are several recent studies, whose themes span multiple areas within the NIDDK research mission. The insights gained through this research can be expected to aid progress in many scientific endeavors, for today's research advances may lead to tomorrow's cures.

MAKING A MARK: HOW NIDDK IS ADVANCING EPIGENOMIC RESEARCH

Epigenetics is an emerging frontier of science, relevant to disorders and diseases under the NIDDK mission. This field—the study of changes in gene activity that are not dependent on DNA sequence—is revealing a dynamic layer of regulation in the cell. Chemical modifications to the DNA or on the proteins that package DNA—both of which are called "epigenetic" marks—can silence a gene or turn it on, and these marks alone or in combination lead to exquisite control of gene activity. The ability of an organism to start as a single cell—with a single genome—and derive a multitude of tissues and cells with different functions to become a multi-cellular organism results from these marks. By progressively restricting the activity of gene sets in various tissues and thereby creating, for example, a liver cell or a fat cell, epigenetic marks regulate an organism's development.

Epigenetic marks can be hereditary and long-term or permanent, or can be newly acquired in reaction to a stimulant. The dynamic and responsive nature of epigenetic marks allows for interaction with the environment, whether it's the hormonal or metabolic environment from cellular signals or the outside environment of chemicals, drugs, or diet. By altering the regulation of genes, epigenetic marks could make people more or less susceptible to developing diseases. Therefore, these marks, their regulation, and their function are the focus of many studies related to human development, health, and a variety of diseases and disorders.

To highlight current efforts and stimulate discussion on the NIDDK's role in this field, in February 2011 the NIDDK held a special forum on epigenomics at the meeting of its National Advisory Council. Whereas epigenetics refers to the study of these chemical marks in a single gene or set of genes, epigenomics refers to more global analysis of epigenetic changes across the entire genome. Because of the enormous potential for epigenetic and epigenomic research to enhance understanding of the biological processes of human health and diseases, the NIDDK and NIH support cutting-edge research in this field, including the Roadmap Epigenomics Program, led by several NIH institutes including the NIDDK. This Program supported five research initiatives to transform epigenomics research and launched the Roadmap Epigenomics Mapping Centers, an effort to develop comprehensive epigenome maps for the benefit of the scientific community.

Where the Human Genome Project produced a comprehensive atlas of all the genes in a human cell, the goal of the Roadmap Epigenomics Program is to build a parallel reference of the epigenome, detailing not only where specific chemical marks are found in the genome, but how they vary across healthy cells in different tissues and individuals. With such reference maps, researchers can then ask questions about how the environment or disease alters the epigenome. Such work is not trivial; technological advances in DNA sequencing as well as the ability to detect specific chemical marks were necessary to propel this effort. There are numerous epigenetic marks and the number grows as new ones are discovered. In addition, the researchers are mapping the marks in progenitor cell types, including induced pluripotent stem cells, cell lines, and cells from various human tissues. Already many maps have been generated, allowing researchers to discover how patterns of specific marks vary in different types of cells. More information on the Roadmap Epigenomics Program can be found at *http://commonfund.nih.gov/epigenomics/*

In addition to participating in the Roadmap Epigenomics Program, the NIDDK supports research in this field by extramural investigators in universities and medical centers, as well as within its own Intramural Research Program. While some epigenetic marks are transitory, others are long-term or permanent and need to be passed on faithfully through cell generations. Research presented at the Council forum described results of investigations into how these marks are maintained and passed on during cell division. Scientists are also looking at how proteins involved in gene regulation are influenced, either in activity or location, by these chemical marks. Patterns of epigenetic marks have been used to identify regions of the genome that do not encode proteins, but that do influence gene regulation—regions previously thought to be inactive and called "junk DNA." Seminal research in NIDDK's Intramural Research Program, presented at the Council forum, identified proteins that bind to these regions, revealing that they act to organize the DNA within a cell's nucleus and coordinate gene activity on a genome-wide level. In other efforts, researchers continue to discover new epigenetic marks and probe their effects on gene regulation. Investigators are also now revisiting previously identified areas of the genome associated with complex diseases to determine whether epigenetic marks and patterns are the key to some of these disease associations.

Exciting NIDDK research has begun to reveal some of the promise that epigenomics holds for understanding human health and disease, and new discoveries are expected as research in this field grows. Scientists are working toward cracking the code—discovering all the ways that epigenetic marks influence gene activity—enabling them eventually to read these annotations on the DNA and better predict biological activity. They are riding a tidal wave of new technologies and wealth of information continuing to rise from epigenomic research. Harnessing all this information toward strategies to promote health, prevent disease, and develop new and improved therapies is a goal of NIDDK research. Advances in the Cross-Cutting chapter and the Diabetes, Endocrinology, and Metabolic Diseases chapter in this compendium highlight some of the progress NIDDK-supported researchers are making in the epigenetics of disorders and diseases.

GENERATING NEW TISSUES FROM STEM AND PROGENITOR CELLS—POTENTIAL FOR FUTURE THERAPIES

Scientists Coax Human Pluripotent Stem Cells To Become Three-dimensional Intestinal Tissue: A group of scientists has succeeded in developing a method for turning human adult stem cells that are pluripotent, or capable of becoming any of a number of cell types, into three-dimensional intestinal tissue in culture. Cell culture (laboratory-grown), or *in vitro*, models that reflect the complexity of human tissues could have several health applications, such as understanding developmental and disease processes, as well as testing new therapies. Scientists have determined how to make pluripotent stem cells develop into a single cell type in culture. However, growing a complex tissue like the human intestine, which is composed of multiple layers of unique cell types, in cell culture had thus far eluded them.

Researchers applied their knowledge of key growth factors that drive the differentiation of cells into particular cell types to solving the problem of developing human intestinal tissue in culture. First,

they treated human pluripotent stem cells with a factor called activin A, which caused the cells to form a single layer of tissue similar to the endoderm, a primitive tissue in the developing embryo that later becomes the intestine and other organs. Next, the researchers added a combination of factors called FGF4 and WNT3A, which are important in intestinal development. They found that these factors acted synergistically in guiding the tissue layer to undergo a series of morphologic changes—rolling up into a tube and budding off to form floating "spheroids" with the appearance of miniature intestines. Then the spheroids were transferred to a three-dimensional, gel-based cell culture system that supports intestinal growth. In this milieu, the spheroids changed again into a complex tissue with hallmarks of intestinal epithelium, such as protrusions (villi) on its inner surface. With more time in culture, the tissue became more similar to mature intestinal tissue, displaying a brush border (the brush-like appearance at the tops of some intestinal cells), all of the major cell types of the intestine, and a capacity for nutrient absorption. With their new method, the scientists were able to generate three-dimensional intestinal tissue from several different types of human stem cells. The researchers used this intestinal tissue model to identify molecular pathways contributing to a human genetic condition associated with loss of an intestinal cell type.

This study is the first to show that human pluripotent stem cells can be coaxed to develop into a three-dimensional tissue with features of the human intestine. The impact of this pioneering work has the potential to radiate out in several new research directions, including elucidating pathways involved in inherited intestinal conditions, testing new drugs for their intestinal absorption, and even generating tissue for transplantation in conditions such as inflammatory bowel diseases, necrotizing enterocolitis, and short-gut syndromes.

Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature 470: 105-109, 2011.

Identification of a Population of Progenitor Cells Capable of Restoring Lost Kidney Function: Scientists have identified a population of progenitor cells in the model organism zebrafish that is capable of forming new filtering units in the kidney following injury.

The nephron is the basic structural and functional unit of the kidney. The cells that comprise the nephron work together to filter blood, removing waste and excess fluid to be excreted as urine. Mammals, including humans, can partly repair damaged nephrons but are incapable of forming new ones. In contrast, fish are able to add new nephrons throughout their lifespan and can regenerate nephrons following injury. In the current study, researchers used zebrafish to study nephron regrowth. Scientists identified small aggregates of cells located throughout the zebrafish kidney that began to proliferate and form new nephrons following drug-induced kidney injury. These progenitor cells were also able to form new nephrons after being transplanted into other fish. Furthermore, real-time imaging of nephron formation in transparent zebrafish larvae showed that the aggregates form when multiple progenitor cells come together and then differentiate into nephrons.

Overall, the process of nephron formation in the zebrafish following injury was similar to that seen in mammalian kidney development. These findings suggest that, if such progenitor cells exist in mammalian kidneys, it might be possible to identify and activate them following nephron loss. The development of a regenerative therapy to restore kidney function in patients with kidney injury or chronic kidney disease would have a significant impact on patient care and well-being.

Diep CQ, Ma D, Deo RC, et al. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. Nature 470: 95-100, 2011.

New Information on How To Make and Maintain Beta Cells: Scientists discovered important steps in the development and maintenance of insulin-producing beta (β) cells. Loss of β cell function plays a role in both type 1 and type 2 diabetes. Therefore efforts to develop functional β cells are important for both forms of the disease. While scientists have made great strides toward generating β cells from stem cells in the laboratory for cell therapies and research, this goal has not yet been achieved. Two studies have made further headway toward this goal by revealing the roles that chemical marks on the genome play in instructing cells to become and remain β cells, and restricting them from other possible cell fates.

In one study, researchers investigated whether there are early chemical marks on the proteins that package DNA that guide certain cells in a choice between becoming pancreas or liver. They examined liver- and pancreas-specific genes in multipotent mouse cells—cells that have the ability to become many types of cells. In these multipotent cells, the liver and pancreas genes are not active, but the scientists found that liver and pancreatic genes have distinct and different "prepatterns" of the chemical marks, depending on whether the cells will go on to become liver or pancreas cells. These marks may help predict which multipotent cells are poised to become β cells, or be a target to coax multipotent cells to become β cells—both of which could help scientists generate β cells in the laboratory.

In the second study, researchers examined whether a specific chemical mark on DNA, known as "methylation," was important to maintain a β cell's identity. Researchers genetically altered mice to lack a protein in β cells responsible for this mark. This led to a loss of β cells and an increase in another pancreas cell type, the α cell. The scientists demonstrated that these new α cells derived from dividing β cells, and that the methylation mark is important to block activation of a gene that would promote α cell development. This discovery—of a mark critical to maintaining β cell identity—provides a new opportunity to promote β cell development. Insights from these two studies arm scientists with critical information toward producing β cells in the laboratory. By manipulating these marks, scientists may be able to coax cells to become and remain β cells.

Xu C-R, Cole PA, Meyers DJ, Kormish J, Dent S, and Zaret KS. Chromatin "prepattern" and histone modifiers in a fate choice for liver and pancreas. Science 332: 963-966, 2011.

Dhawan S, Georgia S, Tschen S, Fan G, and Bhushan A. Pancreatic β cell identity is maintained by DNA methylation-mediated repression of Arx. Dev Cell 20: 419-429, 2011.

Wnt—the Multitasker: Three new studies are providing key insights into the role of the Wnt signaling pathway in several different biological processes: blood stem cell production, regeneration of new bladder cells following injury, and growth of cells in the kidney.

All blood cell types are derived from a population of self-renewing hematopoietic (blood) stem cells (HSCs) that first appear during embryonic development. As *Wnt* genes have been identified as having an important role in some aspects of embryogenesis, researchers sought to better understand the role of Wntl6—a member of the Wnt signaling pathway—in the formation of HSCs. In the first study, researchers examined zebrafish and found that a reduction of the levels of Wnt16 protein during early stage embryonic development blocked the production of HSCs. The results indicate that Wntl6 is required for the production of HSCs during zebrafish development; given that the gene shows similar patterns of expression in mice, it is possible that it has a similar function in mammals.

When the bladder is invaded by bacteria, it initiates a counter-attack, shedding the innermost layer of cells in contact with urine in an effort to prevent invading bacteria from getting a foothold on underlying cells deeper within the bladder wall. Within 24 hours of bacterial infection in mice, remaining bladder cells begin to divide to replenish cells that have been shed. The second study identified a positive feedback loop whereby the bladder cells signal to underlying stromal cells following injury, and the stromal cells stimulate the bladder cells to divide. This growth signal is mediated through Wnt pathways.

Waste removal in the kidney occurs in tiny units called nephrons. During kidney development, the nephron progenitor cell population needs to be precisely controlled to ensure the formation of the appropriate number of nephrons. In the third study, researchers demonstrated in mice that the Wnt9b signaling pathway is active in progenitors and is required for their renewal and proliferation. Wnt9b influences nephron development in conjunction with the transcription factor Six2. When Six2 is present, Wnt9b directs the progenitor cell to remain a progenitor; when Six2 levels are diminished, Wnt9b induces the progenitor cell to develop (differentiate) into a nephron. Thus, the kidney uses Wnt to pre-load a developmental process that is then modulated by the levels of Six2.

Together, these reports provide important information about fundamental molecular and cellular signaling

processes in cell development, growth, and response to injury.

Clements WK, Kim AD, Ong KG, Moore JC, Lawson ND, and Traver D. A somitic Wntl6/Notch pathway specifies haematopoietic stem cells. Nature 474: 220-224, 2011.

Shin K, Lee J, Guo N, et al. Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. Nature 472: 110-114, 2011.

Karner CM, Das A, Ma Z, et al. Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development. Development 138: 1247-1257, 2011.

WHEN THE BODY ATTACKS ITSELF—RESEARCH ON SMALL MOLECULES TO TREAT AUTOIMMUNE DISEASE

Identification of New Class of Compounds That Suppress Autoimmunity: Scientists have developed a novel small molecule that suppressed onset and reduced severity of an autoimmune disease in a mouse model. Autoimmune diseases result from a misguided attack against the body's own organs, tissues, or cells launched by the immune system. These diseases differ in how they manifest; for example, in inflammatory bowel diseases, the misguided immune system action leads to inflammation in the intestines, while in multiple sclerosis, the protective coating that surrounds nerve cells is attacked. Because these diseases result from inappropriate activity of the immune system, current treatments for some autoimmune diseases suppress both harmful and protective aspects of the immune system. These treatments can have toxic side effects and increase a person's risk for infection. Scientists, therefore, are pursuing therapies to more selectively repress specific cells involved in autoimmunity.

In a recent advance, a multidisciplinary team of researchers developed a small molecule, called SR1001, which selectively affects T_H17 cells—immune system cells that have been previously implicated in autoimmune diseases. They demonstrated that SR1001 binds to and represses two proteins, called RORα and RORγt, whose activity is required for the development of T_H17 cells. The scientists discovered that SR1001 inhibited the development of certain mouse cells into T_H17 cells. In addition, when they added SR1001 to existing mouse and human T_H17 cells, they observed a decrease in activation of genes linked to T_H17 cell function.

The scientists also tested whether SR1001 treatment had an effect on a mouse model of multiple sclerosis—one of several autoimmune diseases in which T_H17 cells are known to play a role. Treatment with SR1001 suppressed the clinical severity and onset of autoimmunity in these mice. Importantly, SR1001 was found to target T_H17 cells and not other immune cells, suggesting that the compound may provide a specific way to suppress disease-causing cells without generally suppressing the immune system. Scientists will need to investigate whether SR1001 has potential side effects beyond the immune system and whether it has utility in human disease, but these exciting results identify a new class of compounds—SR1001 and related molecules—that have promise in the treatment of autoimmune diseases.

Solt LA, Kumar N, Nuhant P, et al. Suppression of T_H17 differentiation and autoimmunity by a synthetic ROR ligand. Nature 472: 491-494, 2011.

Potential New Treatment for Graves' Disease: Scientists have identified a small molecule that inhibits the unregulated cellular signaling that gives rise to Graves' disease, also known as toxic diffuse goiter. Graves' disease is the most common cause of overactive thyroid (hyperthyroidism) in the United States. Hyperthyroidism occurs when the thyroid gland makes more thyroid hormone than the body needs. Current treatment choices include radioiodine therapy, surgery, or drugs that block thyroid hormone action and can have rare, but serious, toxicity. The gland makes two hormones, triiodothyronine and thyroxine, that affect nearly every part of the body: metabolism, brain development, heart and nervous system functions, bowels, body temperature, muscle strength, skin and hair, menstrual cycles, weight, and cholesterol levels. Thyroid hormone production is regulated by another hormone called thyroid-stimulating hormone (TSH), which is made by the pituitary gland in the brain. Graves' disease is an autoimmune disease, in which the immune system makes thyroid-stimulating antibodies that activate the TSH receptor (TSHR). These abnormal antibodies mimic the action of TSH and stimulate the thyroid to make too much thyroid hormone.

In new research, scientists synthesized and tested several small molecules, and identified one that directly blocks thyroid-stimulating antibodies from activating the TSHR. The small molecule, called NCG00229600, appears to act by binding to a site on the TSHR independent of where the thyroid-stimulating antibodies (and TSH) bind—an "allosteric" site. Once bound, it most likely prevents antibody-induced changes in the shape of TSHR, which would be required for receptor activation. To test whether NCG00229600 could inhibit signaling by the TSHR, the scientists used two different cell types: an established cultured cell line with high levels of TSHR and cells isolated from human thyroid tissue. They incubated these cells with blood samples that had been collected from 30 individuals with Graves' disease. These blood samples contained the disease-associated thyroid-stimulating antibodies. In the cultured cell line, the presence of NCG00229600 inhibited TSHR signaling by 39 percent; in the thyroid cells, the level of inhibition was 65 percent. NCG00229600 has not yet been tested in clinical trials with people who have Graves' disease, but this exciting research has opened up a new avenue for possibly treating this chronic disease.

Neumann S, Eliseeva E, McCoy JG, et al. A new small-molecule antagonist inhibits Graves' disease antibody activation of the TSH receptor. J Clin Endocrinol Metab 96: 548-554, 2011.

MAPPING THE CELL: PROTEIN STRUCTURE AND GENETIC REARRANGEMENTS

Scientists Paint a "High-Definition" Picture of a Cell Surface Signaling Protein: A detailed description of the three-dimensional structure of the A_{2A} adenosine receptor has shed new light on one of the fundamental elements of how cells in the body communicate with each other. The detailed structure of this receptor was determined to a resolution finer than 3 angstroms—a unit of measurement that is 1/10,000,000,000 (one ten-billionth) of a meter in length. (By way of comparison, a common house fly is approximately 60 million angstroms long.)

To detect and respond to signaling molecules, cells often employ specialized proteins on their surface termed "receptors." Once a signaling molecule binds, the receptor initiates a cascade of events that results in a cellular response. Signaling through the receptor for the molecule adenosine plays an important role in a diverse array of biologic processes, from playing a key role in cardiovascular physiology to mediating coffee's caffeine-driven "buzz." Adenosine receptors are members of a particularly complex family of lengthy proteins that zig-zag back and forth across the cell membrane a total of seven times—the "7TM" receptors. The precise physical and biochemical changes that these receptors undergo upon binding various activators and inhibitors remain a largely unresolved question. In part, this is because their unusual structure has made these proteins poorly suited for traditional methods that have been used to probe structure/function relationships in biomolecules.

While all receptors in the 7TM family share a similar overall structure, there are significant differences among them in several regions, which are hypothesized to contribute to the different responses each receptor elicits when activated. In the current study, the researchers isolated and crystallized a form of the A_{2A} adenosine receptor bound to a synthetic activator. (There are four related receptor subtypes for adenosine.) They described how three of the seven membrane-spanning segments of the receptor tilt, rotate, shift, and "see-saw" when bound to the activator, as compared to their orientation in its absence. Additionally, when the activator was present, one loop of the extracellular portion of the A_{2A} adenosine receptor shifted in a way that the researchers hypothesize is specific to this member of the 7TM family. This enhanced understanding of basic structural and functional changes of the receptor when it is bound to an activator represents a key contribution to researchers' knowledge of this family of molecules. It also demonstrates that it is possible to obtain a stable, activator-bound form of a 7TM receptor at high resolution, which will be helpful in future studies of other members of this family.

Xu F, Wu H, Katritch V, et al. Structure of an agonist-bound human A_{2A} adenosine receptor. Science 332: 322-327, 2011.

A New Map Drives Studies of Genetic Rearrangement: Scientists discovered key features of hotspots associated with genetic rearrangement—the

process by which cells intentionally break, shuffle, and repair their DNA to create new combinations of genes. Genetic rearrangement occurs naturally in cells destined to become sperm and eggs to generate genetic diversity, ensuring that each organism is unique. This process can be beneficial in that new advantageous traits can arise from these genetic rearrangements, but if it goes awry, this process can also generate abnormalities that result in miscarriages, congenital birth defects, and mental retardation. Scientists had known that genetic rearrangements occur more frequently at certain locations in the genome—"hotspots"—but they did not know what makes these locations hotspots. Previous studies focused on individual hotspots, but to determine the common features of mammalian hotspots scientists needed a way to map all of the hotspots in an organism. Researchers in NIDDK's Intramural Research Program developed a molecular approach to identify and catalog hotspots in mice, and generated a high-resolution physical map of these sites in the mouse genome. This map allowed them to identify common characteristics of the mapped hotspots, such as a tendency for hotspots to be found in genes, to be associated with a complex of proteins that package DNA (nucleosomes), and to have a sequence similar to that of the binding site for a protein, called PRDM9, that modifies nucleosomes. The scientists went on to find that hotspots were associated with the specific nucleosome modification produced by PRDM9, hinting at how the process of genetic rearrangement unfolds. In addition to providing a powerful new tool for studies of genetic rearrangement, these findings have the potential to improve the detection of genes linked to disease and to help understand the causes of genetic abnormalities.

Smagulova F, Gregoretti IV, Brick K, Khil P, Camerini-Otero RD, and Petukhova GV. Genome-wide analysis reveals novel molecular features of mouse recombination hotspots. Nature 472: 375-378, 2011.

Dr. David T. Breault and Dr. Jose C. Florez: NIDDK-Supported Scientists Receive Presidential Award

In September 2011, President Barack Obama recognized 94 U.S. scientists, including two supported by the NIDDK, as recipients of the 2010 Presidential Early Career Awards for Scientists and Engineers (PECASE; *www.whitehouse.gov/the-press-office/2011/09/26/president-obama-honors-outstanding-early-career-scientists)*.

PECASE is the highest honor bestowed by the U.S. government on science and engineering professionals in the early stages of their independent research careers. Awardees are selected for their innovative research and their commitment to community service demonstrated through scientific leadership, public education, or community outreach.

Among the 2010 recipients are David T. Breault, M.D., Ph.D. and Jose C. Florez, M.D., Ph.D., both NIDDK extramural grantees.

In addition to Drs. Breault and Florez, 18 other NIH-supported scientists received the award for their research achievements. The NIH has now funded 193 PECASE recipients since the award's inception in 1996. A list of NIH scientists who have received this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

Characterizing Intestinal Stem Cell Populations

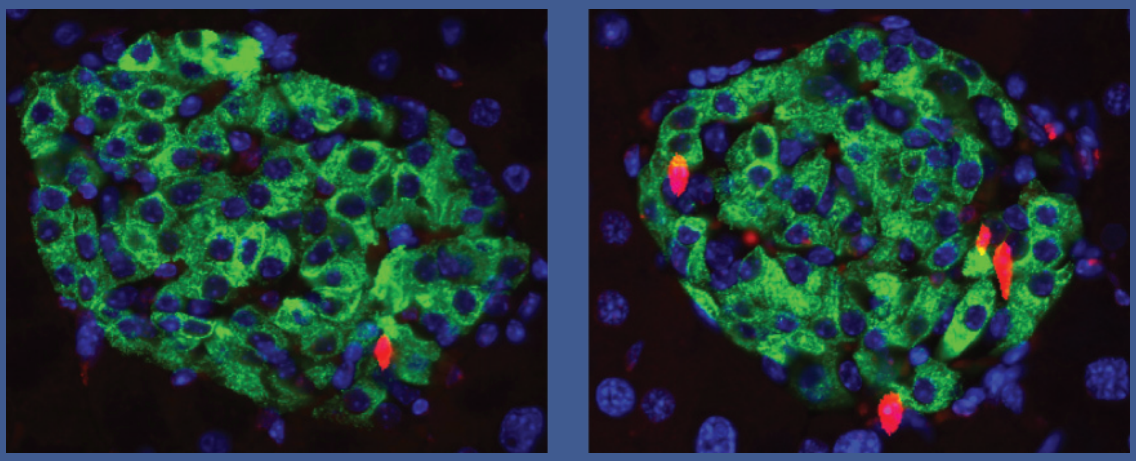
David T. Breault, M.D., Ph.D.

Dr. Breault, a pediatric endocrinologist at Children's Hospital Boston and Harvard Medical School, received a 2010 PECASE award for his research characterizing a subpopulation of intestinal stem cells in mice with unique reparative properties identified using an innovative biomarker. In humans and mice, the lining of the digestive tract undergoes continuous and rapid renewal throughout life, which is sustained by stem cells located within indentations in the intestinal surface called "crypts." This renewal is critical to repopulate lost cells and maintain a barrier against the potentially harmful contents of the digestive tract. In the absence of this renewal, the intestinal tissue may become diseased or inflamed. Therefore, scientists like Dr. Breault are studying intestinal stem cells for clues that could lead to the development of novel therapies for these conditions. Dr. Breault and his colleagues studied a mouse genetically modified so that cells with the biomarker, a specially tagged version of a cellular component called telomerase, are visible under a fluorescent microscope. Telomerase adds special caps to the ends of chromosomes, to protect the cell's genetic material, and is particularly important for cells that must continue to divide, such as stem cells. With this mouse model, Dr. Breault and his colleagues identified a slowly dividing stem cell subpopulation within intestinal crypts. Interestingly, this subpopulation is resistant to injury and contributes to the regenerative response following this injury, suggesting that it may have therapeutic potential. The results of Dr. Breault's pioneering research studying this cell population and its regulatory pathways may give rise to novel therapeutic strategies for gastrointestinal diseases, such as inflammatory bowel diseases, short bowel syndrome, and intestinal cancer.

Learning How Genetics Has an Impact on the Efficacy of Diabetes Drugs

Jose C. Florez, M.D., Ph.D.

Dr. Florez, a geneticist and endocrinologist at Harvard Medical School, received a 2010 PECASE award for his studies to characterize genetic loci associated with type 2 diabetes and related traits in an effort to advance individualized therapy. Although a number of genetic loci have been associated with type 2 diabetes, in many cases the identity of the causal gene and understanding of how it affects disease risk and treatment remain unknown. Dr. Florez's research employs an innovative strategy combining state-of-the-art pharmacogenetic (the study of how genetics influences a drug response) and metabolomic (the study of molecules involved in metabolism) approaches in human studies. In a current study, Dr. Florez and his colleagues will measure the effects of two different diabetes medications—glipizide and metformin—in people at risk for diabetes or with diet-treated diabetes and compare the responses of people who have differences in genetic variants associated with diabetes. In a previous study of the genetics of participants in the NIDDK's Diabetes Prevention Program, Dr. Florez's group found that metformin was ineffective in preventing diabetes in individuals with two copies of a common variant of the *SLC47A1* gene, which encodes a protein that transports metformin, while it was effective for prevention in those with one or no copies of the variant. In addition to furthering understanding of the biology of glucose regulation and how drugs affect the disease, Dr. Florez's research aims to provide new insight into how genetic variants have an impact on an individual's diabetes risk and personalized treatment.



In research described in this chapter, scientists discovered telomere shortening can play a role in metabolic diseases like cardiovascular disease and type 2 diabetes. Telomeres are specialized regions that "cap" the ends of chromosomes to protect critical DNA sequences from being lost each time a cell replicates its DNA and divides. As cells age, telomeres erode, leading to damaging consequences. The effect of telomere shortening is particularly apparent in a mouse model of diabetes. With unaltered telomeres (left), there were relatively few dying cells (red) among the insulin-producing beta cells (green). However, when cells in this mouse model are genetically modified to have short telomeres, scientists observed an increase in cell death (right), as well as loss of beta cell function, and glucose intolerance similar to human diabetes. Short telomeres may increase diabetes risk and serve as a biomarker that can help explore new treatments or prevention strategies.

Images provided by Dr. Mary Armanios, and from Guo N, Parry EM, Li L-S, Kembou F, Lauder N, Hussain MA, Berggren P-O, and Armanios M: Short telomeres compromise β-cell signaling and survival. PLoS One 6: e17858, 2011.

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 25.8 million people in the United States—or 8.3 percent of the total population—and is the seventh leading cause of death.[1] Diabetes lowers average life expectancy by up to 15 years,[2] increases risk of death from cardiovascular disease 2- to 4-fold, and is the leading cause of kidney failure, lower limb amputations, and, in working-age adults, blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the United States in 2007—including costs of medical care, disability, and premature death—was $174 billion.[1] Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.[1]

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 adults and the majority of children and youth with diagnosed diabetes.[1] It most often develops during childhood, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system launches a misguided attack and destroys the insulin-producing beta cells of the pancreas. If left 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, patients require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—in order to regulate their blood glucose levels. Despite vigilance in disease management, with frequent finger sticks to test blood glucose levels and the administration of insulin, it is still impossible for patients to control blood glucose levels to near the normal levels achieved by functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working to develop beta cell replacement therapies 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.[1] Type 2 diabetes is associated with several factors,

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[1] 2011 National Diabetes Fact Sheet. Centers for Disease Control and Prevention. Atlanta, GA.

[2] Portuese E and Orchard T: Mortality in Insulin-Dependent Diabetes. In Diabetes in America (pp. 221-232). Bethesda, MD: National Diabetes Data Group, NIH, 1995.

including older age and a family history of the disease. It is also strongly associated with obesity; more than 80 percent of adults with diabetes are overweight or obese.[3] Type 2 diabetes occurs at elevated rates among minority groups, including African Americans, Hispanic and Latino Americans, American Indians, and Native Hawaiians.[1] Gestational diabetes is also a risk factor: women who have had gestational diabetes have a 35 to 60 percent chance of developing diabetes—mostly type 2 diabetes—in the next 10 to 20 years.[1]

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 79 million adults in the United States who have a condition called "prediabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[1] This population is at high 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. Moreover, follow-up research has shown that this benefit of reduced diabetes risk 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 minority youth. Believed to be related to increasing rates of pediatric obesity, this is an alarming trend for many reasons. First, the onset and severity of disease complications correlate with the duration of diabetes; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. Second, 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 vicious cycle of ever-growing rates of diabetes. Third, diabetes often becomes more difficult to control over time. With longer duration of disease, patients may find it increasingly difficult to strictly control their blood glucose levels and thus prevent or delay the development of complications. 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 Institute's mission; such research will ultimately spur the design of potential new intervention strategies. Exploring interrelationships between some of these diseases is an important and informative facet of this work—for example, diabetes is becoming an increasing problem for people with cystic fibrosis, as life expectancy for these individuals has improved due to advances in cystic fibrosis treatment. In parallel, based on knowledge from past scientific research investments, the Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

MOLECULAR MECHANISMS OF REGULATING THE BODY'S BLOOD GLUCOSE LEVELS

FGF19 Lends Insulin a Hand: A new discovery is reshaping our understanding of the way the body's hormones control blood glucose. While the hormones insulin and glucagon have long been known to respond to the influx of glucose with a meal or to fasting between meals, scientists recently found that another hormone, FGF19, adds a new dimension to this regulation as food makes its way through the digestive tract. During periods of fasting, the pancreas secretes glucagon, which signals the liver to liberate glucose stored there in the compound glycogen, and release it into the bloodstream. When we eat, glucose and other nutrients are transported from the digestive system into

[3] *Eberhardt MS, et al. MMWR 53: 1066-1068, 2004.*

the blood, and the pancreas begins to release insulin. Insulin is a signal to our cells to take up glucose, and in the liver it also suppresses glucose release, induces restoration of the glycogen supply, promotes protein synthesis, and inhibits the action of glucagon. This two hormone picture became a bit richer with the discovery more than a decade ago that GLP-1, a hormone produced by cells of the upper small intestine when food begins to pass through the stomach, serves to signal to the pancreas that a bolus of nutrients is on the way, boosting the insulin response. That discovery led to development and approval of several important new medications for type 2 diabetes.

The new discovery concerns FGF19, a hormone produced by cells in the final portion of the small intestine (the ileum). FGF19 is secreted in response to bile acids—a group of compounds released from the gall bladder to aid in the digestion of fats. The bile acids accompany digesting food through the intestines, so their presence in the ileum indicates that the digestive process is nearing completion. FGF19 induces the gall bladder to rebuild its supply of bile acids, and this was thought to be the hormone's major purpose. Now researchers have shown that, like insulin, FGF19 signals the liver to synthesize glycogen and protein and reduce glucose production and release. Experiments using the human FGF19 hormone in mice show it helps keep blood glucose levels down, restores the liver glycogen supply in mice that have uncontrolled diabetes, and helps moderate blood glucose and insulin levels in mice fed a diet that tends to promote diabetes. In contrast, mice lacking the mouse equivalent of FGF19 have elevated blood glucose and abnormally low liver glycogen supplies. In healthy humans, insulin levels typically reach their highest point within an hour after eating, whereas the signal to produce FGF19 comes relatively late in the digestive process. Therefore, its levels and impact peak about 3 hours after a meal, shortly before liver glycogen supplies typically reach their zenith. Thus, insulin quickly and potently causes glucose uptake and storage at the initiation of a meal, whereas FGF19 provides a later signal to sustain the insulin-initiated response, continuing production of glycogen and protein and repression of liver glucose synthesis until absorption of a meal is complete. The researchers found that FGF19 stimulates glycogen synthesis in a manner that is completely independent of insulin, and that messages from the two hormones are carried into liver cells by distinct sets of "signal transduction" molecules. These findings suggest it may be possible to help people with diabetes control their blood glucose by developing new medications that act through the same mechanism as FGF19.

Kir S, Beddow SA, Samuel VT, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 331: 1621-1624, 2011.

Potthoff MJ, Boney-Montoya J, Choi M, et al. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1α pathway. Cell Metab 13: 729-738, 2011.

New Insight into Liver Glucose Production:

Researchers have uncovered a cellular pathway in mice and fruit flies that may represent a novel target for treating high blood glucose levels. One function of the liver in humans and many animals is to stabilize the body's glucose levels between meals, when glucose isn't being supplied by food, by producing its own glucose and releasing it into the bloodstream. In type 2 diabetes, this function goes awry, contributing to chronically elevated blood glucose. Normally, liver cells detect when glucose levels are falling via glucagon, a pancreatic hormone that counterbalances insulin and signals fasting, and respond by "turning on" genes involved in glucose production. Many of these genes are under the control of gene regulatory factors collectively called FOXO. In a recent study in mice and in isolated cells (from mice and humans), researchers found that three intracellular proteins, called class IIa histone deacetylases (HDACs), regulate activation of FOXO—and thereby help "turn on" glucose production genes—in a glucagon-dependent fashion. This newly identified mechanism significantly expands what is known about glucose production by liver cells. Intriguingly, a second, overlapping research team studying a simpler organism, the fruit fly, uncovered a similar role for class IIa HDAC activation of FOXO and FOXO-controlled metabolic genes in the insect equivalent of fat and liver. Because this molecular mechanism for increasing energy production under fasting conditions appears to be highly conserved from insects to mammals, scientists think it is likely to exist in humans as well. To determine whether the class IIa HDACs play a role in regulating blood glucose, the first research team

studied their activity in diabetic mice. They found that when they experimentally reduced the amounts of these HDACs in the livers of different mouse models of type 2 diabetes—both genetic and diet-induced—the mice showed significant improvements in both blood glucose levels and glucose tolerance tests. The results of these studies thus not only reveal a new and possibly highly conserved molecular pathway regulating glucose production by the liver, but also suggest that, if this pathway exists and functions similarly in humans, targeting the activity of class IIa HDACs could turn out to be a new therapeutic approach for type 2 diabetes.

Mihaylova MM, Vasquez DS, Ravnskjaer K, et al. Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 145: 607-621, 2011.

Wang B, Moya N, Niessen S, et al. A hormone-dependent module regulating energy balance. Cell 145: 596-606, 2011.

New Hypothesis To Explain Type 2 Diabetes—It Might Begin with the Beta Cell: Recent research links diet and obesity to direct effects on insulin production in the pancreas, suggesting a new hypothesis that may help explain how type 2 diabetes develops. The predominant scientific view today is that, in susceptible people, obesity triggers a chronic, low level inflammatory process that induces insulin resistance. Insulin resistance increases the demand on the insulin-producing beta cells of the pancreas. When the beta cells are unable to make sufficient insulin to restore balance, blood glucose levels rise. Thus, the insulin resistance comes first, and its consequences eventually impinge on the beta cell. The new findings turn that view around, by demonstrating how a high-fat diet can inhibit proper beta cell function in both mice and humans, and trigger symptoms associated with type 2 diabetes.

Beta cells secrete insulin in response to glucose molecules passing through glucose transporter proteins located in the cells' outer membranes. These transporter proteins are therefore critical to beta cell function. To remain stably embedded in the membrane and perform their vital task, glucose transporters must be modified by an enzyme called GnT-4a. Previous work determined that mice lacking the *GnT-4a* gene develop diabetes, and also that a high-fat diet reduces GnT-4a levels in the cell. The new research shows that, compared to healthy beta cells, beta cells from mice with diabetes induced by a high-fat diet did not produce enough GnT-4a to keep an adequate supply of glucose transporters in the beta cell membrane. Moreover, GnT-4a and glucose transporter levels were also abnormally low in beta cells obtained from deceased human donors with type 2 diabetes. As expected, these GnT-4a-deficient beta cells from mice and humans did not respond with robust insulin secretion in the presence of elevated glucose. This phenomenon could also be induced in healthy human beta cells by exposing them to a specific form of fat molecule that mimics the effects of a high-fat diet. To further understand the potential impact of reduced GnT-4a in the diabetes disease process, the researchers generated mice with a genetic change that keeps levels of GnT-4a constant in beta cells, even when the mice eat a high-fat diet. Such a diet did cause mice with constant GnT-4a to become obese, but they did not develop diabetes. Although the animals' blood glucose levels rose higher, when fed high-fat food, than those fed a more healthful diet, their glucose levels did not rise as dramatically as those of normal littermates eating high fat. The mice that always produce GnT-4a in their beta cells also did not develop other common features of diabetes. In particular, they did not develop fatty livers or severe insulin resistance. Thus, continued pancreatic production of GnT-4a not only rescued the ability of the mouse pancreas to respond to glucose despite a high-fat diet, but also it improved the response to insulin in the animals' other organs and tissues. While the mechanisms for that effect remain unclear, it suggests that abnormal beta cell function may play an important role earlier in the development of type 2 diabetes than had previously been appreciated. It also highlights the importance of GnT-4a for maintaining healthy blood glucose levels, and suggests the enzyme may potentially be a useful target for pharmaceutical approaches to treating type 2 diabetes.

Ohtsubo K, Chen MZ, Olefsky JM, and Marth JD. Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat Med 17: 1067-1075, 2011.

INSIGHTS INTO THE LINK BETWEEN AGING AND METABOLISM

Molecular Link Between Energy Intake and Lifespan/Aging: Researchers have discovered a molecular connection between energy intake, lifespan, and the aging process. Previous research in animal

models, such as in the roundworm *Caenorhabditis elegans* (*C. elegans*), has shown that reducing calories extends lifespan. Two nutrient- or energy-sensing proteins found inside cells, called AMPK and calcineurin, regulate lifespan in the worm, but it was unknown how they exert their effects.

Using a *C. elegans* model system, researchers identified a critical protein target of AMPK and calcineurin, called CRTC-1. When the scientists depleted CRTC-1 using genetic techniques, the worms lived longer, suggesting that the protein is also a regulator of lifespan. CRTC-1 is a type of protein that controls whether genes are turned on or off. Further experiments demonstrated that AMPK and calcineurin had opposing effects in regulating the extent to which CRTC-1 was chemically modified (phosphorylated), which in turn controlled whether CRTC-1 could turn on its target genes. Additionally, a protein that interacts with CRTC-1, called CREB, was found to be an important component in this lifespan-regulating pathway because depleting the worm's CREB protein also prolonged its life. Because AMPK, CRTC, and CREB are known to play a role in metabolism in mammals, the scientists hypothesized that these proteins were regulating metabolism-related genes to control the worm's lifespan. Surprisingly, this was not the case. Rather, the worm genes regulated by AMPK/CRTC-1/CREB were involved in a type of stress in a cellular component called the endoplasmic reticulum (ER). Research has suggested that aging-related changes to the ER stress response contribute to diseases such as type 2 diabetes, cancer, and neurodegenerative diseases. This observation provides a possible molecular explanation for how nutrient levels could affect the aging process and longevity. Importantly, the pathway involving CRTC-1/CREB is evolutionarily conserved from worms to mammals, suggesting that these findings may be relevant to people. This research provides new insights into the molecular pathways that link energy intake to aging and lifespan, and illuminates new targets for potential intervention against age-related conditions such as type 2 diabetes.

Mair W, Morantte I, Rodrigues APC, et al. Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. Nature 470: 404-408, 2011.

The Long and the Short of It—How Aging Impairs Metabolism: Two recent studies provide insight into how aging leads to decreased organ function and increased risk for certain metabolic diseases, like cardiovascular disease and type 2 diabetes. From decades of research, two theories emerged to explain how aging is linked to metabolic disease and organ failure. In one theory of aging, organs with cells that divide at a high rate are unable to repopulate dead cells, leading to organ failure. A separate theory of aging emerged from studies of mitochondria—the "powerhouses" of the cell that generate most of a cell's energy through metabolic pathways. Mitochondria also have their own DNA, which is different from the rest of a cell's DNA and contains genes needed for mitochondrial function. Aging, it is thought, results from an accumulation of mutations or alterations of the mitochondrial DNA over time, which leads to decreased energy production and damage to the cell. Therefore, in tissues that do not regularly repopulate their cells, such as the heart, liver, and brain, this loss of energy leads to aging.

In a recent study, scientists demonstrated that these two theories about aging-related disease are linked in that they are two facets of the single process of aging. The link comes from telomeres, specialized structures at the ends of DNA. Within a cell, DNA is packed into chromosomes. To ensure that critical DNA sequences near the ends of chromosomes are not lost each time a cell replicates its DNA and divides, telomeres "cap" the ends of chromosomes. Over time, however, these regions are eroded, prompting the cell to stop replicating and even to initiate a death process. To investigate effects of telomere shortening on cell function and aging, scientists studied mice that were genetically engineered to have short telomeres and found that shortened telomeres were associated with a decrease in mitochondrial mass and impaired mitochondrial function. The mice also showed evidence of heart disease and liver dysfunction, characteristics of aging. These important results link the effects of aging on the cell's DNA through telomere dysfunction with decreased function of the mitochondria.

In another study, a different group of researchers looked specifically at the effects of shortened telomeres on β (beta) cells in the pancreas. β cells produce the hormone insulin which helps the body use glucose for energy. Loss of β cell function is a hallmark of diabetes. The scientists found that mice genetically engineered to have short telomeres had impaired secretion of insulin and resulting high levels of glucose in the blood. Importantly, the β cell mass was not decreased in these mice; rather, the short telomeres led to changes in gene expression (whether genes are "on" or "off") that affected multiple cellular processes involved in insulin secretion. This provides critical information about why β cell function declines with age in many people.

Together, these studies identify a role for telomere shortening and resulting mitochondrial dysfunction in age-related metabolic diseases like type 2 diabetes. While these results need to be confirmed in humans, this knowledge could lead to new treatments or prevention strategies for such diseases.

Sahin E, Colla S, Liesa M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 470: 359-365, 2011.

Guo N, Parry EM, Li L-S, et al. Short telomeres compromise β-cell signaling and survival. PLoS One 6: e17858, 2011.

Turning Back Time on Beta Cell Age: Scientists discovered a mechanism for the decline in β (beta) cell proliferation as mice and humans age and identified a potential new strategy to induce β cell regeneration. β cells, which produce the vital hormone insulin, are destroyed by the immune system in people with type 1 diabetes and may not function normally in people with type 2 diabetes. Identifying ways to replace the lost β cells to restore the body's insulin-producing capacity would benefit people with type 1 and type 2 diabetes, and is a major goal of research. As mice and humans age, the ability of their β cells to divide and make new β cells (or "proliferate") diminishes. Therefore, understanding how the β cells lose this ability could lead to strategies to prevent this loss or even to reverse the process and promote expansion of β cells.

A protein called platelet-derived growth factor receptor-α (Pdgfr-α) is found on the surface of cells and is known to regulate cell division and survival; however, its role in β cells has been unknown. In this study, scientists discovered that levels of Pdgfr-α decreased as mouse β cells aged. To further investigate Pdgfr-α's role, the researchers genetically altered the mice, to ask what happened to β cells if Pdgfr-α levels were reduced prematurely. They found that not only did β cell proliferation diminish in these mice, but that they also had decreased β cell mass and impaired glucose control. Because Pdgfr-α was critical to β cell development in young mice, the scientists speculated that the protein might also control β cell regeneration in adult mice. When mice are given a specific chemical, a significant number of their cells are destroyed, but the remaining β cells proliferate and restore the mass, allowing scientists to study β cell regeneration. Using this tool, the researchers observed high levels of Pdgfr-α in the remaining adult β cells, coincident with an increase in proliferation. However, in the mice genetically altered to have reduced Pdgfr-α levels, the remaining adult β cells failed to regenerate, leading the mice to develop severe diabetes. Now knowing that Pdgfr-α is necessary for β cell regeneration, the scientists investigated whether increasing Pdgfr-α activity could reverse the age-dependent β cell loss. Genetically modifying adult mice to have continual Pdgfr-α activity delayed β cell loss, and instead promoted growth of new β cells and increased β cell mass.

Importantly, the scientists demonstrated that Pdgfr-α and other proteins required for its activity are present in human β cells and undergo a similar age-dependent decline in levels. Stimulating human juvenile cultured β cells with a protein that turns on Pdgfr-α activity led to increased β cell proliferation, indicating that Pdgfr-α may regulate new β cell growth in aging β cells in humans as well as mice. The exciting discovery of mechanisms that regulate age-dependent loss of β cells could provide scientists with new avenues for modulating growth of human β cells to promote their regeneration and replace those lost in diabetes.

Chen H, Gu X, Liu Y, et al. PDGF signalling controls age-dependent proliferation in pancreatic β-cells. Nature 478: 349-355, 2011.

IMPROVING DIABETES SCREENING

New Blood Test May Improve Assessment of Type 2 Diabetes Risk: Research in the emerging field of metabolomics has revealed a new approach

for determining who is at greatest risk for developing type 2 diabetes. Several factors are known to increase a person's type 2 diabetes risk. These include overweight or obesity, age, and a family history of the disease. In addition, blood tests can identify people whose blood glucose is intermediate between normal and diabetic levels—people with glucose levels in this range are said to have prediabetes, because they are at significantly higher risk of type 2 diabetes than peers with lower blood glucose. More than a third of Americans—roughly 79 million people—are considered to have prediabetes by this definition, according to 2011 estimates. These intermediate levels of blood glucose, however, are not a perfect predictor of disease risk; indeed, many people with prediabetes will not go on to develop diabetes in their lifetimes. So a better test for diabetes risk would be invaluable for helping direct scarce preventive health care resources to those who need them most.

To address that need, researchers have turned to the new field of metabolomics, the study of the many chemical compounds our bodies produce in the course of daily life. Looking for a chemical signature of diabetes risk, the researchers compared the blood from a group of 189 people who developed type 2 diabetes over the course of an earlier, 12-year study, to blood from 189 people in the same study who did not develop the disease, but who were otherwise similar in terms of age, blood glucose levels, and degree of overweight. The researchers discovered that blood levels of five different amino acids were higher in people who went on to develop diabetes years later, and confirmed the result in a second set of people. (Amino acids are the building blocks of protein molecules.) By combining tests for three of the five amino acids, they were able to identify a subgroup of people whose risk of diabetes is about six times greater than that of people with otherwise similar risk factors, but lower amino acid levels. The scientists then confirmed the relationship of the amino acid levels to diabetes risk by performing the test in two more sets of people. This re-test not only confirmed the relationship of the amino acids to diabetes risk, it showed that it holds true even in people with comparatively low blood glucose, who are therefore considered to be at relatively low risk of diabetes based on blood glucose alone. This research raises intriguing questions as to the molecular mechanism that ties these amino acids to development of diabetes. If an affordable clinical version of the test is developed, it may one day greatly improve the ability of health care providers to identify people most likely to benefit from diabetes prevention therapy.

Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 17: 448-453, 2011.

Health and Cost Benefits of Genetic Testing for Neonatal Diabetes Mellitus: Research suggests that genetic testing for neonatal diabetes mellitus would not only improve patients' quality of life, but also result in cost savings. Neonatal diabetes mellitus (NDM) is a rare form of diabetes that occurs in the first 6 months of life. It is called "monogenic" diabetes because it is caused by defects in a single gene (as compared to type 1 and type 2 diabetes, in which risk is related to multiple genes). In about one-half of babies with NDM, the condition is transient and disappears during infancy but can reappear later in life; in the other half, the condition is lifelong and is called permanent neonatal diabetes mellitus (PNDM). Research on the genetics of PNDM has shown that over 40 percent of the cases result from defects in one of two different genes (*KCNJ11* or *ABCC8*). Knowledge about how those genes function in the body led to the discovery that people with defects in one of those genes could be treated with oral sulfonylurea therapy rather than injections of insulin—a less burdensome treatment approach that results in better glucose control. However, PNDM can be misdiagnosed as the more common type 1 diabetes, so some patients who could be taking sulfonylureas are instead put on insulin therapy. Thus, scientists are considering whether routine genetic testing—particularly for children under 6 months of age who have been diagnosed with diabetes—should be done to identify individuals who carry one of the PNDM-causing genes so that they are correctly diagnosed with this form of the disease.

In new research, scientists used a conceptual model comparing a policy of routine genetic testing with no testing, to develop estimates of total medical costs and health outcomes over 30 years resulting from correctly diagnosing children with PNDM or misdiagnosing them with type 1 diabetes. The analysis showed that routine genetic testing could result in quality of life benefits that increase over time, and also save approximately $12,500 over 10 years, and $30,400 over 30 years. Improved quality of life is due to factors such as using a less

burdensome therapy and having a reduced lifetime risk for complications. Reduced costs stem from factors such as lower health care costs for treating complications. This research suggests that genetic testing in neonatal diabetes is a medical advance that would improve patients' quality of life and save money. It also highlights the potential economic benefits of implementing personalized genetic medicine approaches.

Greeley SAW, John PM, Winn AN, et al. The cost-effectiveness of personalized genetic medicine: the case of genetic testing in neonatal diabetes. Diabetes Care 34: 622-627, 2011.

New Insights on When To Screen Children for Type 1 Diabetes Risk Factors: Researchers have defined a time period during which to screen children for risk of developing type 1 diabetes. In the disease, a misguided attack of the immune system—known generally as "autoimmunity"—leads to destruction of the insulin-producing β (beta) cells of the pancreas. A feature of the onset of autoimmunity is the body's development of antibodies to β cell proteins. These antibodies are called "autoantibodies" because they recognize proteins within the body, rather than invading pathogens. Importantly, they typically appear before overt symptoms of type 1 diabetes and thus serve as useful clinical predictors of the disease. Blood tests for the presence of autoantibodies can accurately identify relatives of people with type 1 diabetes who are at high or moderate risk of developing the disease within 5 years. At-risk people identified by screening may be eligible for trials to test promising prevention strategies.

Although it is known that diabetes autoantibodies frequently develop in childhood, less is known about the relationship between age and onset of autoimmunity. To gain insights into this relationship, scientists studied children 3 to 18 years of age who participated in the NIDDK's Diabetes Prevention Trial-Type 1 (DPT-1). All of the children were relatives of people with type 1 diabetes. In the trial, the children were initially screened for the presence of autoantibodies. Of the 42,447 children screened, 3,235 had autoantibodies. Those who did not have autoantibodies were invited to return for re-screens. Of the 11,813 children who returned, 469 (4 percent) had autoantibodies. Further analysis showed that risk for developing autoimmunity was highest in early childhood, with risk decreasing by 5 percent each year. If children tested positive for autoantibodies, most of them (75 percent) did so by age 13. These findings suggest that, in relatives of people with type 1 diabetes, annual screenings should begin in early childhood and continue through early adolescence to identify the majority of children who are at increased risk for developing the disease and who may be eligible for prevention trials.

Vehik K, Haller MJ, Beam CA, et al. Islet autoantibody seroconversion in the DPT-1 study: justification for repeat screening throughout childhood. Diabetes Care 34: 358-362, 2011.

TYPING DIABETES IN YOUTH

New Approach to Characterization of Pediatric Diabetes Type: Scientists developed a marker-based approach to classifying diabetes type in youth. Current classification of diabetes type predominantly falls into one of two categories: type 1 or type 2 diabetes. Although both of these are metabolic disorders, they have different causes and different options for treatment. In type 1 diabetes, a misguided autoimmune attack specifically targets the insulin-producing beta cells of the pancreas and destroys them. People with type 1 diabetes, therefore, do not produce insulin and require insulin injections. In type 2 diabetes, the body becomes less sensitive to the action of insulin and may eventually stop producing insulin. Therefore, approaches to treating people with type 2 diabetes who continue to produce some insulin of their own include improving their response to insulin, increasing its production by the pancreas, reducing release of glucose by the liver, and other approaches. Previously, diagnosis of diabetes type was generally driven by the age of onset, since onset of type 1 diabetes most often occurs in children and adolescents, among whom type 2 diabetes was once unknown. However, type 2 diabetes is now increasingly being diagnosed in children and adolescents. In addition, the obesity epidemic has clouded classification, as the presence of obesity no longer automatically indicates type 2 diabetes, since youth with type 1 diabetes are increasingly obese. Finally, cases of "hybrid" diabetes—with aspects of both type 1 and type 2—have been reported. Diabetes classification is therefore particularly challenging in young people. Moreover, an increasing number of treatment options are available, particularly for people with type 2 diabetes, and some

people with type 2 diabetes require insulin treatment. An accurate understanding of the underlying disease characteristics is needed to ensure that people receive the proper treatment.

To improve diabetes classification and better understand these disorders, scientists in the SEARCH for Diabetes in Youth Study measured markers of autoimmunity and insulin sensitivity in over 2,000 individuals under 20 years of age with recently diagnosed diabetes. The presence or absence of the markers led to the recognition of four groups of youth with diabetes, and allowed the researchers to explore how other factors varied across these groups. As expected, young people whose diabetes was identified as autoimmune and insulin-sensitive had been considered to have type 1 diabetes by their physicians and had traditional characteristics of type 1 diabetes: an average age of onset at 9.3 years; relatively low prevalence of obesity; treatment with insulin; and mostly of non-Hispanic white origin. Those whose diabetes was identified as nonautoimmune and non-insulin sensitive had been diagnosed with type 2 diabetes by their physicians and had traditional characteristics of type 2 diabetes: a higher probability of belonging to a racial/ethnic minority; a typically later age of onset (after onset of puberty); and a high prevalence of obesity.

Youth in the autoimmune and non-insulin sensitive group mostly had been diagnosed by their own doctors as having type 1 diabetes, although they had an older average onset age (12.9 years), a smaller proportion of non-Hispanic white ethnicity, and a higher prevalence of obesity. As this group did not differ significantly in other measures from youth with autoimmunity who are insulin-sensitive, the scientists hypothesize that this group represents individuals with type 1 diabetes who are obese, which is accompanied by abnormal metabolic function causing insulin resistance. It would be valuable to study further this group to determine whether their clinical course is different from insulin-sensitive youth with type 1 diabetes, including the development of diabetes-related complications. Most of the participants in the final group, nonautoimmune and insulin-sensitive, had originally been diagnosed with type 1 diabetes. These youth had many characteristics consistent with this diagnosis: early age of onset (9.4 years); mostly non-Hispanic white origin; and a low prevalence of obesity. The scientists hypothesize that this group could represent people with type 1 diabetes who lacked the traditional markers of autoimmunity at the time of testing.

The results of this study offer researchers and clinicians a marker-based method for diabetes classification and provide definitions of diabetes type using this approach. In this study, most pediatric diabetes fell into groups that align with traditional descriptions of type 1 and 2 diabetes, and provider classification agreed well with this approach. In addition to providing information to improve diabetes type diagnosis, this approach will allow scientists to better understand the course of diabetes in youth.

Dabelea D, Pihoker C, Talton JW, et al. Etiological approach to characterization of diabetes type: the SEARCH for Diabetes in Youth Study. Diabetes Care 34: 1628-1633, 2011.

GENETICS OF TYPE 2 DIABETES INFORMS PREVENTION STRATEGY

Gene Variants Influence the Effectiveness of a Diabetes Prevention Approach: Geneticists have recently shown that variants of a gene called *SLC47A1* can have a significant impact on the ability of the medication metformin to prevent diabetes. Metformin is a well-tolerated, generic medicine that has for decades been the first-line treatment for most people with type 2 diabetes. The NIDDK's landmark Diabetes Prevention Program (DPP) clinical trial tested two approaches to preventing type 2 diabetes in a multi-ethnic group of people at high risk for the disease: metformin, and a lifestyle modification approach designed to achieve 7 percent weight loss through diet and exercise. The DPP found that both interventions were effective at delaying or preventing the development of diabetes. The lifestyle intervention was particularly so, reducing the rate of diabetes by 58 percent compared to a control group that received standard counseling and a placebo. Metformin reduced risk of diabetes by 31 percent relative to the same control group. Recent advances in understanding the genetics of type 2 diabetes now provide the opportunity to assess the interaction between risk genes and the DPP interventions, which may one day enable personalized care to optimize prevention of the disease in those at risk.

The new study investigated genetic variations in (or near) any of 40 genes that had been previously identified as affecting risk for diabetes, as well as genes in which mutations cause rare forms of diabetes and genes involved in drug metabolism. Specifically, the researchers determined whether any of the genes had a significant impact on which DPP participants developed diabetes, and whether any influenced the effectiveness of metformin or the lifestyle intervention. Although they found that several of these gene variants seemed to be increased in the patients that went on to develop diabetes, none achieved clear statistical significance. Although this finding may seem to be at odds with previous research, there are several reasons why this was not unexpected. Because the cohort of the DPP was known to be at particularly high risk of diabetes, for example, as a group they were expected to be enriched in high-risk variants of many of the genes. And importantly, most of the participants in the DPP received interventions that helped prevent diabetes, effectively blunting the impact of genes which ordinarily contribute only modestly to the risk of diabetes.

The researchers also found that the lifestyle intervention was effective regardless of genetic risk factors. Thus, even people with a high genetic risk of type 2 diabetes can reduce their likelihood of developing the disease by maintaining a healthful lifestyle. But intriguingly, the researchers found that while metformin was quite effective for preventing diabetes in the majority of the DPP participants, about a third of the group, those with a distinct version of *SLC47A1*, appeared to receive no diabetes protection at all from the medication. *SLC47A1* encodes a protein known to be involved in clearing metformin from the body, and, in a prior study, the gene had been found to influence the ability of metformin to control diabetes in people who already have the disease. The new research supports and extends that result, showing the gene also can affect diabetes prevention. Taken together with the earlier study, this research suggests that while metformin is highly effective for lowering blood glucose and/or preventing diabetes in the majority of people to whom it is prescribed, almost a third of patients may have a response to metformin that is less robust. A genetic test may one day be available to help tailor diabetes prevention or therapeutic approaches to specific people, and the characterization of genes associated with diabetes may lead to new therapeutic strategies. Importantly, no genetic or demographic characteristic has yet been found to render the DPP lifestyle intervention ineffective: research continues to show that it is a powerful approach for preventing type 2 diabetes in people at high risk for the disease.

Jablonski KA, McAteer JB, de Bakker PIW, et al. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the Diabetes Prevention Program. Diabetes 59: 2672-2681, 2010.

RESEARCH ON NEW THERAPIES FOR TYPE 2 DIABETES

Designing New and Improved Type 2 Diabetes Drugs: Researchers have identified experimental drugs that are as effective in mice as current medications for type 2 diabetes, but cause fewer side effects. A hallmark of type 2 diabetes is that cells become resistant, or less sensitive, to the action of insulin. Some current type 2 diabetes drugs target a protein called PPARγ, which is known to regulate insulin sensitivity. The drugs make the body more sensitive to insulin but come with unwanted side effects, such as fluid retention, weight gain, bone loss, and an increased risk of heart failure. In previous research, scientists discovered that a specific chemical modification (phosphorylation) to PPARγ leads to the abnormal regulation of a number of genes related to obesity and insulin sensitivity in mice. They also found that the current diabetes drugs block this modification in people with type 2 diabetes, thus countering insulin resistance. However, the drugs also broadly stimulate PPARγ, which the scientists thought was responsible for the negative side effects.

In new research, the scientists built on this finding to search for drugs that block phosphorylation of PPARγ without broadly stimulating it, with the hope that the drugs would improve insulin sensitivity without causing the unwanted side effects. Toward that goal, the scientists selected a compound that binds strongly to PPARγ, but does not broadly stimulate it. Next, they generated a series of similar compounds and searched for ones that blocked phosphorylation of PPARγ. They focused on one promising compound that, when tested in diabetic mice, improved insulin sensitivity without

causing fluid retention or weight gain. The compound also had no detrimental effect on bone formation in cultured laboratory cells, whereas current drugs do interfere with bone formation in the cells. Although additional research is needed before potential new drugs could be tested in people, this exciting finding suggests that it may be possible to develop a new class of type 2 diabetes drugs that improve insulin sensitivity but that have fewer negative side effects.

Choi JH, Banks AS, Kamenecka TM, et al. Antidiabetic actions of a non-agonist PPARγ ligand blocking Cdk5-mediated phosphorylation. Nature 477: 477-481, 2011.

Natural Product Has Potential To Combat Type 2 Diabetes: Scientists have identified a natural product that improves metabolic abnormalities associated with type 2 diabetes in mice, and are now testing the product in people. Fatty liver is strongly associated with insulin resistance and type 2 diabetes. One possible approach to reduce fatty liver is through promoting bile acid synthesis. Bile acids are made in the liver and have a role in processing dietary fats; high levels of bile acids reduce fatty liver. A protein called LRH-1 is known to regulate bile acid synthesis; it is one of a group of proteins called nuclear receptors, which regulate diverse aspects of metabolism. Researchers looked for compounds that could activate LRH-1 and potentially promote bile acid synthesis and reduce fatty liver. Through screening, they identified compounds called phosphatidylcholines (PCs) that activated LRH-1. PCs are a type of lipid (fat) and a major component of cellular membranes; they are found in many foods such as eggs and soy. Although not all PCs activated bile acid synthesis, one particular PC, called DLPC, did, and the scientists also further studied it because it is a natural product. In two mouse models of insulin resistance, DLPC treatment increased bile acid levels, decreased fatty liver, and increased insulin sensitivity. In contrast, DLPC treatment had no effect on mice that were genetically engineered to lack LRH-1 in their livers, suggesting that DLPC was exerting its beneficial effects through LRH-1 either directly or indirectly. While the precise mechanism underlying DLPC's effects remains to be determined, the research suggests that DLPC is a promising compound for the prevention or treatment of metabolic disorders, such as type 2 diabetes. Based on the findings, the researchers have begun a human clinical trial to explore the potential benefits of DLPC treatment in people with prediabetes.

Lee JM, Lee YK, Mamrosh JL, et al. A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. Nature 474: 506-510, 2011.

Vitamin D May Help More Than Just Bones—It May Also Help Prevent Diabetes: Results from a clinical trial suggest that vitamin D supplementation could aid efforts to delay development of type 2 diabetes in people at high risk. In addition to known risk factors such as family history, older age, and obesity, nutrient status may contribute to the development of type 2 diabetes. For example, several studies had previously suggested that lower vitamin D status or lower calcium intake may be associated with greater risk for diabetes. However, whether improving levels of these nutrients could have any effect on risk reduction had not been rigorously tested. In a recent clinical trial, researchers investigated whether, in adults at high risk for type 2 diabetes, short-term vitamin D and/or calcium supplementation could improve three indicators of diabetes risk: an assessment of pancreatic beta cell function; insulin sensitivity; and blood glucose measures such as HbA1c, a test that indicates average blood glucose levels over the previous 2 to 3 months.

Ninety-two women and men were randomly assigned to one of four daily supplement groups. People in these groups received either (1) placebo, (2) vitamin D supplements only, (3) calcium supplements only, or (4) both vitamin D and calcium supplements for 16 weeks. During that time, participants were asked to maintain their normal diets, but to avoid taking vitamin D, calcium, or other supplements of their own, beyond what was provided for the study. When the researchers compared measurements taken at the start of the trial period to those at the end, they found that the "disposition index"—a calculation that is used to assess how well the insulin-producing beta cells compensate for altered insulin sensitivity—not only improved significantly in participants who received vitamin D supplements, but it also worsened in those who did not. Changes in insulin sensitivity and in HbA1c did not differ significantly between the groups during the course of the trial, although researchers observed a slight trend toward improved HbA1c in people who received vitamin D. The use or lack of calcium supplements

did not appear to affect any of the risk indicators. Because declining beta cell function is an early step in progression to type 2 diabetes, therapies that improve or preserve function could help stave off disease.

While longer-term, larger studies will be necessary to further assess the effects of vitamin D and to determine levels of supplementation that are potentially both safe and effective, the results of this study suggest that addition of vitamin D supplements might turn out to be a simple intervention that can help prevent or delay progression to type 2 diabetes in people at high risk.

Mitri J, Dawson-Hughes B, Hu FB, and Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. Am J Clin Nutr 94: 486-494, 2011.

DELAYING PROGRESSION OF TYPE 1 DIABETES

Results from Clinical Trials Testing Strategies To Halt Type 1 Diabetes: Researchers in the NIDDK's Type 1 Diabetes TrialNet reported results from two clinical trials aimed at slowing progression of type 1 diabetes. Both trials tested agents that could potentially intervene in the immune attack that destroys insulin-producing β (beta) cells. The goal of the trials was to stop the immune system from destroying remaining β cells—and thus preserve insulin production—in people with newly diagnosed type 1 diabetes. This goal is important because preservation of insulin production is associated with reduced risk for diabetes complications.

The first trial tested a drug called abatacept, which affects immune system cells (T cells) that are known to be involved in type 1 diabetes. Scientists tested whether administering infusions of the drug over a 2-year period could slow progression of the disease in people with new-onset type 1 diabetes. The results showed that abatacept slowed disease progression for 6 to 9 months compared to placebo. After that time, the effect of the drug diminished, and rate of loss of insulin production was similar in the abatacept and placebo groups. However, because of the initial beneficial effects of the drug, after 2 years, people in the abatacept group produced 59 percent more C-peptide, a marker of insulin production, compared to people in the placebo group. These results suggest that abatacept slowed disease progression and preserved patients' β cell function. A second trial focused on a protein called GAD, which is a known target in the immune system attack on β cells in type 1 diabetes. Researchers tested a vaccine against GAD to see if it could dampen the immune response and slow disease progression in newly diagnosed patients. The results showed no difference in C-peptide production after 1 year in people who received the vaccine compared to people who received placebo, demonstrating that the GAD vaccine did not slow type 1 diabetes disease progression.

These results show that, in people with new-onset type 1 diabetes, abatacept slowed disease progression and preserved patients' insulin production, while a GAD vaccine had no effect. Because additional research is needed to understand better the importance of the benefits compared to the risks of treatment, abatacept is not recommended for treating type 1 diabetes in clinical practice at this time. However, the abatacept trial shed light on the biology of type 1 diabetes which could lead to more effective and safer future therapies. For example, the observation that abatacept was effective in the first 6 to 9 months after disease diagnosis suggests that it may be valuable to explore therapies affecting T cells early in the course of disease. It also suggests that combination therapy, which may involve the use of different agents at different stages of disease progression, may be beneficial. Thus, in addition to identifying a possible new therapy for type 1 diabetes, the results may also help to inform the design of future clinical trials to combat the disease.

Wherrett DK, Bundy B, Becker DJ, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. Lancet 378: 319-327, 2011.

Orban T, Bundy B, Becker DJ, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. Lancet 378: 412-419, 2011.

UNDERSTANDING BARRIERS TO TYPE 1 DIABETES CARE

Finding Ways To Combat Self-imposed Insulin Restriction in Women with Type 1 Diabetes:

Researchers have identified factors linked with some women's decisions to take insufficient insulin to properly manage their type 1 diabetes (insulin restriction). Some women with type 1 diabetes intentionally take less insulin than prescribed by their doctors—usually because of the fear of gaining weight and problems with diabetes self-care—and thus do not achieve optimal blood glucose control. Insulin restriction is a serious health issue because good blood glucose control reduces the risk of long-term diabetes complications. Indeed, researchers conducted an 11-year follow-up study of women with type 1 diabetes, 30 percent of whom reported restricting insulin at baseline. In this previous research, they discovered that insulin restriction was associated not only with increased rates of diabetes complications, but also with increased risk of premature death. These findings underscore the need to find ways to prevent or help women stop insulin restriction to improve their health.

In new research, the same group of scientists examined the same population of women, but looked at factors associated with decisions to start or stop insulin restriction over the 11-year follow-up period. The results showed that women who stopped restriction reported improved diabetes self-care, fewer problems with diabetes self-management, and less fear that taking insulin would cause weight gain. In contrast, women who started insulin restriction were fearful of weight gain. Overall, fear of weight gain and problems with diabetes self-care were core issues associated with women's decisions to start or stop insulin restriction. The researchers also looked at actual weight gain and found that the result was the exact opposite of the fears: women who stopped restricting did not gain weight, while the insulin restrictors gained weight. This observation could potentially be used by doctors as a tool to help women with type 1 diabetes manage concerns about weight gain, and thus help avoid insulin restriction. Overall, this study sheds light on core issues surrounding women's decisions to restrict insulin and highlights the importance of health care providers' assessing and addressing insulin management as well as any weight concerns or symptoms of eating disorders when treating women with type 1 diabetes. These insights could also inform future research to develop and test new intervention strategies that could more effectively prevent insulin restriction and help those who use this practice to return to healthier insulin administration and to address related weight and other concerns, so that they could enjoy better health.

Goebel-Fabbri AE, Anderson BJ, Fikkan J, Franko DL, Pearson K, and Weinger K. Improvement and emergence of insulin restriction in women with type 1 diabetes. Diabetes Care 34: 545-550, 2011.

DIABETES COMPLICATIONS

Predicting and Slowing Development of Cardiovascular Disease in Type 1 Diabetes:

Researchers have made advances in identifying biological predictors and a strategy to slow progression of cardiovascular disease in people with type 1 diabetes. Type 1 diabetes, like type 2 diabetes, is associated with an array of serious, long-term complications, such as cardiovascular disease. In people with and without diabetes, plaque—made of fat, cholesterol, calcium, and other molecules found in blood—can build up in a person's arteries, leading to a condition called atherosclerosis. As the plaque collects and hardens, it can narrow the arteries, reduce the flow of oxygen-rich blood to parts of the body, and potentially lead to heart attack or stroke. People with diabetes have a greatly increased risk for cardiovascular disease.

In a recent study, researchers investigated the long-term effects of intensive blood glucose control on the progression of atherosclerosis in participants of the NIDDK's landmark Diabetes Control and Complications Trial (DCCT) and its follow-up study, called the Epidemiology of Diabetes Interventions and Complications (EDIC). The DCCT showed that intensive control of blood glucose levels reduced the risk of complications of the kidneys, eyes, and nerves in people with type 1 diabetes. Previous results from EDIC demonstrated that people who had been intensively treated during the DCCT had fewer than half the number of cardiovascular disease events than those who received treatment that was conventional at the time. In the recent study, the researchers found

that people in the intensive glucose control group had slowed atherosclerosis progression 6 years after the end of DCCT compared to the conventional group, but that this slowing did not continue in years 6-12. Since development of atherosclerosis in the intensive group progressed more slowly in years 1-6 following the end of the DCCT, this group still demonstrated an overall beneficial effect of the prior intensive treatment 13 years after the DCCT. These results support the recommendation that people with type 1 diabetes implement early and continued intensive blood glucose control to slow atherosclerosis.

In other recent studies, researchers examined whether the presence of a marker called "oxidized low-density lipoprotein," or oxLDL, could predict risk for cardiovascular events. LDL, sometimes called "bad" cholesterol, carries fats in the blood to parts of the body. Modified forms of LDL are recognized by immune system proteins which bind them; these complexes are known as "oxLDL-IC." In both studies, the researchers measured oxLDL-IC levels in samples collected from a subset of participants in the DCCT when they first joined the trial. Then, they examined whether there was a correlation between oxLDL-IC levels and signs of atherosclerosis that were measured in the same people after 8 to 20 years as part of EDIC. Both studies found that increased levels of oxLDL-IC were predictive of later atherosclerosis in people with type 1 diabetes. In one of the studies, the researchers found that another immune complex with a differently modified LDL was also associated with signs of later atherosclerosis. Not only do these studies suggest a pathogenic role for modified LDL immune complexes, but they also indicate that measures of these factors may help to identify people at high risk for cardiovascular disease.

After the DCCT ended, more than 95 percent of the participants enrolled in the EDIC follow-up study. As reported in these three advances, because of the long-term commitment of these participants, researchers are adding new knowledge to the understanding of cardiovascular disease and demonstrating the importance of intensive blood glucose control in people with type 1 diabetes.

Lopes-Virella MF, Baker NL, Hunt KJ, et al. Oxidized LDL immune complexes and coronary artery calcification in type 1 diabetes. Atherosclerosis 214: 462-467, 2011.

Lopes-Virella MF, Hunt KJ, Baker NL, et al. Levels of oxidized LDL and advanced glycation end products-modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes. Diabetes 60: 582-589, 2011.

Polak JF, Backlund J-YC, Cleary PA, et al. Progression of carotid artery intima-media thickness during 12 years in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study. Diabetes 60: 607-613, 2011.

Study Links Prediabetes with Periodontal Disease: Dentists have long known that their patients with diabetes (both type 1 and type 2 diabetes) have an elevated risk of periodontitis, defined as chronic inflammation of the soft tissue around the teeth and deterioration of the network of the fibers that connect teeth to the surrounding bone. In addition to the swollen, tender gums that bleed easily and are the first manifestations of periodontitis, the progressive diminishment of the attaching fibers can lead to tooth loss. It is not yet known why diabetes and periodontitis are frequently associated with one another. One possible explanation is that elevated glucose levels in oral tissues might create an environment conducive to promoting gum disease. Alternatively, the inflammation that is characteristic of periodontitis might promote insulin resistance. Indeed, both of these hypotheses could be correct, with the two diseases reinforcing one another. Interestingly, new research shows that periodontitis is also associated with increased levels of glucose below the threshold for diabetes. To better understand the relationship between oral health and elevated blood glucose, researchers assessed the periodontal health and fasting plasma glucose levels of more than 12,000 participants in the National Health and Nutrition Examination Survey III. The examiners used two different measures of oral health that are commonly used to diagnose periodontitis—"clinical attachment loss" and "pocket depth"—and stratified the participants into equally sized groups ranging from very healthy to serious periodontitis for each measure. Then they looked for the presence of type 2 diabetes and prediabetes (which refers to blood glucose levels that are elevated, but not as high as in diabetes, a condition associated with increased risk

of later developing type 2 diabetes) in each group. As expected, diabetes was most prevalent in the group with the most serious periodontitis (by either measure). Interestingly, there was also a strong correlation between prediabetes and periodontitis. Although it is tempting to conclude from this that periodontitis often precedes and may therefore promote diabetes, it is important to note that this study did not follow the participants over time, so the researchers could not determine whether fasting glucose levels rose from prediabetic to diabetic levels more quickly in people with periodontitis than in those without it. In any case, the results underline the importance of determining the biological link between these chronic inflammatory diseases through further research. The findings are also of clinical importance, because they suggest that screening for periodontal disease, along with a concerted effort to improve and maintain oral health, may be particularly important not only for people with diabetes, but also for those with prediabetes. Further, because periodontitis is now clearly known to be a risk factor for elevated blood glucose levels, it may be advisable for people with the disease to consider testing for undiagnosed diabetes or prediabetes.

Choi Y-H, McKeown RE, Mayer-Davis EJ, et al. Association between periodontitis and impaired fasting glucose and diabetes. Diabetes Care 34: 381-386, 2011.

NEW INSIGHTS ON RARE METABOLIC DISEASE

Potential New Treatment for Niemann-Pick Type C: Scientists have identified a class of drugs that corrects a defect in cells from people with a rare genetic disease called Neimann-Pick type C (NPC). NPC is a disease in which the body cannot properly break down lipids, which include cholesterol and other fats. This leads to too much cholesterol in the liver and spleen, and excessive amounts of other fats in the brain; NPC is thus referred to as a lipid storage disease. NPC may be diagnosed at any age, but is most often diagnosed in middle to late childhood. NPC is a fatal disease, with children often living only until their teenage or early adult years. Previous research identified two genes—*NPC1* and *NPC2*—that are linked to NPC; defects in the *NPC1* gene account for about 90 percent of NPC cases. In new research, scientists focused on a class of drugs called histone deacetylase (HDAC) inhibitors to examine if they could prevent or reduce the excess fat accumulation in NPC cells. Many HDAC inhibitors have been tested in people with a variety of diseases and have been found to be safe; two HDAC inhibitors have been approved by the U.S. Food and Drug Administration for treating certain forms of cancer. In the study, researchers used cultured human cells that had a mutation in the *NPC1* gene. Before treatment with HDAC inhibitors, cholesterol was trapped within the cells. Dramatically, after treatment, cholesterol was no longer trapped, and the cells appeared to function like normal, unaffected cells. In contrast, HDAC inhibitors were ineffective in treating human cells carrying a defect in the *NPC2* gene, suggesting that the beneficial effect may be specific to cells with an *NPC1* gene defect. HDAC inhibitor therapy will have to be tested in clinical trials of NPC patients, but this exciting research has opened up a new avenue for possibly treating some people with this rare, devastating disease.

Pipalia NH, Cosner CC, Huang A, et al. Histone deacetylase inhibitor treatment dramatically reduces cholesterol accumulation in Niemann-Pick type C1 mutant human fibroblasts. Proc Natl Acad Sci USA 108: 5620-5625, 2011.

Diabetes Research Strategic Plan: Building on Advances, Seizing Opportunities

In late February 2011, the NIDDK announced the publication of a new blueprint for diabetes research supported by the NIH and other federal agencies. Entitled *Advances and Emerging Opportunities in Diabetes Research: A Strategic Planning Report of the Diabetes Mellitus Interagency Coordinating Committee*, this multi-faceted strategic plan identifies compelling opportunities for research over the next decade on diabetes and its complications. Spanning basic, clinical, behavioral, and translational research, the report features advances, key questions, and future directions in 10 major diabetes research areas. It also addresses the need for technological and human resources to accelerate discovery. Developed with input from researchers, patient advocates, and the public, the report is a guide for research efforts that can benefit the tens of millions of Americans who are living with, or at risk for, diabetes and its complications.

Organization and Purpose

The *Strategic Plan* is framed around major scientific areas representing important opportunities in research on all forms of diabetes. These 10 areas are overlapping but complementary in scope:

- Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications
- Type 1 Diabetes and Autoimmunity
- The Beta Cell
- Type 2 Diabetes As a Multi-Dimensional Disease
- Obesity
- Bioengineering Approaches for the Development of an Artificial Pancreas To Improve Management of Glycemia
- Clinical Research and Clinical Trials
- Special Needs for Special Populations
- Diabetes Complications
- Clinical Research to Practice: Translational Research

Each of the 10 chapters in the *Strategic Plan* addressing these areas of scientific opportunity includes an introduction, summaries of recent research advances, key questions and future directions (goals) for research, and a closing section describing how the research directions outlined in the chapter may transform the health of people with or at risk of diabetes. The *Strategic Plan* also includes a chapter that outlines resource and infrastructure development needs to support the implementation of the future directions for diabetes research.

Tackling diabetes from multiple angles, the goal of the *Strategic Plan* is to accelerate discovery on several fronts, including the relationship between obesity and type 2 diabetes, and how both conditions are affected by genetics and environment; the autoimmune mechanisms at work in type 1 diabetes, and the emerging role of the immune system in type 2 diabetes; the biology of beta cells, which release insulin in the pancreas and represent potential for therapy or a cure; the development of artificial pancreas technologies that could help to alleviate the burden of therapy on people with type 1 diabetes while preserving their health; prevention

of diabetes health complications that affect the heart, eyes, kidneys, nervous system, and other organs; and reduction of the impact of type 2 diabetes on groups disproportionately affected by the disease, including the elderly and racial and ethnic minorities in the United States. Through seizing upon opportunities outlined in the *Strategic Plan*, the hope is to more quickly erase the scourge of diabetes and improve the health of patients, their families, and the Nation.

Origin of the *Diabetes Research Strategic Plan*

In 1999, the congressionally established Diabetes Research Working Group issued a comprehensive plan for diabetes research, entitled *Conquering Diabetes: A Strategic Plan for the 21st Century*. This report was the culmination of the vigorous efforts of the Working Group, a committee of leading extramural diabetes experts. In the years since that plan was released, there have been major advances in the understanding of diabetes, new tools and technologies have been developed, and strategies for diabetes prevention and treatment have been expanded. Many of these scientific successes are directly linked to ideas and goals set forth in the 1999 plan, which has served to guide the NIH's planning activities for fostering discovery in diabetes. In 2002, the NIDDK published *A Scientific Progress Report on the Diabetes Research Working Group's Strategic Plan*, which highlighted program efforts, research advances, and scientific opportunities.

In 2006, the NIH published *Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan*, a report that described opportunities that would inform research on many areas of commonality between type 1 and type 2 diabetes, but with a primary focus on type 1 diabetes research. This report was developed under the auspices of the Diabetes Mellitus Interagency Coordinating Committee (DMICC), a statutory committee established in 1974. Chaired by the NIDDK, the DMICC facilitates cooperation, communication, and collaboration on diabetes efforts across the federal government.

In late 2008, the DMICC determined that the time was right to embark on an update of both previous diabetes research plans by identifying the most promising, up-to-date, high-priority opportunities for diabetes research that could build on recent advances and be accomplished over the next 5 to 10 years. The ensuing effort resulted in the new *Diabetes Research Strategic Plan*.

A Collaborative Planning Process

The *Strategic Plan* was developed with broad input from a diverse and talented group of researchers and lay experts dedicated to advancing diabetes research. These volunteers were assembled into working groups to address each of 10 scientific areas of important opportunity related to diabetes. An additional working group composed of representatives from each of the other 10 groups addressed overarching needs for scientific expertise, tools, technologies, and shared research resources. Each working group was chaired by a scientist external to the NIH and was composed of additional external scientific experts, as well as representatives of DMICC member organizations and diabetes voluntary organizations. Working groups met through conference calls and electronic exchanges to assess the state of the science and identify advances and emerging opportunities in their scientific areas.

An overarching *Diabetes Research Strategic Plan* Leadership Group was formed of the chairs of the 11 working groups and representatives from the federal government and diabetes voluntary organizations. This overarching working group met in person on July 7, 2009, to review progress of the scientific working groups and ensure that the *Strategic Plan* was comprehensive and addressed the most compelling opportunities for prevention, therapy, and cure of diabetes and its complications. In 2010, a draft of the *Strategic Plan* was posted on the NIDDK web-site to provide an opportunity for broad public input prior to completion and publication of the final plan.

Implementation of the *Diabetes Research Strategic Plan*

The *Diabetes Research Strategic Plan* is part of a dynamic planning process that involves collaboration among numerous stakeholders to ensure that research progress is regularly assessed and that new and emerging opportunities for diabetes research are identified. The DMICC will continue to play a key role by assessing progress toward the research goals described

in the *Strategic Plan*. The NIH will also continue to solicit broad external input from the scientific, lay, and patient advocacy communities to inform its planning efforts. The NIH, other DMICC member organizations, and the scientific community will use the research questions and future directions described in the *Strategic Plan* as a scientific guidepost to enhance fundamental understanding of diabetes, improve current treatment strategies, and identify ways to prevent or cure diabetes and its complications.

Advances and Emerging Opportunities in Diabetes Research: A Strategic Planning Report of the Diabetes Mellitus Interagency Coordinating Committee is available electronically on the NIDDK web-site at *http://diabetesplan.niddk.nih.gov*

Hard copies of this *Strategic Plan* may also be ordered from the National Diabetes Information Clearinghouse at *http://catalog.niddk.nih.gov*

The Special Statutory Funding Program for Type 1 Diabetes Research

The *Special Statutory Funding Program for Type 1 Diabetes Research (Program)* is a special appropriation dedicated to supporting research on the prevention and cure of type 1 diabetes. On behalf of the Secretary of the U.S. Department of Health and Human Services (HHS), the NIDDK administers the *Program* in collaboration with multiple NIH Institutes and Centers and the Centers for Disease Control and Prevention (CDC). This funding program augments regularly appropriated funds that the NIH receives for diabetes research.

NIDDK Director Dr. Griffin Rodgers received the 2011 JDRF Children's Congress Hero Award—the JDRF's top honor—for his steadfast commitment to research on type 1 diabetes. Pictured with Dr. Rodgers (center) are JDRF Chairman, Board of Directors Mr. Frank Ingrassia (left) and JDRF President and CEO Mr. Jeffrey Brewer (right).
Photo credit: Juvenile Diabetes Research Foundation International

Renewal of the *Program*—
New Research Opportunities

Special funding for type 1 diabetes research was initially provided in fiscal year (FY) 1998 for $30 million a year, and has since been renewed and augmented. In December 2010, the *Program* was renewed for $150 million each year for FY 2012 and 2013. Since 1998, the *Program* has provided $1.89 billion for research on type 1 diabetes.

To receive input on emerging research opportunities that could be pursued with the recent *Program* reauthorization and funding renewal, the NIDDK convened a panel of scientists from across the country and others with expertise in type 1 diabetes in May 2011. The panel was asked to provide input on draft concepts, put forth by the NIH and CDC, for initiatives that could be supported with the funds. Based on input from the panel, and with consideration of research opportunities outlined in the recent *Diabetes Research Strategic Plan* (see this chapter), the NIH plans to pursue several new initiative concepts in FY 2012-2013.

Evaluating the *Program*—
Research Advances and Innovation

The law that previously renewed the *Program* also mandated that an evaluation of the *Program* be submitted to the Congress by January 1, 2011. To meet this requirement, the NIDDK submitted the evaluation report to the Congress in December 2010. This report describes the unique, innovative, and collaborative research consortia and clinical trials networks enabled by the *Program*, as well as the scientific accomplishments that have emerged.

As described in the evaluation report, assessment of the *Program* indicated that it has produced significant scientific advances; yielded robust scientific output; led to issuance of new patents; promoted development of resources for use by the broad scientific community; fostered clinical research; and attracted new scientists to the study of type 1 diabetes. In addition, the evaluation report contains profiles of people participating in clinical research supported by the *Program* and profiles of scientists whose studies have been supported by the special funds, including the late Dr. Mark Pescovitz. This report can be found at *www.t1diabetes.nih.gov/evaluation2010*

NIDDK Director Dr. Griffin Rodgers Testifies to Congress on Type 1 Diabetes Research

On June 22, 2011, NIDDK Director Dr. Griffin P. Rodgers was invited to testify about progress in type 1 diabetes research before the Senate Committee on Homeland Security and Governmental Affairs. The hearing, entitled "Transforming Lives Through Diabetes Research," was chaired by Senators Joseph Lieberman and Susan Collins. Dr. Rodgers spoke of research made possible by the *Program*, including progress from studies to identify the environmental factors that cause type 1 diabetes, and advances in developing and testing artificial pancreas systems. A hearing on type 1 diabetes research is held every 2 years in conjunction with the Juvenile Diabetes Research Foundation International (JDRF) Children's Congress. The previous day, Dr. Rodgers received the 2011 JDRF Children's Congress Hero Award for his work in advancing type 1 diabetes research and improving the lives of people affected by the disease.

At a June 2011 congressional hearing on type 1 diabetes, JDRF Children's Congress delegates (foreground) listened to testimony from (at table, left to right) JDRF Celebrity Advocate Co-chair, and actor, Mr. Kevin Kline; NIDDK Director Dr. Griffin Rodgers; and Chair of the FDA Artificial Pancreas Critical Path Initiative, Dr. Charles Zimliki. Several of the children also spoke at the hearing, describing their experiences with this disease and the importance of research (see Patient Profile in this chapter).
Photo credit: Juvenile Diabetes Research Foundation International

Coordinating the Coordinators: NIDDK Brings Together Type 1 Diabetes Study Coordinators To Exchange Ideas and Best Practices

People who have or are at risk for type 1 diabetes have made enormous contributions to research on this disease by participating in clinical studies. Their efforts clearly demonstrate a commitment to finding a way to prevent or cure the disease and to helping others who are or may be diagnosed. Alongside these passionate participants work another group of people dedicated to this cause—the type 1 diabetes clinical study coordinators. The NIDDK broke new ground in September 2011 with its first joint study coordinator meeting for several type 1 diabetes clinical research studies supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*. Coordinators from The Environmental Determinants of Diabetes in the Young (TEDDY); the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study (DCCT/EDIC); Type 1 Diabetes TrialNet; Search for Diabetes in Youth (SEARCH); and Trial to Reduce IDDM in the Genetically at Risk (TRIGR) met to exchange ideas and best practices, share challenges, and discuss professional development.

Although each of these studies aims to change the course of type 1 diabetes in the United States and around the world, they have different goals. For example:

- SEARCH is providing nationwide data on the percent or proportion of children with diabetes (prevalence), the rates of development of childhood diabetes (incidence), and whether these rates and the clinical course of diabetes in children and youth are changing over time.
- The TEDDY and TRIGR studies aim to identify the environmental triggers of type 1 diabetes, to inform the development of prevention approaches. These two studies have enrolled newborns who are at high genetic risk for the disease, and will evaluate potential triggers such as viruses and hygiene (TEDDY) and a protein in cow's milk (TRIGR).
- TrialNet is testing strategies to prevent or delay progression of type 1 diabetes in people at high risk for or newly diagnosed with type 1 diabetes.
- DCCT/EDIC continues to examine the benefit of intensive blood glucose control on the development of complications in people who have had type 1 diabetes for more than 30 years.

Though these studies include people at different stages of disease progression and of varying ages, they are all long-term studies that are dependent on participants to achieve their goals.

The study coordinator is an integral component of any type 1 diabetes clinical study. These studies require dedicated staff to find, recruit, screen, and enroll individuals. For example, studies related to type 1 diabetes prevention require screening tens or hundreds of thousands of people to identify those eligible for enrollment. Once participants are enrolled, it is critical that they remain active in the study and carry out the protocol as planned. Coordinators wear any number of different hats, working tirelessly to recruit and retain study volunteers, collecting key samples and data from participant visits, maintaining excellent data quality, providing critical input to protocol development, and even designing ancillary studies.

For DCCT/EDIC coordinators specifically, this effort has been nearly 3 decades of dedication—a key reason why about 95 percent of the original participants of the DCCT are involved in its EDIC follow-up study. The landmark DCCT/EDIC demonstrated that intensive control of blood glucose levels can have long-lasting effects toward reducing the onset and progression of diabetes complications involving the kidney, eyes, nerves, and heart. These findings revolutionized clinical management of type 1 diabetes and translated into dramatic health benefits for all people with type 1 diabetes. At the conclusion of DCCT, participants were recruited to EDIC. EDIC study coordinators have faced significant challenges in conducting this long-term investigation. For example, participants move geographically and "burn out" with the demands of participating in a study, and EDIC staff

changes over time. However, EDIC study coordinators have found ways to meet these challenges, such as by developing flexible scheduling, maintaining strong participant-staff bonds, and always leaving the door open so that participants who need to take a break from the study can return at any time. The role of the EDIC study coordinator has evolved as well; study coordinators have a dynamic role and are voting members of the study group, chair study committees, and serve as co-investigators on several ancillary studies. This increased autonomy, accountability, and responsibility have contributed to high job satisfaction, high-quality data collection, and exemplary patient retention, making EDIC an excellent model for other clinical studies in type 1 diabetes.

The other studies represented at the September 2011 meeting also face significant challenges. Study protocols can be demanding, requiring study coordinators to encourage volunteers to remain engaged. For example, participation in TEDDY requires families to dedicate a significant investment of time and effort, which can become overwhelming. Parents need to stay motivated in collecting their child's stool samples and taking their child for blood draws on a regular basis, which are key components of the study. Indeed, hundreds of thousands of samples have already been collected for analysis or storage for future analysis of possible triggers of type 1 diabetes. Some TrialNet protocols require participants to receive multiple infusions of a drug over 2 years; others involve daily infusions for 14 consecutive days and still others call for overnight stays in the clinic. Additionally, these studies are long term, requiring participants to stay involved with the study for years, even decades. For example, SEARCH asks children to return for follow-up clinical visits 1, 2, and 5 years after the initial visit. TRIGR is following children until age 10 with annual clinical visits after 2 years, whereas TEDDY is following children until age 15 with continued sample collection and clinical visits. The study coordinators also have an important role in easing anxiety experienced by study participants and their families. For instance, in TrialNet, volunteers are asked to participate in repeat screenings and to return year after year even if they currently have no signs of the disease, in order to identify individuals at risk for developing type 1 diabetes, and to offer them an opportunity to participate in prevention trials. This annual screening can be anxiety provoking, with the possibility of finding out that the individual or their child has signs of type 1 diabetes. However, the study coordinators are there to help families cope with anxiety. The challenges of keeping participants motivated in demanding protocols, retaining volunteers for decades of follow up, and providing support through stressful procedures are important considerations for the study coordinators.

The meeting provided an opportunity for coordinators to share their studies' resources and creative solutions to problems and develop ways to improve efficiency, cooperation, and, ultimately, study success. In breakout sessions, they brainstormed new recruitment and retention tools like social media, the role study coordinators can play in study design, and professional development strategies. Group discussions highlighted several opportunities for enrichment and coordination, including the formation of a network of type 1 diabetes study coordinators to increase communication and collaboration among the studies. The study coordinators also suggested future meeting topics, reflecting the synergy generated by meeting face-to-face. By convening the meeting to create opportunities to improve the recruitment and retention of participants and to invigorate the study coordinators, the NIDDK recognizes the value and commitment of each and every participant and study coordinator, and remains dedicated to the goals of preventing and curing type 1 diabetes.

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HEALTHY Schools, Healthier Students

Thirty years ago, type 2 diabetes, which often develops in people with overweight or obesity, was referred to as "adult onset" diabetes because it most often develops in people who are middle-aged or elderly. In fact, at the time, type 2 diabetes was unknown in children; when diabetes struck in childhood, it was type 1 diabetes, often referred to as "juvenile" diabetes. Unfortunately, the trend in this country toward larger waistlines is not restricted to adults. National data indicate that about 17 percent of children between 6 and 19 years of age are obese, and another 15 percent are overweight. Rates of obesity are even higher in those who are economically disadvantaged and in ethnic minority groups. Type 2 diabetes now represents 33 percent of newly diagnosed diabetes cases in 10 to 19 year olds, and accounts for the majority of new cases of diabetes among adolescents in certain racial/ethnic groups. With the hope that extensive changes throughout schools and related communications efforts could help improve adolescents' food choices and physical activity levels—and thus reduce risk factors for type 2 diabetes, including obesity—the NIDDK spearheaded a large study with thousands of participating students. The results of this study, called HEALTHY, showed that the school-based intervention had the most benefits for those students who had been the most at risk.

Childhood Obesity: A Worrisome Development

As part of the extensive planning and development of the HEALTHY study, researchers considered the many serious health issues associated with type 2 diabetes and obesity. The development of type 2 diabetes among young people has significant personal and public health consequences, as these youth are likely to develop the eye, kidney, nerve, and cardiovascular complications of diabetes during what should be the most productive period of their lives. An additional concern for young women with the disease is that diabetes during pregnancy significantly increases the risk of complications for both mothers and their babies; and children born to women with either type 2 diabetes or gestational diabetes are themselves at significantly elevated risk for developing type 2 diabetes, an effect which may potentially reinforce the increasing rate of type 2 diabetes among youth. Further, the recent NIH-supported Hypoglycemia and Adverse Pregnancy Outcomes Study showed that risk for many of the adverse pregnancy outcomes associated with gestational diabetes is increased even below the threshold blood glucose level considered to define diabetes. Rather, those risks are lowest at the lowest blood glucose levels, and rise continuously with increasing blood glucose levels. These findings suggest that it will benefit the health of future mothers (and their children) to reach their child-bearing years with weight, glucose levels, and other diabetes risk factors as close as possible to normal. And ominously, a growing body of research evidence is showing that being obese during childhood increases the risk for serious health problems throughout a person's life. For example, obese young people who do not develop type 2 diabetes as children remain at substantially elevated risk of developing the disease as adults. Also, as they enter middle age they are more likely than their lower body mass index (BMI) peers to develop conditions such as high blood pressure and other aspects of the metabolic syndrome, and atherosclerosis (thickening of arterial walls, sometimes called "hardening of the arteries"), a hallmark of heart disease that can presage heart attacks and strokes.

Going to School To Promote Health

Researchers and public health professionals who are focused on the childhood obesity problem are seeking practical approaches to help as many children as possible avoid obesity and the many serious problems that can stem from it. From this perspective, schools

have great potential to play a role in reducing pediatric diabetes risk, because no other institution has as much contact time with youth. Schools can add class curricula aimed at enhancing knowledge about science and health, and they can implement environmental changes that target modifiable risk factors for obesity and type 2 diabetes, such as diet and physical activity. Several studies in recent years have sought to measure the effects of school-implemented changes to diet, activity, and the curriculum.

Working with teachers and administrators in schools with students of several age groups, researchers have examined efforts that restricted availability of sugared beverages, lowered fat content in school lunches, changed physical education curricula, and introduced science and nutrition education materials. Several studies have tested interventions with multiple components, from the lunch room, to the classroom, to the gymnasium. Although some shorter studies (*i.e.*, interventions lasting for a few months) seemed to at least temporarily improve one or more measures of pediatric health, longer studies have generally demonstrated only modest effects.

Yet, if a practical and effective program to improve student health can be developed, middle schools may be a key place to implement it. Children in the sixth through the eighth grades are generally 11 to 14 years old and in early adolescence. This is, of course, a period of physical maturation and metabolic change as well as emotional and mental growth and development. Research has shown that as youth of this age group progress through puberty, hormonal changes not only alter their body composition, but also tend to lower their insulin sensitivity, suggesting this may be a key period along a potential pathway to type 2 diabetes. Students at this age are developmentally capable of beginning to assume personal responsibility for behavior and choices, and indeed diet and physical activity behaviors are often in flux during adolescence. Thus, middle school may afford a key opportunity to encourage healthful behaviors.

Making HEALTHY Changes at Schools

The NIDDK, with supplementary support from the American Diabetes Association, worked to develop and test a comprehensive but practical approach schools could take to try to improve the health of their students. The result was HEALTHY, a study which evaluated a 3-year, multi-component, school-based program to decrease risk factors for type 2 diabetes. Because children from lower income households or who are from racial/ethnic minorities are at substantially elevated risk for both obesity and type 2 diabetes compared to higher income and/or white peers, schools selected for HEALTHY either had to have at least 50 percent of their students eligible for federally subsidized, free or reduced-price meals, or be made up of at least 50 percent minority students. In fact, the averages for participating schools were much higher: over 70 percent of the students who were in the HEALTHY cohort were eligible for free or reduced-price meals, and over 70 percent were minorities.

During the fall semester of 2006, the researchers recorded the initial height and weight of more than 6,000 sixth graders from the participating schools who wished to participate and whose parents consented, and also measured the students' fasting insulin and blood glucose levels. The results of those tests were sobering, and highlight health disparities in risk factors for pediatric type 2 diabetes. Sixteen percent of the students had relatively high levels of blood glucose while fasting (prediabetes), and almost 7 percent had elevated fasting insulin levels. The highest percentage of prediabetes and elevated fasting insulin levels was observed in Hispanic American students. Overall, nearly half of the sixth-grade students in schools participating in the HEALTHY study were considered overweight or obese according to their BMI. This is higher than the national average of U.S. children, but similar to rates observed in other predominantly minority populations. Among the students in the

study, the Hispanic American children had the greatest percentage of overweight/obese individuals, followed by African American children.

The HEALTHY intervention consisted of four integrated components—nutrition, physical activity, behavioral knowledge and skills, and communications and social marketing—each of which was carefully pilot-tested before the formal study began. The nutrition component targeted the quantity and nutritional quality of foods and beverages that were served throughout the school environment (cafeteria, vending machines, à la carte options, snack bars, school stores, fundraisers, and classroom celebrations). The physical education component was designed to increase the amount of time students spent in moderate-to-vigorous physical activity, defined as activity sufficient to raise the heart rate to 130 beats or more per minute. Behavioral knowledge and skills were promoted with the use of a classroom-based program, FLASH (Fun Learning Activities for Student Health), which targeted self awareness, knowledge, behavioral skills, and peer involvement to promote behavior change. The various components of the intervention were integrated by having a theme each semester that targeted specific behaviors (*e.g.*, drink more water and fewer sweetened beverages; engage in more physical activity and less sedentary activity) that could be addressed across all intervention components. The study worked with marketing and creative design experts to develop a brand, logo, activities, and materials that effectively linked these behavior messages. In the latter half of the study, students worked with study staff to produce photographs, artwork, audio messages, and video clips that reinforced the intervention in a way that reflected student perspectives and local sensibilities. Rounding out the communications approach, HEALTHY researchers worked to engage the entire school staff in promoting healthy behaviors.

Of the 42 qualifying schools that participated, 21 were randomly assigned to receive the intervention, while the remaining 21 served as comparison ("control") schools. HEALTHY targeted sixth graders, who received the intervention through the end of the eighth grade. All students in the sixth grade in the intervention schools were exposed to the intervention. However, the study only collected data from students who provided their assent, as well as the written consent of a parent. The intervention began in the spring of 2006, and proceeded through the end of eighth grade, in 2009, when outcome data were again collected in all schools. Except for data collection, the study sponsored no activities in the comparison schools. Over 6,000 students—59 percent of the student body—agreed to participate in the sixth grade and most (over 4,600 students) were re-assessed at the end of the study in eighth grade. (The great majority of those who were measured only in sixth grade had moved to other schools before the end of the study.)

A Trove of Intriguing and Encouraging Findings

The results of HEALTHY were reported in the *New England Journal of Medicine*. The Physician Section of the American School Health Association and the American Academy of Pediatrics Council on School Health later declared the paper to be among the 13 most important school health papers published in 2010. Study investigators reported the surprising finding that the intervention and comparison schools both saw a reduction of about 4 percent in the overall proportion of students who were either overweight or obese. The HEALTHY program also resulted in statistically significant reductions in other risk factors for type 2 diabetes in the intervention schools, including elevated levels of insulin in the blood, and a waist circumference above the 90th percentile. Despite the difference in insulin levels, the study did not find a difference in average blood glucose levels between the two groups of schools.

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Perhaps the most exciting outcome of the study, however, was that the intervention significantly lowered the obesity rate in the children at highest risk for type 2 diabetes—those who were already overweight or obese in the sixth grade: these students had a 21 percent lower risk for being obese at the end of the eighth grade if they were in the intervention schools than if they were in the comparison schools. In the HEALTHY study, the children who were obese at the outset were the most likely to have also begun with elevated levels of both glucose and insulin. Encouragingly, this group of children seems to have particularly benefited from the HEALTHY program: fasting insulin and waist size fell to a significantly greater degree in intervention schools compared to control schools among students who began the study overweight or obese.

The HEALTHY results are particularly notable given the mixed results seen with other school-based interventions. Indeed, the reduction in obesity achieved by the schools using the HEALTHY program in the children who were most at-risk stands in sharp contrast to the modest obesity effects generally observed in intensive, clinic-based, behavioral-treatment programs. Importantly, the HEALTHY program achieved these successes in a cohort consisting largely of children whose race, ethnicity, and socioeconomic status are risk factors for obesity and diabetes. That success may be related to the comprehensive approach HEALTHY took to target both diet and physical activity, strategically incorporating multiple environmental and behavioral strategies.

There may be other reasons, too, that the intervention worked well for those students who were overweight or obese at the beginning of the study. Before HEALTHY began, study investigators expected that this group would be the hardest to reach. However, it is possible that the intervention resulted in greater improvements in diet and activity in these high-risk children, since they may well have had higher food consumption and lower levels of physical activity before enrollment than did the children who were not overweight. Also, HEALTHY provided the intervention to the entire school, perhaps preventing some of the stigmatization that would have occurred if the intervention had been targeted selectively to the most overweight and obese children. In addition, HEALTHY's schoolwide changes may have made it easier for these children to make healthful choices than interventions that have been less comprehensive about addressing environmental factors that promote obesity. Although parents were not the target of the HEALTHY intervention, newsletters and other messaging were sent home to families. Perhaps parents of overweight and obese children may have been more responsive to intervention messages. Whatever the reason for the enhanced effect in this subgroup, wide-scale implementation of the approach could have significant benefits for children at highest risk for type 2 diabetes.

Understanding Results Observed at Comparison Schools

Certainly among the most intriguing HEALTHY study observations was the finding of an overall decrease in the number of overweight and obese children in both the control and intervention schools, when considering all of the children, including those who were normal weight at the outset of the trial. This was surprising, because in previous studies, rates of overweight and obesity generally continued to rise in control/comparison groups. Moreover, national statistics at the time that HEALTHY was being planned suggested that obesity rates were rising among youth. More recent data, however, indicate that childhood obesity rates may have leveled off in the United States. Also, although HEALTHY implemented no school-based activities at the control schools, all students who participated in data collection in sixth grade received a health "report card," with advice about seeking medical follow-up if there were abnormalities in weight, blood pressure and glucose,

insulin or lipid values. It is possible that parents in comparison schools acted on these results.

This potential explanation is bolstered by a report from Arkansas that suggested a leveling off of obesity rates among children after implementing BMI screening in the schools by school nurses, and notifying parents of the results. A recent report from California, however, did not show any change in BMI or obesity with BMI reporting, so its impact and influence in the HEALTHY study remain unclear. Numerous local, state, and federal mandates addressing childhood obesity during the HEALTHY study period may also have influenced the results in both control and intervention schools. Enhanced public concern about obesity among children may well have resulted in significant programming changes in the comparison schools. Indeed, physical education class time did increase in the HEALTHY control schools during the course of the study, and an equal number of intervention and comparison schools eliminated vending machines by the end of the study. Further research is needed to better understand factors currently affecting, and potentially stabilizing, rates of overweight and obesity among youth.

Offering HEALTHY Changes to More Schools

The HEALTHY program can potentially be implemented in other middle schools. Importantly, the physical education and FLASH components of the intervention were taught by the regular teachers, although the research team provided training. Cafeteria staff members also received training and were encouraged to promote messaging about nutritional quality. All HEALTHY materials for each of the five intervention semesters, including the physical education curriculum, FLASH student and teacher workbooks, nutrition messaging, and all posters and other social marketing materials are available at no cost at *www.healthystudy.org* These materials may provide helpful resources to staff at other schools seeking to implement environmental changes or school programs that promote messaging and behaviors that support healthy lifestyles among youth.

Genomic Variation and the Inherited Basis of Type 2 Diabetes

Dr. David Altshuler

Dr. David Altshuler is a world leader in the study of human genetic variation, using tools and information from the Human Genome Project to discover the underlying causes of type 2 diabetes and other common diseases. Dr. Altshuler earned his Ph.D. in 1993 from Harvard University and his M.D. in 1994 from Harvard Medical School. He completed his internship, residency, and clinical fellowship in Endocrinology at the Massachusetts General Hospital. A founding member of the Broad Institute of Harvard and MIT, he serves as a Director of the Broad Program in Medical and Population Genetics, as well as the Broad Institute's first Deputy Director and Chief Academic Officer. He is also a Professor of Genetics and Medicine at Harvard Medical School in the Department of Molecular Biology at the Center for Human Genetics Research, as well as the Diabetes Unit at the Massachusetts General Hospital.

Dr. Altshuler has been a lead investigator in multiple public-private partnerships that have established a foundation for disease genetic research: the Single Nucleotide Polymorphism (SNP) Consortium, the International HapMap Project, and the 1000 Genomes Project. His work has contributed to the discovery of over 100 gene variants that are associated with the risk of type 2 diabetes, cholesterol levels, heart attack, prostate cancer, systemic lupus erythematosus, and rheumatoid arthritis. In 2011, Dr. Altshuler received the prestigious Stern Award from the American Society for Human Genetics, which is given in recognition of major scientific achievement in human genetics that has occurred in the last 10 years. At the September 2011 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Altshuler described the research his team is pursuing, in collaboration with researchers around the world, to identify the causes of type 2 diabetes, its fundamental biology, and ultimately to develop more effective approaches to prevention and therapy. While most NIDDK-supported research has been and continues to be "hypothesis-driven" research, Dr. Altshuler described how new, transformative technologies can be used for discovery research to uncover new and unexpected disease pathways.

Rationale for a Human Genetic Approach: From the Patient to Biology and Medicine

Why use a human genetic approach to understand and ultimately guide treatment of disease? Dr. Altshuler focused on human genetics as a method for discovery—uncovering new causes of disease not previously discovered by other approaches.

Dr. Altshuler started by noting the vast majority of research is (appropriately) hypothesis-driven, building on previous observations. In today's world, these observations most often derive not from patients, but from experiments in cells and animal models. Moreover, drug development focuses to a large extent on these well-characterized hypotheses. And yet, experience has shown that the vast majority of candidate therapies that enter human clinical trials fail due to lack of efficacy, toxicity in humans, or other challenges. Dr. Altshuler argued that hypothesis-driven research is inherently hypothesis-limited research—and, to the extent that many important biological processes and disease mechanisms in humans remain to be identified, our ability to understand and treat disease will similarly be limited.

He posited that a new approach is needed to improve our knowledge of disease mechanisms in humans, and that this will ultimately boost the success rate for candidate therapeutics. Dr. Altshuler explained

that human genetic sequence data, technologies, and methods have recently become sufficiently advanced to identify robust genetic risk factors for common diseases. Starting with the human patient, scientists cast a wide and unbiased net across the genome, searching for whatever genes contribute to disease risk. These genetic risk factors point to new components of pathophysiology, and Dr. Altshuler argued that, however new and unexpected they may be, their relevance to humans argues for their being understood and pursued. The major challenge going forward is to invent methods to harness the resulting discoveries to develop diagnostic tools and therapeutic targets.

Dr. Altshuler next described his and colleagues' work to apply these approaches to type 2 diabetes.

Mapping of Genes and Variants Associated with Type 2 Diabetes—Background and Update

Within a population, about half of an individual's risk for type 2 diabetes is thought to be due to genetic factors inherited from his or her parents. So, how do researchers discover the specific genes and gene variants responsible for this inherited risk? Dr. Altshuler explained that, for many years, it was assumed that the genetic mutations that underlie inherited disease were rare and had very large effects. This was based on experience with single gene disorders such as cystic fibrosis and muscular dystrophy. In such cases, researchers developed a highly successful approach called family-based linkage studies to discover gene variants that tracked with disease. That approach had been successful in understanding the genes underlying literally thousands of rare single gene disorders. But, type 2 diabetes is not a single disease. Rather, it is a constellation of disease syndromes, or forms, some very rare, all leading to a final common diagnostic marker—hyperglycemia, or high levels of glucose in the blood. Only in rare families is diabetes caused by a single gene mutation. These include perhaps 10 genes contributing to rare families with early onset and/or severe forms of type 2 diabetes. Mutations in these genes explained only a percentage or two of the cases of type 2 diabetes seen in the clinic.

Based on the analogy to these rare single gene disorders, in the 1990s investigators applied family based linkage studies to the common forms of type 2 diabetes. Unfortunately, these studies failed to identify robust and reproducible discoveries of specific genes that contribute to the common forms of type 2 diabetes.

Around the year 2000, data from the NIH-led Human Genome Project (HGP) and the technologies developed to bring it to fruition helped launch a new approach to discovering genetic contributors to type 2 diabetes and other complex diseases. Rather than drilling down in multiple stages to find single genes, researchers sought to look out across the entire genome and ask: are there genetic variations that are more (or less) common in people with the disease as compared to people without the disease? These studies are called Genome Wide Association (GWA) studies.

Such studies required researchers to compare variation in the genetic blueprint among thousands of people. Each copy of the human genome is made up of three billion chemical units—the nucleotides adenine, thymine, cytosine, and guanine, represented by the letters A, T, C, and G. Researchers have known for decades that 99.9 percent of these nucleotides are the same when any two people are compared—that is, about one in every one thousand letters in one person's genome will differ from that of another person. Dr. Altshuler explained that the vast majority of the genetic variation in each individual human genome is common, not rare. That is, there are specific sites, spread across the human genome, that differ at a high frequency between human beings. This limited number of common variant sites could be catalogued and then tested for association with disease. In such studies, a skew in the frequency of

a particular common variant (*e.g.*, a higher frequency of "G" vs "A" at a particular site in the genome) could serve as a clue that a specific gene(s) in that region is somehow contributing to the disease.

In efforts facilitated by the HGP and new technologies, Dr. Altshuler has been a leader of NIH-supported projects such as the International HapMap Project and the 1000 Genomes Project. These projects were established to comprehensively catalogue common genetic variations between human beings. A database of human DNA variations, called dbSNP, is maintained by NCBI of the National Library of Medicine at NIH. Dr. Altshuler explained that 10 years ago, when the database was first created, it contained less than 1 percent of the DNA variations present in any individual. Today, over 98 percent of the DNA variations present in an individual are in this catalog.

Over the last 5 years, Dr. Altshuler and other investigators have used this information to search for genes contributing to risk of type 2 diabetes. As recently as 2005, there were only a couple of genetic variants known to contribute to the common forms of type 2 diabetes. Since then, over 40 new genetic variants have been discovered and shown to be reproducibly associated with disease risk.[1] In aggregate, these common variants account for about 10 percent of inherited risk of type 2 diabetes—far from complete, but a major increase as compared to the 1 percent or so of inherited risk that was explained 10 years ago.

Dr. Altshuler described an intriguing outcome of the GWA studies of diabetes-associated variants: before embarking on these studies, in 2006 he and his collaborators attempted to compile a list of all known candidate genes that had been hypothesized (through any of a variety of methods) as possibly relevant to type 2 diabetes. Over 600 such genes were identified based on reading papers and examining the results of other types of research.

Later, GWA studies identified a number of gene variants associated with blood glucose and insulin levels in healthy people without diabetes.[2] As expected, the vast majority of the genetic risk factors were in or near genes that were already on candidate gene list. In other words, the previous methods had done a good job of identifying genes that contribute to the inherited variation in blood glucose and insulin in individuals without diabetes.

In contrast, when Dr. Altshuler and colleagues examined the results of the GWA study approach for type 2 diabetes, they found little overlap with the list of 600 candidate genes. These results suggest that the genes underpinning normal glucose metabolism are not necessarily the same genes that contribute to the abnormalities that ultimately lead to type 2 diabetes. Dr. Altshuler proposed that the approaches that generated the pre-2006 list of candidate genes for type 2 diabetes—mostly studies in cells and animal models of glucose metabolism—were very successful at illuminating genes involved in human glucose metabolism in people without diabetes. But, it is possible that these approaches did not fully recapitulate the biological processes that go awry in human type 2 diabetes. Thus, focusing only on genes involved in normal glucose metabolism could limit investigators' ability to discover the genes and processes that contribute to this disease in people.

Can knowledge of genetic variants be employed clinically to help predict disease in individuals? Interesting results have emerged from leveraging clinical trial data. A key advance in diabetes from the last decade was the finding that type 2 diabetes can be prevented or delayed in people with prediabetes through medication or intensive lifestyle change (a program of diet and moderate exercise to induce 5 to 7 percent weight loss). This finding emerged from the Diabetes Prevention Program (DPP), a clinical trial spearheaded by the NIDDK. Now that gene variants affecting diabetes and other related traits have been identified, Dr. Altshuler and colleagues, led by Dr. Jose Florez of

Harvard Medical School and the Massachusetts General Hospital, have been analyzing data from the DPP to ask a key question: are people who have these genetic variants more likely to develop diabetes, or more or less likely to benefit from the DPP interventions?

Beginning with a study of variants in the *TCF7L2* gene[3] and followed by additional investigations of other high-risk variants, it turns out that the intensive lifestyle intervention was, on average, equally effective in DPP participants regardless of whether or not they had a high-risk variant—meaning that a high-risk genotype indicated by these variants can be overcome. Dr. Altshuler observed, however, that there does appear to be a genetic interaction between genotype and interventions that creates a continuum of risk. That is, if a gene variant increases diabetes risk and an intervention lowers it, an individual's risk ends up being intermediate. This continuum of risk versus an absolute "yes/no" risk may turn out to be typical for genetics of complex traits, such that genetics will end up being similar to familiar predictors of the sort that have existed in medicine for a long time—*e.g.*, cholesterol and blood pressure levels as measures of risk for heart disease.

While conducting human genetic studies offers the exciting prospect of new discoveries highly germane to human disease, Dr. Altshuler highlighted three major challenges to characterizing the genetic variant contributions to type 2 diabetes. First, the vast majority of the genes implicated by GWA studies are novel and thus previously unstudied. Second, the body tissue or tissues in which each gene might act are not yet clear —thus, researchers do not know what tissue to start with to get at how the gene functions, and how variants contribute to disease. Third, most of the genetic variants found through GWA studies are "non-coding," that is, the genome sequence variant does not appear to be within the part of the gene that codes for a protein product. Thus, it is difficult to pinpoint what is the actual causal gene—*i.e.*, the gene affected by the variant (either a nearby gene, or a gene that is otherwise affected by the variant). Researchers will need to overcome these challenges as they work to connect novel genetic variants to the biology of type 2 diabetes.

Dr. Altshuler reported that even as the results of the GWA studies he described are being interpreted, a second technological revolution is under way that makes it possible to use sequencing to find not only common variants within a population, but also less common and so-called "private" variants that are specific to an individual or family. Two NIDDK-supported, international diabetes projects are leading the way in the application of these new "next generation" sequencing technologies to try to further illuminate this disease. One, "GoT2D," is a research consortium established to develop and evaluate a variety of next generation sequencing approaches and methods for gene discovery in type 2 diabetes. The study, co-funded by the Wellcome Trust, the NIDDK, and the NIH's National Human Genome Research Institute (NHGRI), with additional support from the American Recovery and Reinvestment Act, is now examining genetic data from 2,800 people of European ancestry chosen from the extremes of diabetes risk. The other project is "Type 2 Diabetes Genetic Exploration by Next-generation sequencing in multiEthnic Samples," or T2D-GENES, a research consortium supported by the NIDDK and the NHGRI. Because the vast majority of genetic variant studies have been performed in populations of European ancestry, T2D-GENES is probing both the genetic differences that exist in different racial and ethnic groups and the role of different environments, different behaviors, *etc.*, in interacting with genetic risk for diabetes, by conducting DNA sequencing and other studies across a variety of samples from people of different ancestries and current geographic locations.

Dr. Altshuler noted that the pace of discovery in this field has been remarkable. Only 4 years ago, there had been few discoveries in type 2 diabetes, while now, with next generation sequencing, the

number is poised to expand rapidly beyond the over 40 candidates found in just the last few years. Researchers studying other diseases have made similar rapid increases in gene discovery within this short time period. With such a steep increase in information and new clues to disease, it will likely be 5 or 10 years until researchers have sorted through the enormous amounts of data to get a clearer picture of how genetic variants affect disease and the insights they can provide.

From Genes and Genetic Variants to Biology and Medicine

For diabetes and other diseases, what should researchers do with the genetic variants and associated genes that have already emerged from the exploratory approach of modern human genetic studies, and those that appear to be forthcoming? Dr. Altshuler emphasized that researchers need to turn their focus to understanding the biological functions of these genes and how they influence disease. Dr. Altshuler described three ways the research community can follow up on the wealth of data that has come from GWA and other sequencing studies:

- Generate biological hypotheses from the genetic findings to discover connections between genes and diseases, and pursue them.
- View sequence variations as "nature's randomized trial" for disease biomarkers that are potentially being targeted for therapeutics. That is, researchers could investigate whether or not genetically driven perturbation of a biologic factor affects disease outcome, thereby informing the likelihood that a drug developed to do the same thing might work.
- Investigate where and how prediction based on DNA information can improve or augment what researchers and clinicians can do today with other measures.

In terms of discovering connections between genes and pathophysiology, leveraging research findings from different lines of inquiry will help to make these connections more rapidly. For example, if a human genetic variant of interest for type 2 diabetes discovered by GWA studies or next generation sequencing is associated with a gene already being studied mechanistically in animal models, research teams can collaborate to see if the mouse model shows similar diabetes-related traits that will make it worth investigating as a model system for processes important in human diabetes.[4] Dr. Altshuler described one such project already under way that is showing promise for understanding the role of a gene, called *IMP2*, in type 2 diabetes. Variants in other genes that interact with *IMP2* have also been associated with type 2 diabetes in GWA studies and show interesting effects on glucose metabolism in non-diabetic humans,[1] strengthening the case for fleshing out the biology of these genes and the mechanism(s) by which they are contributing to diabetes risk.

Looking to the Future

Dr. Altshuler concluded by reasserting that human genetics offers an approach to develop new hypotheses about human biology not only in the context of rare syndromes linked to one or a few genes, but also for common diseases in which the genetic contributions are myriad and complex. While for decades the absolutely rate-limiting step has been the inability to go from a disease to a gene, recent advances in knowledge and technologies have helped scientists make very substantial progress in that regard for complex diseases—and, with the new sequencing technologies, it appears the field is poised for even more rapid growth. The challenge of the next 10 years for human genetic studies for type 2 diabetes and other common, complex diseases will be how to choose which follow-up experiments to do, which genes to study, and what approaches to take—a process that will take the effort and collaboration of many researchers.

[1] *Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 42: 579-589, 2010.*

[2] *Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42: 105-116, 2010.*

[3] *Florez JC, Jablonski KA, Bayley N, et al. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355: 241-250, 2006.*

[4] *Zhu H, Shyh-Chang N, Segrè AV, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell 147: 81-94, 2011.*

PATIENT PROFILE

Jack Schmittlein

Living with Type 1 Diabetes–A Disease That "Never Takes a Vacation"

Jack Schmittlein

On October 4, 2004, Jack Schmittlein's life changed forever. It was on that day that Jack, at age 6, was diagnosed with type 1 diabetes.

"Instead of being a carefree kindergartner, I was faced with pricking my fingers 8 to 10 times a day, counting carbs [carbohydrates], and taking insulin shots," says Jack, now an articulate 13 year old. So articulate, in fact, that he was invited by Congress to testify at a hearing entitled "Transforming Lives Through Diabetes Research" in June 2011. The hearing was held in conjunction with the Juvenile Diabetes Research Foundation International's (JDRF) Children's Congress.

What was it like to testify before members of the U.S. Congress? "Awesome!" says Jack. "It was a once-in-a-lifetime experience, and I felt honored to be speaking for all the other kids who have type 1 diabetes." Jack gave eloquent testimony about what it is like living with type 1 diabetes and the importance of pursuing research toward a cure for the disease.

"Instead of being a carefree kindergartner, I was faced with pricking my fingers 8 to 10 times a day, counting carbs [carbohydrates], and taking insulin shots," says Jack.

About Type 1 Diabetes

Type 1 diabetes is an autoimmune disease in which the immune system destroys cells in the pancreas that make insulin, a hormone required by the body to use sugar from food as a cellular fuel. People with type 1 diabetes must carefully monitor blood sugar levels, which Jack does by pricking his finger to get a small drop of blood to be tested. They must also administer insulin, either through injections or an insulin pump.

A constant challenge faced by people with the disease is matching food intake (which is why Jack counts carbs), physical activity, and insulin doses in order to maintain healthy blood sugar levels. Dramatic rises and drops in blood sugar can have immediate and life-threatening consequences, as Jack and his family are well aware. At the same time, NIDDK-supported research has shown that carefully controlling blood sugar levels over the long term is crucial to help prevent serious complications of diabetes, such as diabetic eye, kidney, nerve, and heart disease. Therefore, managing diabetes is a difficult and delicate balancing act of trying to control blood sugar levels, particularly in terms of avoiding dangerous bouts of very high or low blood sugar.

PATIENT PROFILE

A Disease That "Never Takes a Vacation"
The constant burden of managing type 1 diabetes means that the disease permeates every facet of a family's life.

"Every night my dad takes the late shift to check my blood sugar level after I've gone to sleep," says Jack. "And every morning my mom is there to wake me up and make me check my sugar level as the first thing I do each and every morning." In other words, "Managing diabetes is hard work that lasts 24 hours a day, every day," explains Jack.

"There are days when we're just not in charge of this disease," says Jack's mom, Denise. "Despite how controlled we try to keep it, diabetes wins a lot of the time."

A couple of years ago, the Schmittleins went on a vacation that included an excursion trip to swim with stingrays. Jack was excited. When they arrived by boat at the designated spot, "I jumped into the water," he says. He suddenly felt his blood sugar go extremely low. He immediately became dizzy and couldn't swim. Fortunately, his mom, Denise, was able to get him safely back into the boat while dad, Marc, stayed with Jack's younger brother, Cole. "My sister, Alexis, got left in the deep water with a bunch of stingrays coming at her. She still hasn't forgiven me," Jack says with a brotherly chuckle.

But the day only got worse. The Schmittleins discovered that Jack's insulin pump had broken, so the entire family needed to return to shore. They spent the next 2 days scrambling to get a replacement pump while Jack instead had to take insulin shots every time he ate. "Diabetes never takes a vacation!" says Jack.

"There are days when we're just not in charge of this disease," says Jack's mom, Denise. "Despite how controlled we try to keep it, diabetes wins a lot of the time."

Raising Awareness About Type 1 Diabetes
Jack has been actively involved in raising awareness about type 1 diabetes, and his outreach efforts have been remarkable. In addition to testifying before Congress, he was selected by the JDRF to be its 2010 Youth Ambassador for Connecticut and Western Massachusetts. He has spoken at JDRF diabetes fundraising events, including the Promise Ball, where he was the keynote speaker to an audience of more than 750 people, and Walk for a Cure, where he has spoken twice and been a team captain four times. He's also helped to organize a fundraising walk at school for 600 of his fellow students.

Why does Jack work so hard at raising awareness about type 1 diabetes and advocating for a cure? "I can only imagine what it would be like to not have to check my sugar as much, not have to measure everything I eat, and not have to tell my insulin pump how much insulin I need," he says. It would also mean that Jack, who loves sports, wouldn't have to come out in the middle of his basketball or football games to test his blood sugar. In other words, he is waiting for the day when he can take a permanent vacation from having type 1 diabetes.

Another motivation for Jack has been his best friend since second grade, who would keep him company on walks to the school nurse's office every day for blood sugar checks. "On the way, we'd try to guess what my sugar numbers would be," recalls Jack. When they were in fourth grade, Jack's best friend was also diagnosed with type 1 diabetes. Jack says that his friend's diagnosis is "just one more reason why I work to raise awareness about type 1 diabetes."

Hope Through Research
Right now, Jack is putting a lot of hope in what is called an artificial pancreas, or a "closed-looped system," still under development, in which a computer would

PATIENT PROFILE

calculate insulin dose based on blood sugar levels and deliver insulin automatically through an insulin pump, mimicking the function of a real pancreas. This type of system could reduce or eliminate much of the everyday burden of managing type 1 diabetes. The NIDDK is vigorously supporting research toward the development of an artificial pancreas that could help Jack and other people with type 1 diabetes.

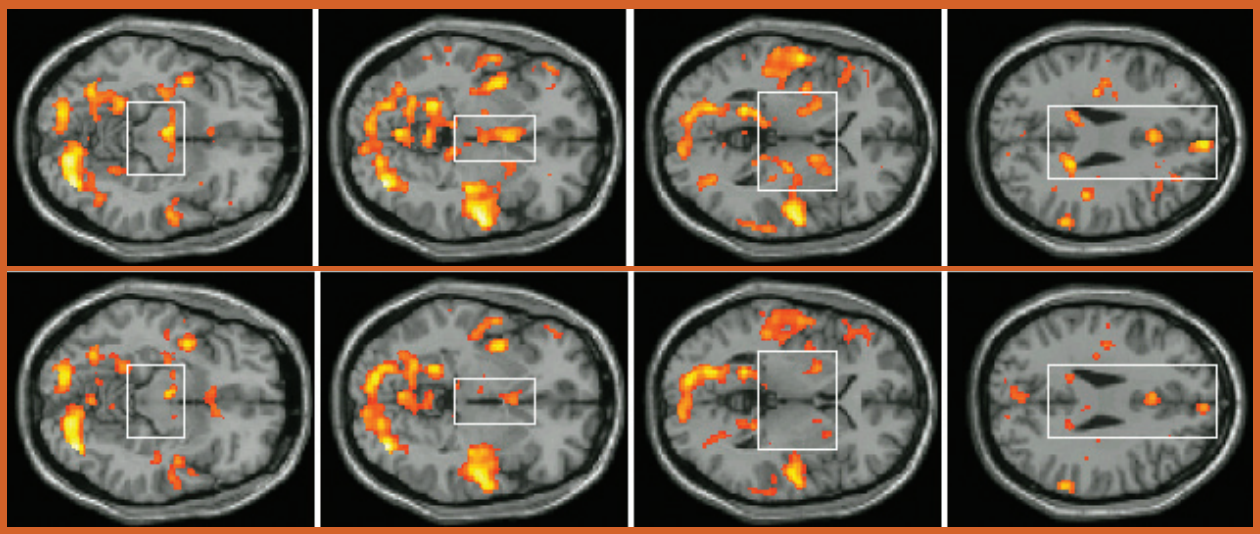
"I'm really looking forward to the day when I can say, 'I used to have diabetes.'"

"An artificial pancreas would help prevent my blood sugar from dropping and give me insulin if my blood sugar gets too high," says Jack. "It's going to be the next best thing to a cure. It will give me my life back so I can just feel like a kid, instead of a kid with diabetes," he adds hopefully.

In the meantime, Jack and his family will need to be vigilant and continue to cope with type 1 diabetes each and every day. "This disease is all consuming," says Denise. But, she notes that many things have helped her family cope with the disease, including getting support from other families living with type 1 diabetes, having a good relationship with Jack's doctor, and remaining optimistic that Jack can function with this disease and live a full and long life. Her advice to other parents is: "Don't let this disease define your child. Let them be a kid!"

As for Jack, his advice to his peers is: "If you're a kid and get diagnosed with type 1 diabetes, don't overreact…just be responsible for yourself and you'll be fine."

But he quickly adds, "I'm really looking forward to the day when I can say, 'I used to have diabetes.'"



Gastric bypass surgery, a treatment for extreme obesity, is performed on the digestive tract. Its effects, however, also reach to the brain to reduce the appeal of high-calorie food. In a recent study described in this chapter, researchers recruited volunteers who were planning to have gastric bypass surgery, and used functional magnetic resonance imaging to scan their brains before surgery and 1 month after surgery. During the imaging sessions, volunteers were shown pictures of calorie-dense foods (pizza and cake) and low-calorie foods (raw vegetables) to see whether the pictures, along with recordings of the names of the foods, would activate areas of the brain. The researchers found that brain responses to all food, but especially high-calorie foods, were notably diminished after surgery, particularly in areas known to process decisions to pursue rewarding, enjoyable experiences. Images left to right each show a different area of the brain (depicted in gray); images in the top row represent responses to high-calorie foods, whereas those in the bottom row represent responses to lower-calorie foods, with each showing the same area of the brain as the image directly above. The bright orange and yellow colors highlight areas with greater brain activity prior to surgery compared to a month after surgery. That is, if brain activity differed before and after surgery, the area where there was a difference is marked in yellow/orange. Within the boxed areas (comparing each image to the one directly below it), the yellow/orange spots are larger in the top row than in the bottom row, reflecting the fact that the surgery changed brain responses to high-calorie foods more than to lower-calorie foods. When asked how the pictures of foods made them feel, the volunteers reported less inclination to eat after surgery, and they no longer preferred high-calorie foods, consistent with the patterns of activity in the brain images. While gastric bypass surgery is designed to restrict calorie intake by modifying the structure of the digestive tract, these new findings help explain an additional way in which this surgery may lead to substantial weight loss—through loss of preference for high-calorie foods.

Images provided by Dr. Allan Geliebter, and reprinted from Ochner CN, Kwok Y, Conceição E, Pantazatos SP, Puma LM, Carnell S, Teixeira J, Hirsch J, and Geliebter A. Selective reduction in neural responses to high calorie foods following gastric bypass surgery. Annals of Surgery 253(3): 502-507, 2011; permission conveyed through Copyright Clearance Center.

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.

Approximately one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.[1,2] Nearly 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.[3] Obesity disproportionately affects people from certain racial and ethnic groups and those who are socio-economically 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. 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 for preventing and treating obesity span behavioral and environmental approaches in families, schools, and other community settings; medical interventions; and combinations of these strategies. In parallel, Institute-supported investigations into the biologic processes associated with body weight will spark new ideas for intervention approaches. To help bring research results to health care providers and the public, the Institute also sponsors education and information programs.

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. In 2011, the Task Force released an updated *Strategic Plan for NIH Obesity Research*, which was developed with extensive external input from researchers across the country, professional and other health-focused organizations, and others. The new *Strategic Plan* reflects the exciting opportunities that have emerged from research progress in the years since the NIH developed its first strategic plan on this major public health challenge.

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

GENETICS OF BODY WEIGHT AND ASSOCIATED CONDITIONS

Further Unraveling the Genetic Basis of Obesity: Scientists have uncovered 18 new genetic variants that predispose people to increased body mass index (BMI), a commonly used measure of obesity. A variety of factors contribute to risk for obesity: behavior, environment, and biology—including

[1] *Statistics Related to Overweight and Obesity. http://win.niddk.nih.gov/statistics/index.htm*

[2] *Flegal KM, et al. JAMA 303: 235-241, 2010.*

[3] *Ogden CL, et al. JAMA 303: 242-249, 2010. 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).*

genetic factors. Over the past few years, several genes have been discovered that influence body weight, but together only account for a fraction of all of the genetic determinants of obesity. To gain further understanding of the genes that regulate body weight, researchers sought to identify new genetic variants that associate with BMI—a calculation of weight relative to height—which is a simple and noninvasive measure of obesity. Scientists used several genome-wide association (GWA) studies to scan 2.8 million individual genetic variants in the DNA of nearly 250,000 people of European ancestry to find variants that associate with BMI. When the researchers analyzed data from GWA studies, they confirmed all 10 genetic variants that had previously been shown to associate with BMI, and also uncovered 22 new variants. Four of these 22 genomic regions were known from prior studies to be associated with obesity-related characteristics, such as body weight or increased waist circumference, narrowing the list to 18 new genetic variants that were not known previously to be associated with obesity. The genomic regions found to be associated with BMI implicate a range of biological processes and pathways in obesity risk, including gene regulation, brain function, and immunity.

Importantly, the researchers found that some BMI-associated genetic variants were also linked to metabolic traits such as insulin resistance, elevated blood lipid levels, and type 2 diabetes. One particularly interesting gene associated with increased BMI, *GIPR*, was also associated with increased glucose uptake by the body after carbohydrate ingestion. This link was surprising because often increased BMI is linked to reduced glucose uptake, indicating insulin resistance. These findings emphasize the complex genetic foundation for obesity, metabolism, and disease, with many genes providing varying degrees of influence. Researchers believe that this newly expanded list of genetic variants associated with BMI still only accounts for a small fraction of the genes that affect obesity. Therefore, additional avenues of research may help attain a more comprehensive understanding of body weight genetics.

Speliotes EK, Willer CJ, Berndt SI, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet 42: 937-948, 2010.

Genetic Variants Help Determine Body Fat Distribution: Scientists have identified 13 new genetic variants associated with waist-hip ratio, several of which appear to exert effects only in women. While obesity can be characterized as a condition of excess body weight, there are many approaches to measuring obesity, including BMI, or weight relative to height; overall body fat percentage; waist circumference; and waist-hip ratio (WHR), which reflects body fat distribution. A larger waist-hip ratio has been associated with serious health conditions such as type 2 diabetes and cardiovascular disease, and these increased health risks may occur even if a person's BMI is within the normal range. Analyses from previous obesity studies have suggested that the genetic factors that influence WHR are largely distinct from those that affect other measures of obesity, such as BMI. Prior studies have also shown that WHR is strongly influenced by genetics, but specific regions of the genome contributing to WHR remain largely undiscovered.

In this study, researchers surveyed the DNA from hundreds of thousands of individuals, using data from many large-scale GWA studies, and examined the links between WHR and each of millions of regions of the genome. Through their extensive analyses, scientists found that 14 genetic variants associated with WHR, although one of these had been previously identified. Of the 14 genetic variants, only four exhibited an association with BMI, suggesting that the genetic factors contributing to these two measures of obesity are largely independent of one another. Seven of these new genetic variants had large effects only in women. The scientists found that the genetic variants were also associated with increased circulating fats, harmful cholesterol, and insulin resistance—all indicators of metabolic disease. Eleven of the genetic variants were also associated with type 2 diabetes.

When examining the functions of genes within these genomic regions, the scientists found that they were involved in a range of biological processes, including fat cell development, regulation of fat molecule production, embryonic development, blood vessel formation, and insulin response. Interestingly, five of the genes identified in this study were turned on differently in fat tissue from the buttocks or the waist, suggesting that the differential action of the genes in

specific regions of the body could be linked to variation in body fat distribution. Together with results from GWA studies investigating other measures of obesity (also described in this chapter), research scientists are gaining a more detailed picture of the genetic foundation of this complex condition.

Heid IM, Jackson AU, Randall JC, et al. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat Genet 42: 949-960, 2010.

Risk Variations Discovered for Nonalcoholic Fatty Liver Disease: Research scientists studying the heritability of nonalcoholic fatty liver disease (NAFLD) have identified genetic variants that increase disease susceptibility. NAFLD ranges in severity from accumulation of fat in the liver, without injury, to the presence of liver fat with varying degrees of inflammation and scarring, which are associated with liver fibrosis and cirrhosis and which can progress to liver failure and the need for liver transplantation. This study looked at the heritability of NAFLD, identified risk variants associated with NAFLD, and defined the metabolic consequences of these variants.

Although evidence indicating that genetic factors contribute to NAFLD existed, heritability for this disease needed to be established and quantified, and few risk variants had been identified thus far. The researchers began this study with data collected by the Genetics of Obesity-related Liver Disease (GOLD) consortium, which consists of cohorts from four existing consortia, three of which include families. The members of the GOLD patient cohort had been diagnosed with NAFLD using imaging by computed tomography (CT), a noninvasive method to quantitatively measure liver fat. Analysis of data from the three family-based GOLD cohorts confirmed that CT-diagnosed NAFLD does, indeed, have a genetic component that is estimated to be between 26 percent and 27 percent. Next, the researchers used data from all four GOLD cohorts to search for NAFLD susceptibility variants. Genome-wide association studies were conducted separately on samples from each cohort. The results of the four studies were combined in a "meta-analysis" that identified three variants significantly associated with NAFLD—including one variant that had been identified previously. Additional analysis uncovered other possible variants. To study these potential variants further, the scientists sought to determine whether any were associated with another measure of NAFLD, based on biopsies collected in the NIDDK's Nonalcoholic Steatohepatitis (NASH) Clinical Research Network. This NASH network consists of patients who have fatty liver disease with fibrosis. Association studies with the NASH cohort and NAFLD variants showed that four of the variants identified in the GOLD cohorts also were associated with this liver disease in the NASH cohort; a fifth was only associated with CT-measured disease. Because earlier epidemiology studies linked NAFLD to metabolic traits, the variants were examined individually for effects related to these traits, including cholesterol, triglycerides, blood sugar, insulin resistance—a risk factor for diabetes, and obesity. The researchers found that three of the variants had effects on specific metabolic traits. The distinctive patterns of these metabolic effects suggest that the variants involve different metabolic pathways.

This study demonstrated that NAFLD is a genetic disease, determined the extent of its heritability, and identified five risk variants. The researchers combined study results with new technologies in inventive ways to determine which genes may be influenced by the variants and to elucidate the distinct patterns of metabolic traits affected. These patterns indicate involvement of different metabolic pathways and suggest that the pathways may provide multiple targets for drug development.

Speliotes EK, Yerges-Armstrong, LM, Wu J, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 7: e1001324, 2011.

Gene Variant Links Low Body Fat Percentage to Increased Diabetes Risk in Men: Using DNA from tens of thousands of individuals, researchers have identified genetic variants at two regions of the genome that are associated with reduced body fat percentage, one of which was also linked to increased risk for type 2 diabetes and cardiovascular disease in men. In addition to behaviors like dietary habits and exercise frequency, genetic factors are known contributors to obesity. Recent GWA studies have uncovered many

genes associated with BMI, which has been used as a surrogate for obesity. Since body weight is a sum of both lean and fat mass, genes associated with weight may not reflect an association with excess fat. Scientists had not looked as extensively for genes that specifically affect the amount of body fat.

In this study, scientists took advantage of several large-scale GWA studies, including more than 76,000 individuals, to find genetic variants that are associated with body fat percentage. The researchers looked for variants in approximately 2.5 million regions of the genome, and found 14 that seemed to have some association with body fat percentage. Further analysis narrowed their focus to three of these variants, including one that had previously been identified as associated with obesity and body fat. The other two variants were in genes not previously known to play a role in body fat, and both were associated with reduced body fat percentage. The researchers investigated links between the two newly identified gene variants and a variety of metabolic traits. The variant of the first gene, *IRS1*, exhibited a complex pattern of associations: it was linked to lower body fat percentage, but surprisingly also to increased circulating fat and glucose, and to reduced levels of "good" cholesterol. The *IRS1* variant was also linked to increased insulin resistance—an adverse condition which elevates risk of type 2 diabetes and heart disease. In addition, the *IRS1* variant was associated with a higher proportion of fat surrounding the internal abdominal organs (visceral fat), which confers higher risk for disease, as compared to fat just beneath the skin (subcutaneous fat). Many of these associations, including body fat percentage and several of the metabolic traits that were analyzed, had stronger effects in men. The variant of the second gene associated with reduced body fat percentage, *SPRY2*, was linked to higher insulin sensitivity, as would be expected. While the *SPRY2* variant did not show any differences between men and women, it was linked to body fat percentage in individuals of European descent, but not in those of Indian-Asian descent.

These two genes, *IRS1* and *SPRY2*, join a growing list of genetic factors that affect body weight, body fat percentage, and other obesity indicators such as waist-to-hip ratio. The findings in this study show that the genetic contributions to body weight are complex, with specific gene variants exhibiting different influences on obesity and diabetes risk between genders, as well as among different ethnic populations. A greater understanding of these complex genetic interactions could lead to personalized intervention strategies to reduce the elevated risk of diabetes and cardiovascular disease seen in people who are overweight and obese, as well as in some people who are lean.

Kilpeläinen TO, Zillikens MC, Stančákova A, et al. Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. Nat Genet 43: 753-760, 2011.

MOLECULAR MECHANISMS REGULATING FAT METABOLISM

Fat-saving Gene May Be an Achilles Heel in Efforts To Achieve Energy Balance: A molecule found in fat cells may hinder the body's ability to burn off fat, a finding that could yield new insight into the development of obesity. Normally, the body seeks a state of balance between energy consumption (eating food), energy storage (fat), and energy expenditure (burning fat to fuel activity and generate body heat). If this "energy balance" is disrupted, excess fat accumulation and obesity can result. Scientists believe they have found an unwitting contributor to this disruption in a cellular molecule called "CREB regulated transcriptional coactivator 3" (CRTC3), one of several CRTCs involved in metabolism. To mobilize stored fat for burning, the body can send signals through the nervous system to adipose (fat) cells, triggering a program of intracellular changes involved in the breakdown of fat. Interestingly, researchers found that these signals also activate CRTC3 and, surprisingly, that CRTC3 limits fat burning. Experiments in a mouse model revealed that, compared to mice possessing normal amounts of CRTC3, mice genetically engineered to lack CRTC3 had an apparent metabolic advantage—they were not only slimmer than normal mice when on a regular diet, but they also were resistant to weight gain and other negative effects of a high-fat diet, such as inflammation and insulin resistance. Intriguingly, these mice did not eat less or exercise more than their normal counterparts, nor did they appear to have problems with storing fat. Instead, the scientists found that mice lacking CRTC3 were breaking down and burning off fat as heat at a

much higher rate than usual. They appeared to do this by increasing initial fat breakdown in "white" adipose tissue, the body's most abundant form of fat tissue, and also by boosting the numbers of "brown" fat cells, which burn fat to generate heat. These experiments suggest that, normally, CRTC3 applies a "check" against the signals that stimulate fat burning, limiting their effect. Knowing, from previous research, that CRTC3 helps turn certain genes "on" or "off," the scientists performed additional experiments and discovered that, once activated, CRTC3 turns on a gene that limits fat breakdown. While these results come from studies in mice and mouse cells, it is possible that increases in CRTC3 activity relative to signals promoting fat burning could help explain disruptions in energy balance that contribute to obesity in humans. Supporting this hypothesis, the researchers found an association between a genetic variant of CRTC3, which confers an abnormally high level of CRTC3 activity, and increased risk of obesity in Mexican Americans. Armed with these new insights into the effect of CRTC3 on energy expenditure, scientists can now further explore the contribution of CRTC3 to obesity and possibly to the development of insulin resistance and type 2 diabetes.

Song Y, Altarejos J, Goodarzi MO, et al. CRTC3 links catecholamine signalling to energy balance. Nature 468: 933-939, 2010.

New Study Reveals Molecular Relationship Between Circadian Rhythm and Fat Metabolism: Results from a new study may help explain how metabolic problems develop when circadian rhythms are disrupted. In many animals, including humans, biological "circadian clocks" regulate many behaviors and bodily processes to harmonize these activities with daily, rhythmic changes in the environment, such as day/night cycles. Circadian rhythms also appear to be intricately linked with the daily regulation of metabolism. In humans, misaligning normal circadian rhythms with behaviors such as sleep and eating—for example, by working the night shift—increases vulnerability to diabetes, obesity, and other metabolic problems. However, the molecular mechanisms underlying this vulnerability are not yet clear. Because of its vital role in fat and glucose metabolism, the liver is a key target in metabolic dysfunction. Researchers have now found evidence that fat metabolism in the liver is set to a circadian rhythm by two interacting factors. One of these factors, histone deacetylase 3 (HDAC3), is an enzyme that chemically modifies structural proteins associated with DNA in a way that helps regulate gene expression (whether genes are turned "off" or "on"). When HDAC3 is recruited to sites in the genome, genes at those sites tend to be turned "off," and when it is absent, those genes are free to be turned "on."

Evidence is emerging that cells use HDAC3 as part of a nimble strategy to shift patterns of gene expression across the genome, including those important to circadian rhythms and metabolism. In the current study, researchers compared the presence of HDAC3 at sites across the mouse liver genome at two different points in the circadian cycle—once during the normal rest and fasting time for mice (day) and once during the active, feeding period (night). They found that, while HDAC3 occupied over 14,000 sites during the day, all but 120 of these sites are empty of HDAC3 at night. Chemical modifications and changes in gene expression "machinery" followed this rhythm of HDAC3 occupation in a way suggesting that gene expression at these sites was cycling from being "off" during the day to being "on" at night. In contrast, when liver cells were depleted of HDAC3, rhythmic control of gene expression at these sites was disrupted. The researchers found that the level of HDAC3 in liver cells does not itself vary significantly over the course of the day, suggesting that the rhythm of HDAC3 recruitment to the genomic sites is driven by some other factor. Indeed, they found that a "clock protein" called Rev-erbα, which is expressed in a circadian manner, is required for driving HDAC3 recruitment to its binding sites. Interestingly, the genomic sites bound by both HDAC3 and the clock protein during the day are enriched for genes involved in metabolism, particularly metabolism of lipids (chemical components of fat), including genes controlling lipid synthesis. The metabolic implications of this observation became clear when the researchers compared mouse livers depleted of HDAC3 to those with normal amounts of HDAC3. After 2 weeks of HDAC3 depletion, mouse livers had accumulated nearly 10-fold more fat than was found in livers from mice with normal amounts of HDAC3. Similar results were seen in mice lacking the clock protein: these animals had nearly twice as much liver fat as their normal counterparts.

These experiments provide an explanation for findings from other studies showing that fat synthesis in the mouse liver is higher at night, when mice are active and feeding, than during the day, when they are at rest and should be utilizing fat stores. While these studies were performed in mice, they elucidate a mechanism linking circadian rhythm with fat storage in the liver that could potentially help explain how disruption of circadian rhythm leads to metabolic dysfunction in people.

Feng D, Liu T, Sun Z, et al. A circadian rhythm orchestrated by histone deactylase 3 controls hepatic lipid metabolism. Science 331: 1315-1319, 2011.

INFLAMMATION AND OBESITY

A Double-edged Sword—The Body's Own Defenses Contribute to Insulin Resistance Associated with Obesity: New research further unraveled the complex contributions of the immune system to insulin resistance. Insulin resistance is a condition in which the body produces insulin—a hormone that helps the body use glucose for energy—but does not use it properly. Insulin resistance increases the risk for type 2 diabetes and heart disease; therefore understanding how it develops is critical toward efforts to prevent or reverse it. During excess weight gain, specific immune cells, called macrophages, migrate into and accumulate in adipose (fat) tissue and promote chronic, low-grade inflammation, which contributes to the development of insulin resistance. Although many genes and molecular pathways have been implicated in the immune response to excess weight gain, how macrophages, and other immune cells including certain T cells, promote inflammation and subsequent insulin resistance remains poorly understood. Several recent studies sought to elucidate how immune cells are activated in response to fat and turn on genes that lead to inflammation.

Two studies revealed new insights into how immune cells "sense" obesity. Proteins that reside on the surface of macrophages normally recognize foreign invaders in the body and initiate an immune response, which leads to inflammation. However, these proteins can also recognize molecules that are associated with excess weight gain and initiate a response that eventually leads to insulin resistance. In one study, scientists determined that a protein called Nlrp3 promotes insulin resistance, in part through recognition of a specific type of complex fat molecule called ceramide. When fed a high-fat diet, mice normally develop insulin resistance. Mice genetically engineered to lack Nlrp3, however, were protected from this effect. To investigate whether Nlrp3 played a similar role in humans, the researchers examined the responses of people who were obese with type 2 diabetes to weight loss through dietary changes and increased physical activity. They found that weight loss in these people led to reduced *Nlrp3* gene activity in fat tissue, and improved their sensitivity to insulin. The link between ceramide and insulin resistance was further explored in another study, in which researchers studied the effects of the hormone adiponectin on ceramide. Adiponectin, a circulating protein that promotes insulin sensitivity and reduces inflammation, interacts with a pair of protein partners (AdipoR1 and AdipoR2) on the surface of many types of cells throughout the body. The scientists discovered that AdipoR1 and AdipoR2 cause the breakdown of ceramide in the cell. Mice genetically engineered to produce increased levels of AdipoR1 and AdipoR2 in the liver had improved insulin sensitivity. Together, these findings suggest a central role for ceramide in the development of insulin resistance.

Other efforts uncovered the mechanisms by which immune cells turn on a program of immune response genes to initiate inflammation—what happens after immune cells sense obesity. In one study, scientists focused on understanding the role of FoxO1—a protein that can turn some genes on and off. The scientists showed that FoxO1 directly interacted with specific genes in macrophages, thereby activating a genetic program that induced inflammation. Importantly, when they reduced FoxO1 levels in mice, the researchers found that the inflammatory response, which was normally induced upon immune system stimulation, was abrogated. Another team of scientists dissected the function of a protein called Coronin 2A, finding that it acts in opposition to FoxO1; it associated with proteins that function to turn off genes in macrophages, including ones involved in the inflammatory response. However, when macrophages were stimulated, the scientists found that Coronin 2A facilitated the removal of these proteins from some of the inflammation genes, allowing them to be turned on. These studies have identified two key molecular factors that coordinate

the program of genes turned on during the macrophage inflammatory response.

Lastly, new results added information about how excess weight gain affects other immune cells in the development of insulin resistance. In addition to macrophages, another type of immune cell, the regulatory T (Treg) cell, is known to play a role in insulin resistance. Previous research found that Treg cells reside in the adipose tissue of lean mice, but not in that of overweight mice, and the presence of Treg cells in fat tissue helps to protect mice from developing insulin resistance. In a recent study, scientists found that, during excess weight gain in mice, macrophages in the adipose tissue send molecular signals that inhibit the production of Treg cells. This leads to depletion of Tregs in fat tissue in mice. To translate this finding to humans, the scientists showed that Treg cells were relatively abundant in human fat tissue from lean people, but observed a modest depletion of Treg cells in adipose tissue from obese individuals. This interaction between the two immune cells—macrophages and Treg cells—in fat tissue demonstrates a complex relationship between the immune system and the development of obesity.

These findings add key new knowledge to understanding the complicated immune response to obesity. Some of these recent results have been confirmed in humans, but additional research will determine whether the pathways studied in mice function similarly in people. These studies also suggest that numerous molecular pathways could serve as targets for the development of therapeutics aimed at reducing fat tissue inflammation, with the goal of preventing insulin resistance and other adverse health consequences of obesity.

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Fan W, Morinaga H, Kim JJ, et al. FoxO1 regulates Tlr4 inflammatory pathway signalling in macrophages. EMBO J 29: 4223-4236, 2010.

Holland WL, Miller RA, Wang ZV, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med 17: 55-63, 2011.

Huang W, Ghisletti S, Saijo K, et al. Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. Nature 470: 414-418, 2011.

Vandanmagsar B, Youm YH, Ravussin A, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17: 179-188, 2011.

HOW THE BRAIN AFFECTS EATING BEHAVIOR AND METABOLISM

A Gut Feeling in the Brain—How the Hormone GLP-1 Signals the Brain To Reduce Food Intake and Body Weight: Researchers gained new insights into the body's control of eating with discoveries about the signals sent by a powerful molecule, GLP-1, to cells in the brain—and in particular, a part of the brain that has a direct line of communication with the gut. The body produces GLP-1 primarily in the brain and in the intestines. At meal time, GLP-1 responds to the influx of food by triggering the beta cells of the pancreas to produce insulin, which then prompts various cells in the body to take up sugar. Because of GLP-1's role in balancing blood sugar levels, scientists have developed medications, currently available, which mimic or increase GLP-1 activity as a means of treating type 2 diabetes. GLP-1 also functions to help people feel full longer, and as a result, eat less. A team of researchers recently investigated how GLP-1 exerts its effects on brain cells in rats. Based on knowledge of GLP-1's action in beta cells, the researchers focused on three signaling proteins that they thought might respond to GLP-1 in the brain. These proteins are known by their acronyms, PKA, AMPK, and MAPK—with the "K" in each standing for a type of chemical-tagging activity (kinase) common to proteins that relay important signals within cells. To facilitate their studies, the researchers used a particularly stable version of GLP-1 (called Exendin-4). They administered it directly to the rats' brains, and found that it modulated the activity of each of these signaling proteins. The rats also lost weight. With a machine the scientists refer to as a "feedometer," which recorded how much the rats were eating every minute, the researchers discovered that administering the stable version of GLP-1 into the rats' brains caused them to eat fewer meals, although the size of each meal did not change. This finding likely explains why the rats

lost weight. It is also intriguing because previous research had shown that GLP-1 administered elsewhere in the body has a different, but complementary, effect: it does not change the number of meals but does reduce the amount of food eaten in each. With more precise experiments, the researchers were able to pinpoint a key region of the brain that responds to GLP-1. This region is within an area of the brain that can communicate with the digestive tract, by sending signals through nerves that reach from the brain to the gut and back. These findings may lead to the development of new drugs that target GLP-1 signaling in the brain.

Hayes MR, Leichner TM, Zhao S, et al. Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation. Cell Metab 13: 320-330, 2011.

A Protein in the Brain Regulates Fat Tissue Composition in the Body: Researchers have discovered that in rats, a protein in the brain determines the makeup of fat tissue and influences body weight, physical activity, and metabolism. For decades, scientists have known that damage to the hypothalamus—a part of the brain that functions to connect the nervous system to the endocrine system—leads to changes in hunger, satiety, and physical activity. These changes alter the balance between calories (energy) taken in and calories burned, or energy balance. The reasons for these changes, however, have remained unclear. Neuropeptide Y (NPY), a protein produced in different regions of the hypothalamus, is known to influence energy balance, but its function in a specific region, called the dorsomedial hypothalamus (DMH), has not been defined. To address this question, research scientists depleted NPY protein levels in the DMH of rats and examined the animals' body weight, fat composition, and metabolism. Lower NPY levels in the DMH led to reduced weight and decreased amounts of inguinal fat—a specific fat depot in the lower abdomen. When the researchers looked carefully at the cellular makeup of the inguinal fat tissue, they discovered a change in its composition. Typically, fat tissue under the skin consists largely of "white fat" cells, which store fat molecules, called lipids. Another type of fat tissue, called "brown fat," actually burns calories in order to generate heat. Reduction of NPY in the DMH led to a loss of white fat and the development of active brown fat in the inguinal tissue. The researchers also found that genes involved in breaking down lipids were turned on in the inguinal fat tissue. These effects were reduced when, prior to depleting NPY, the scientists injected a chemical into inguinal fat to destroy the nerve connections that mediate the DMH signals. In addition, when NPY levels were reduced in the rats' DMH, the scientists observed increased physical activity, energy expenditure, and body heat production in response to cold temperatures. To further understand how NPY affects obesity, rats were fed a high-fat diet, which led to weight gain, increased feeding and accumulation of fat, and increased insulin resistance (a condition associated with diabetes and prediabetes), in rats that had normal levels of NPY. Reduction of NPY in the DMH, however, lessened all of these effects of high-fat diet consumption, supporting a role for NPY in diabetes risk and obesity. These findings suggest that normally, NPY in the DMH region of the brain blocks the formation of brown fat, promotes weight gain and inactivity, and slows metabolism. This study thus adds new insights into the complicated connection between the brain and energy balance. If NPY is found to have a similar function in people, it could provide a molecular target for strategies to address obesity and associated conditions, such as type 2 diabetes.

Chao PT, Yang L, Aja S, Moran TH, and Bi S. Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. Cell Metab 13: 573-583, 2011.

To Eat or Not To Eat—How Bariatric Surgery for Obesity Affects the Brain: Used for treating extreme obesity, gastric bypass surgery not only changes the digestive tract, but it also appears to affect the brain in ways that reduce the appeal of high-calorie food, as researchers discovered from brain imaging studies. Gastric bypass surgery leads to substantial weight loss by making the stomach smaller and by routing food on a shortcut through the small intestine to bypass an area that would otherwise absorb some of the calories. Yet, these changes do not appear to account for all of the weight loss.

Intriguingly, this surgery may also lead to changes in the brain. While people who lose weight from dieting often report an increase in appetite that makes it hard to keep the weight off, individuals who lose weight

from gastric bypass surgery do not seem to have the same increased desire to eat. To better understand how the surgical procedure affects the brain, researchers recruited people who were planning to have bariatric surgery, performed brain imaging (functional magnetic resonance imaging) on the volunteers 1 month before and 1 month after surgery, and compared the results. The volunteers, 10 women from diverse racial and ethnic groups, were all extremely obese prior to surgery. During each imaging session, the scientists showed the volunteers pictures of calorie-dense foods, such as pizza and cake; lower-calorie foods, such as raw vegetables; and, as a control, office supplies. They also provided recordings so that the study volunteers would hear such tempting phrases as "chocolate brownie," along with words for the other items. The brain scans revealed significant differences pre- and post-surgery. Although the pictorial and verbal food cues activated a number of regions of the brain, surgery dampened these effects, most noticeably reducing brain responses to calorie-dense foods. Moreover, the largest changes were in areas of the brain known to process rewarding experiences and pleasure-seeking behavior, such as the decision to eat highly appetizing food. When asked how they felt in response to the pictures and verbal descriptions of foods, the volunteers reported less of an inclination to eat, particularly calorie-dense foods, after surgery. Fortunately, their interest in eating vegetables did not diminish to the same extent.

This study shows that gastric bypass surgery affects brain activation and reduces the desire to eat high-calorie foods. The researchers hypothesize that one way in which surgery may have these effects could be through changes in various signaling molecules in the body that are known to influence appetite. With further research, scientists may elucidate the biological mechanisms for these effects of surgery, and potentially develop new medications to achieve the same results.

Ochner CN, Kwok Y, Conceição E, et al. Selective reduction in neural responses to high calorie foods following gastric bypass surgery. Ann Surg 253: 502-507, 2011.

It's All (Well, Partly) in Your Head—The Brain and Metabolism: Studies from several laboratories are bringing into greater focus the critical impact of the brain on regulation of body weight, and considerable evidence suggests that signals from the central nervous system also have important effects on glucose levels via the liver and other tissues. New research shows that the brain exerts its influence on glucose levels and body weight through several distinct neural and hormonal pathways, with implications for diabetes and obesity therapies.

For example, the hormone leptin—best known for its appetite-suppressing action—can also normalize glucose levels when delivered into the brains of rats with uncontrolled diabetes. Recently, in experiments in rats, researchers found that this was the result of a profound drop in the amount of glucose released into the bloodstream by the liver, as well as an increase in glucose uptake by muscle and other tissues. They found that these effects did not rely on the pancreatic hormone insulin, which lowers blood glucose through effects on liver and other tissues, and leptin's actions also did not rely entirely on reduction of glucagon, the pancreatic hormone which signals the liver to release glucose. Thus, this study helps define a novel mechanism by which leptin action in the brain can modulate glucose levels independently of these two major glucose-regulating hormones. Because this work defines an insulin-independent mechanism by which the body affects blood glucose levels, the findings may have implications for treating both major forms of this diabetes.

Another group of researchers has helped define the role of a protein called the melanocortin-4 receptor (MC4R) in controlling the liver's uptake and secretion of glucose. A mutation in the *MC4R* gene is known to cause severe obesity in humans. Mice lacking MC4R are also severely obese, with greatly elevated blood glucose and insulin levels. In studying these mice, the researchers tested the effects of restoring MC4R function only to a subset of nerve cells that usually contain MC4R; this subset is referred to as cholinergic neurons based on the type of signaling molecule (neurotransmitter) they use. Although no other tissues in the mice contained MC4R, blood glucose and insulin levels were normalized, the former partly through suppression of liver glucose production. These animals were slightly less obese than those without any MC4R at all, and further experiments showed that the modest effects on body weight likely resulted from increased calorie burning (energy expenditure), although the

mice still consumed more food than normal mice. Interestingly, previous research had shown that MC4R in other types of brain cells does affect food intake. This study therefore adds to growing evidence that MC4R has independent effects on weight and glucose levels—information that will be useful in attempts to develop obesity therapeutics targeting MC4R.

Similarly, another research team identified a key glucose modulating role for a group of nerve cells that use a different receptor protein (a serotonin receptor) and neurotransmitter. Working in mouse models, they found that these nerve cells, which are located in the hypothalamus—a part of the brain that has important effects on appetite and energy regulation in the body—also help control the liver's response to insulin.

Intriguingly, the hypothalamus contains nerve cells that can "sense" glucose in a similar fashion to pancreatic beta cells. Another research team investigated a subset of these nerve cells to see if they play a role in regulating blood glucose levels in the body. Because the nerve cells they studied are directly stimulated by glucose, the researchers developed experimental methods in mice to make the nerve cells more sensitive so that even low levels of glucose stimulate them to fire, or less sensitive, firing only when glucose reaches higher than normal levels. These experiments helped identify molecules in the nerve cells important for glucose sensing. Additionally, they found that the effects extended beyond the brain: making the nerve cells more sensitive led to lower blood glucose levels, while lower sensitivity led to higher glucose levels, indicating that impulses from these nerve cells have a key role in modulating blood glucose levels.

Each of these discoveries helps in understanding the way the brain affects metabolic processes elsewhere in the body, and each represents a potential target for intervention to restore healthy glucose levels in people with diabetes.

German JP, Thaler JP, Wisse BE, et al. Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia. Endocrinology 152: 394-404, 2011.

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Xu Y, Berglund ED, Sohn J-W, et al. 5-HT2CRs expressed by pro-opiomelanocortin neurons regulate insulin sensitivity in liver. Nat Neurosci 13: 1457-1459, 2010.

Kong D, Vong L, Parton LE, et al. Glucose stimulation of hypothalamic MCH neurons involves K(ATP) channels, is modulated by UCP2, and regulates peripheral glucose homeostasis. Cell Metab 12: 545-552, 2010.

RESEARCH TO FIGHT CHILDHOOD OBESITY

Examining How Maternal Glucose Levels May Influence Childhood Obesity: New studies are providing insight into how nutritional exposures in the womb may affect children's risk for overweight and obesity as they grow. Concerns have been rising about the effects on offspring of gestational exposure to elevated maternal blood glucose levels. Previous research had established that maternal diabetes during pregnancy not only increases likelihood of complications during gestation and delivery, but that it also increases risk of obesity and type 2 diabetes in the offspring. However, more recently, a major clinical study showed that elevated maternal blood glucose levels below those diagnostic of gestational diabetes still incurred risk for serious pregnancy, birth, and neonatal complications. Now, researchers are trying not only to better understand these short-term risks, but also to ascertain whether even moderately elevated maternal blood glucose levels affect future obesity risk in offspring.

Two recent studies tackling this question have found a positive correlation. In one study, researchers recruited over 260 women, a subset of over 1,000 who had participated in a large pregnancy clinical study and whose babies were now toddlers. They looked to see if there was a relationship between maternal blood glucose levels in women not diagnosed as diabetic during pregnancy and their child's body mass index (BMI) at 3 years of age. The study results suggest that high maternal glucose levels during pregnancy correlate with increased risk of overweight and obesity in the children. Another research team pursued this question in 27 older children, between 5 and 10 years of age, whose mothers' blood glucose levels during pregnancy ranged from low to diabetic. In this study,

not only was the children's body composition—both fat and lean mass—determined, but also their resting energy use and activity levels, which could affect their body composition, particularly their amount of body fat. The results suggest that the higher a mother's glucose levels during pregnancy, the more likely the child will have both higher fat and lean mass, and that this correlation may be independent of how active the child is, their energy use at rest, and their diet. These studies were small and had other limitations, so additional research is needed to understand the long-term effects of prenatal exposure to elevated maternal glucose levels on children's risk of obesity. Nonetheless, both studies suggest avenues for further investigation toward the goal of promoting healthy outcomes for the next generations.

Chandler-Laney PC, Bush NC, Rouse DJ, Mancuso MS, and Gower BA. Maternal glucose concentration during pregnancy predicts fat and lean mass of prepubertal offspring. Diabetes Care 34: 741-745, 2011.

Deierlein AL, Siega-Riz AM, Chantala K, and Herring AH. The association between maternal glucose concentration and child BMI at age 3 years. Diabetes Care 34: 480-484, 2011.

Lifestyle Intervention Program Targeting Obesity Shows Promise in Ethnically Diverse Children: New research has shown that an intensive, family-based lifestyle intervention program can lead to sustained reductions in body weight and indicators of diabetes risk in ethnically diverse children. Because the high rate of childhood obesity and overweight has been a challenging problem, many previous studies had focused on body weight management through lifestyle intervention. While some of these studies led to reductions in body weight, they were limited in scope because they often involved small numbers of predominantly Caucasian, middle-class participants who were not as obese as the children in the current study, and limited follow-up times after the intervention.

In order to extend and broaden the scope of previous research, scientists in this intervention study included larger cohorts of ethnically diverse (African American and Hispanic) inner-city children from predominantly lower-income families—populations that tend to be at high risk for obesity and overweight. Study cohorts included children, ages 8 to 16, who were obese, as defined by a body mass index (BMI, a measure of weight relative to height) at or above the 95th percentile. Participants were randomly assigned to either an intervention or control group. The intervention group received an extensive family-based program that included: a twice-weekly exercise regimen of intense aerobic games and other physical activities (*e.g.*, obstacle courses, basketball, and flag football); nutrition education that promoted healthy foods and moderate consumption; and behavior modification instruction that provided training in self-awareness and goal-setting. Children in the intervention group were also encouraged to maintain a healthy lifestyle after the program was completed. In contrast, the control group received general information on diet and exercise, along with psychosocial counseling, every 6 months for the duration of the year. Although the active portion of the study ended after 12 months, researchers followed all participants for an additional 12 months to determine longer-term effectiveness of the interventions. Evaluation measures included physical traits (height, weight, BMI, percent body fat, blood pressure) and levels of blood components (insulin, glucose, circulating fats, and cholesterol).

The results demonstrated that the average BMI in the intervention group was reduced within 6 months and sustained for 24 months—1 year after the end of the intervention program. In addition, significant reductions in insulin resistance—a measure of the body's inability to utilize the hormone insulin to promote the uptake of sugar, and an indicator of risk for diabetes—were also observed at 24 months. The results from this study indicate that intensive lifestyle intervention programs can sustainably lower excess body weight and improve other health measures in children from diverse ethnic and socioeconomic backgrounds, potentially leading to a reduced burden of obesity-related health problems into adulthood.

Savoye M, Nowicka P, Shaw M, et al. Long-term results of an obesity program in an ethnically diverse pediatric population. Pediatrics 127: 402-410, 2011.

It All Adds Up—New Mathematical Model Helps Explain Why Dietary Strategies Have Not Worked Well for Many People

Researchers have found that the widely accepted dietary paradigm for weight loss—that reduction of 3,500 calories will shed one pound of weight—is incorrect, and have developed a mathematical model (and an accompanying online weight simulation tool) that more accurately predicts weight loss for adults. The commonly held belief that eating 3,500 fewer calories, or burning them off exercising, will always result in a pound of weight loss assumes that all people respond to caloric change in the same way. A recent study conducted by scientists in the NIDDK Intramural Research Program has challenged this static model and shown it to be overly simplistic. Many diets fail because people have unreasonable expectations based on the static model: they expect to see results rapidly, and feel discouraged when weight loss slows over time despite adherence to a specific diet. The new model developed by this research team reveals that this pattern of anticipated weight loss is unrealistic, leading many people to abandon their diet and exercise strategies.

In order to address complex, multi-faceted health conditions, a range of conceptual and technological approaches must be employed by scientists. Dr. Kevin Hall, trained physicist and research scientist at the NIDDK, decided to tackle the health burden of obesity by utilizing his strong mathematical skills to develop a more comprehensive, and much more realistic, model for weight loss and metabolism. Computer-based simulations developed by his lab more accurately reflect changes in a person's body during weight loss by taking into consideration differences between people, such as gender, age, height, weight, amount of body fat, and resting metabolic rate. The team's complex mathematical "dynamic" model and internet-based weight simulation tool (found at *http://bwsimulator.niddk.nih.gov*) incorporates these various parameters; the model also accounts for changes in metabolism during weight loss, and the variation in these changes among people. "This research helps us understand why one person may lose weight faster or slower than another, even when they eat the same diet and do the same exercise," explains Dr. Hall. "Our computer simulations can then be used to help design personalized weight management programs to address individual needs and goals."

To test their model, the scientists compared expected weight changes to actual changes in people. They found that the new model more accurately predicted weight loss, suggesting that many factors must be integrated to fully understand an individual's response to diet and exercise. These simulations revealed many physiological complexities associated with weight loss. For example, the team found that people's bodies adapt slowly to changes in dietary intake. They also found that heavier people can expect greater weight change with the same change in diet, though reaching a stable body weight will take them longer than people with less fat. The previous static model overestimated weight loss because it failed to account for how metabolism changes during weight loss.

The model makes specific predictions about weight loss, as illustrated by making assumptions for an average overweight adult. In this case, for every pound of weight loss desired, 10 calories per day must be permanently cut from the current intake. At that rate, it will take about 1 year to achieve half of the total weight loss, and almost all of the weight loss will have occurred by 3 years. Researchers can use the web simulation tool to plan for an initial phase of more-rapid weight loss followed by a weight maintenance phase. "By using our model to track progress, clinicians can help people re-evaluate their goals and ability to achieve them at the pace they want," Dr. Hall said. "It's a good reality check for how long weight loss takes, and what changes in eating and exercise are required to achieve and maintain goal weight."

The effective use of mathematical modeling to address real-world health problems—in this case obesity—highlights the need to explore inventive research directions. "This research illustrates how the interdisciplinary skills of NIH scientists, like a physicist doing obesity research, can help lead to innovative

ways to test, understand, and treat a major public health epidemic," said NIDDK Director Dr. Griffin P. Rodgers, "Advancing research from the laboratory to the bedside enables us to make the discoveries that can better people's lives." The NIDDK has been committed to advancing research in a range of health issues through computational approaches. For example, scientists in the NIDDK's Laboratory of Biological Modeling pioneered the field of computational neuroscience. By investigating the behavior of oscillating signals, these scientists have applied modeling to research fields like neuronal signaling and insulin secretion from the beta cells of the pancreas.

Despite its validity in a controlled research setting, the computer simulation of metabolism is intended to be a research tool, not a specific weight-loss guide for the public. The computer program can run simulations for changes in calories or exercise that would never be recommended for healthy weight loss, and people should consult with their physician prior to embarking on a diet plan. Additionally, the researchers point out that their current mathematical model was developed for adults, and would not predict weight change in children and adolescents because it does not account for biological changes associated with growth.

Current research by Dr. Hall's team seeks to improve the computational modeling tool to make it more useful for a variety of applications. To more rigorously test the model, Dr. Hall and his team are taking advantage of NIDDK's Metabolic Clinical Research Unit—a state-of-the-art facility that allows researchers to carefully measure energy intake (how many calories individuals eat) and expenditure (how many calories people burn to fuel basic life functions and physical activity). Efforts are also underway to use and validate this computational tool in clinical trials. The model is currently being tested in an NIDDK-funded clinical trial aimed at understanding links between specific foods and human physiology, including brain reward pathways. The trial will provide metabolic data from participants undergoing weight loss, which will then be used to test the model through personalized computer simulations.

The new model developed by Dr. Hall and his colleagues shows that weight loss in adults happens slowly over longer periods of time than previously expected. This finding could help explain why many dieters observing initial weight loss relax their diets, only to find that the weight comes back due to altered metabolism. The model can also be used to help simulate potential effects of policy changes aimed at addressing obesity. Dr. Hall and his team hope to use the knowledge gained from developing the model and from clinical trials in people to refine the tool so everyone can develop a healthy and realistic personal strategy for long-term weight management.

Hall KD, Sacks G, Chandramohan D, et al. Quantification of the effect of energy imbalance on bodyweight. Lancet 378: 826-837, 2011.

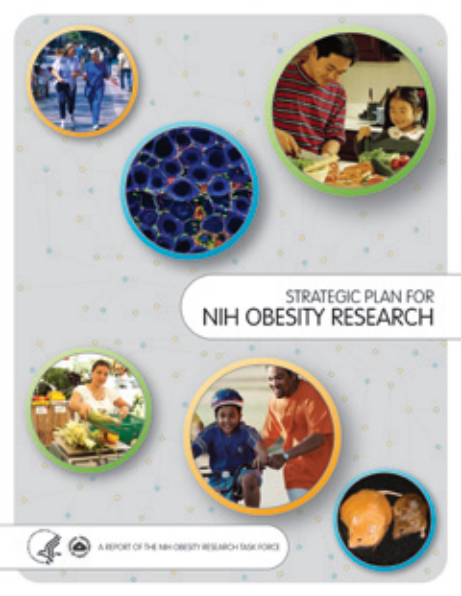
Strategic Plan for NIH Obesity Research

In 2011, the NIH published an updated *Strategic Plan for NIH Obesity Research*. The new *Strategic Plan* reflects the exciting scientific advances and opportunities that have emerged in the years since the NIH published its first strategic plan for research on this major public health challenge. Obesity is a major contributor to type 2 diabetes, cardiovascular disease, many forms of cancer, and numerous other diseases and conditions in adults and children. Because obesity is a multi-faceted problem, the new *Strategic Plan* outlines a multi-faceted research agenda to illuminate the causes and consequences of obesity, develop and evaluate diverse prevention and treatment strategies, explore ways to implement and scale up promising approaches, and provide an evidence base to inform policy-making. Integral to all areas of the *Strategic Plan* are studies to identify and reduce health disparities, including research focused on populations at disproportionate risk for obesity and its serious health consequences. Also emphasized is translational research—bridging scientific discovery to improvements in public health.

The *Strategic Plan* is framed around the following overarching themes:

- Discover fundamental biologic processes that regulate body weight and influence behavior
- Understand the factors that contribute to obesity and its consequences
- Design and test new interventions for achieving and maintaining a healthy weight
- Evaluate promising strategies for obesity prevention and treatment in real-world settings and diverse populations
- Harness technology and tools to advance obesity research and improve health care delivery
- Facilitate integration of research results into community programs and medical practice

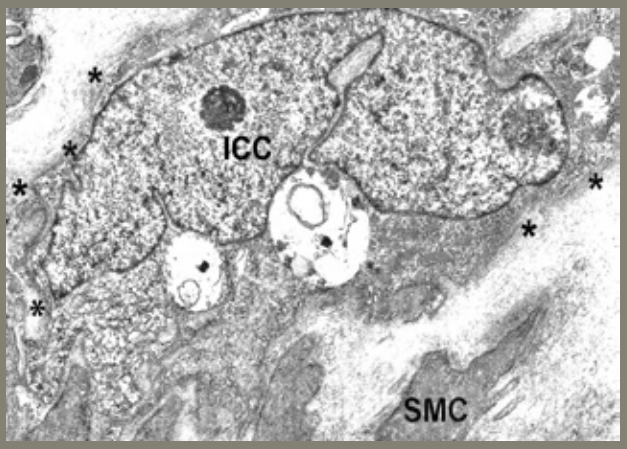
The trans-NIH Obesity Research Task Force developed the *Strategic Plan* with crucial input from researchers across the nation, professional and other health-focused

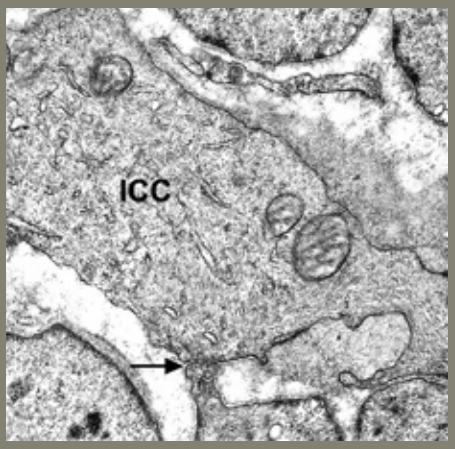


organizations, health care providers, and the public. As one of the lead Institutes on the Task Force, the NIDDK had a major role in the development of the *Strategic Plan*. Co-leading the Plan's development with the NIDDK were the National Heart, Lung, and Blood Institute, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, and the National Cancer Institute; and many other NIH components participated.

Importantly, the *Strategic Plan* is intended to be dynamic. NIH research planning will continue to build on emerging discoveries and knowledge, catalyze the development of new and more effective obesity prevention and treatment approaches and expand those that work, so that people can look forward to healthier lives.

Two versions of the *Strategic Plan for NIH Obesity Research* are available: a full version targeted to the scientific community, and a non-technical summary. Both can be accessed on the NIH web-site at *http://obesityresearch.nih.gov/About/strategic-plan.aspx*





Researchers have found that cellular changes take place in the stomachs of patients with gastroparesis—a gastrointestinal disorder in which food is delayed in leaving the stomach. This digestive disorder is thought to result from damage to nerves that control the muscular contractions needed for moving food through the stomach. Gastroparesis can be linked to diabetes or be of undetermined cause. These images, taken as part of a study described in this chapter, use transmission electron microscopy to visualize changes that occur in some of the cell types found in the stomach, such as interstitial cells of Cajal (ICCs), which act as "pacemakers" controlling smooth muscle cell contraction in the stomach. In a sample of cells from a person with diabetic gastroparesis, the ICC has some unusual features (left image), including large empty holes or "vacuoles" inside, as well as a thickened outer membrane with some gaps (asterisks). Also, the ICC from the person with gastroparesis does not make contact with the neighboring smooth muscle cell (SMC; left), as it does in a tissue sample from an individual without this disorder (right, see arrow). Identifying these cellular changes helps scientists to better understand how diabetic and other forms of gastroparesis develop and can lead to new therapeutic targets.

Images provided by Dr. Pankaj J. Pasricha, Dr. Gianrico Farrugia, and Mr. Peter R. Strege, and reprinted from Gastroenterology, 140, Grover M, Farrugia G, Lurken MS, Bernard CE, Faussone-Pellegrini MS, Smyrk TC, Parkman HP, Abell TL, Snape WJ, Hasler WL, Ünalp-Arida A, Nguyen L, Koch KL, Calles J, Lee L, Tonascia J, Hamilton FA, Pasricha PJ, Cellular changes in diabetic and idiopathic gastroparesis, 1575-1585, copyright 2011, with permission from Elsevier.

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. In 2004, more than 35 percent of all emergency and outpatient hospital visits—some 100 million—were associated with a diagnosis of a digestive disease.[1] While some digestive diseases are common and others quite rare, collectively, they exact 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. To reduce the public health burden associated with digestive diseases, NIDDK-supported scientists are vigorously pursuing research to better understand how widespread these diseases are across the United States and in specific population groups, to identify the causes of these diseases and how they progress, and to test new interventions for prevention and treatment of these costly diseases, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by destructive 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 may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to grow intestinal tissue in cell culture will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

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

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[1] *Everhart JE, editor. The burden of digestive diseases in the United States. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: U.S. Government Printing Office, 2008; NIH Publication No. 09-6443.*

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 results in damage to 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. The greater challenge now facing patients and their health care providers is to improve methods capable of diagnosing celiac disease early, before damage occurs or other conditions develop. 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 microorganisms that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These microbes can affect intestinal health 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 microorganisms influence the development and function of the digestive tract, as well as other systems throughout the body, such as those with immune and metabolic functions.

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 may include gallstones, heavy alcohol use, and inherited genetic factors. In both forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation and severe pain. Research has elucidated genetic 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, as well as distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse effects on health and can sometimes lead 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) known as nonalcoholic steatohepatitis. In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. While some forms of liver disease are caused by viral infection such as hepatitis B and C, 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 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 of critical importance to identify liver disease early, preserve liver function in people with liver disease, and develop new treatment options, including transplants performed with liver tissue from living donors.

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, 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, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss 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.

NEW DISCOVERIES ABOUT BACTERIA IN THE GUT

"Microbial Signatures" Identified for Irritable Bowel Syndrome in Children: Researchers have found that certain mixes of intestinal bacteria are associated with pediatric irritable bowel syndrome (IBS). IBS symptoms, which include abdominal pain, constipation, gas, and bloating, can make this a difficult and debilitating syndrome for children. At this time, the cause of IBS is unknown, IBS is less defined in children than adults, and there are no satisfactory treatments for either adults or children. Researchers have now looked to children's intestinal bacteria for clues to the cause and possible cure of this syndrome.

For this study, the researchers compared the intestinal bacteria populations of 22 children with IBS to those of 22 healthy children. All of the children were between the ages of 7 and 12 years. Over a 2-week period, the children collected stool samples for the study and entered a description of each stool and any pain associated with the stool in diaries. From the stool samples, the researchers isolated and sequenced the DNA of all the bacteria present, which is known as the "microbiome." Analyses of the bacterial DNA sequences in each sample provided the researchers with information on the number of total bacteria, identified the bacterial species that were represented, and determined the relative size of each type of bacterial population. Comparisons of the microbiomes showed that the children with IBS and the healthy children had similar total numbers of intestinal bacteria; however, the relative abundance of bacterial types differed. For example, the microbiomes of children with IBS were characterized by significantly greater percentages of the class gamma-proteobacteria and much smaller percentages of several *Bacteroides* species than the microbiomes of healthy children. In addition, the researchers were able to distinguish between two pediatric IBS subtypes—IBS with constipation and IBS unsubtyped—by analyzing the composition of the bacteria in samples from children with IBS and the pain that they described in their diaries. Based on differences in microbial composition and recurrent abdominal pain associated with pediatric IBS, the researchers developed "microbial signatures" associated with IBS in children and its unique subtypes. This pioneering study presents important insights into the relationship of pediatric IBS, a painful condition with no known cause, and intestinal bacteria. The scientific community can now use these microbial signatures of pediatric IBS as clues to uncovering the mysteries of this syndrome.

Saulnier DM, Riehle K, Mistretta T-A, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. Gastroenterology 141: 1782-1791, 2011.

Gut Immune Cells Need a Little Help from Their Bacterial "Friends": Scientists have found that a "friendly" bacterium, one of the many that lives in the human intestine, actively engages with gut immune cells through molecular communication processes to maintain the "tolerance" response required for a symbiotic (mutually beneficial) relationship. The immune system senses both harmful and friendly bacteria in the gut and normally mounts an immune reaction only to harmful bacteria, while remaining tolerant of friendly bacteria that perform beneficial functions for their human host. One of the main mechanisms the immune system uses to accomplish this task is mediated through Toll-like receptors (TLRs) on host immune cells that recognize patterns of molecules on the surface of both harmful and beneficial bacteria. However, if the TLRs recognize both "good" and "bad" bacteria, how do immune cells distinguish the "good" ones and react appropriately by peacefully co-existing with them?

Scientists aimed to solve this mystery by studying how the immune system interacts with a prominent member of the community of helpful gut bacteria called *Bacteroides fragilis*. *B. fragilis* is known to use a molecule on its surface called polysaccharide A (PSA) to shape the host immune response. Using a number of animal and *in vitro* models, the researchers selectively manipulated elements of the host immune cells and the gut bacterial community, including *B. fragilis* and its production of PSA, to reveal how *B. fragilis* bacteria let intestinal cells know that they are helpful, not harmful. They found that some *B. fragilis* live very close to cells lining the colon. There, in close proximity to the cells of the intestinal immune system, the bacteria communicate through PSA binding directly to a TLR on the surface of a host immune cell, causing molecular signals to be transmitted within and between immune

cells that ultimately suppress an inflammatory immune response. PSA is unique in its ability to activate the TLR in such a way that an immune response is suppressed; by contrast, molecules on harmful bacteria have been shown to activate immune responses.

These findings provide fresh insights into how the intestinal immune system has apparently co-evolved with gut bacteria to exchange molecular signals that distinguish helpful from harmful microbes. The results of this study show that friendly microbes contribute directly to establishing immune tolerance in the host gut cells, which is needed for a symbiotic relationship. This research suggests that in humans, intestinal immune cells may need help from the bacteria themselves in reacting appropriately to these microbes as "friend" rather than "foe," a finding with important implications for diseases in which these interactions are abnormal, such as inflammatory bowel diseases.

Round JL, Lee SM, Li J, et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science 332: 974-977, 2011.

Communication and Protein "Fences" Make Good Neighbors of Gut and Bacteria: Scientists have shown how a microbe-fighting protein helps create a protective buffer zone between the inner walls of the small intestine and the bacteria contained within. Scientists have also found that communication between cells lining the intestinal wall (epithelial cells) and immune cells is necessary to sense potentially harmful bacteria nearby and release this antimicrobial protein. These findings may explain how, in a healthy state, helpful bacteria can survive in the digestive tract without triggering an immune attack, while harmful bacteria are appropriately targeted and neutralized.

Researchers have long known that about 100 trillion bacteria reside in the intestines of the human body. Many of these microbes help us digest food and absorb important nutrients, while others can have ill effects. In the colon, a dense layer of mucus creates a physical barrier between the gut's walls and microbes. The mucus helps to prevent bacterial infection and immune attack. However, in the small intestine, the mucus layer is thin and permeable to allow absorption of nutrients. Scientists have puzzled over how the mutually beneficial relationship between bacteria and host is maintained.

This research group suspected that certain immune mechanisms might work in partnership with the mucus layer in the small intestine to keep bacteria at bay. They first assessed in mice how microbes are naturally positioned relative to the intestinal lining. Using a technique that makes bacteria glow green and intestinal walls blue, the scientists found a zone of separation between the bacteria and the lining. The researchers then studied mice lacking the protein MyD88, which activates a branch of the immune system that recognizes certain molecular patterns in disease-causing microbes. With this portion of the immune system impaired, bacteria invaded the protective zone and came into direct contact with the intestinal lining. MyD88 also controls the production of several antimicrobial proteins in specialized cells in the intestine's lining. Following this clue, the researchers identified a protein called RegIIIγ as responsible for the bacteria-free zone. Mice lacking RegIIIγ lacked the buffer zone between bacteria and the intestinal wall. The intestinal lining had increased numbers of bacteria adhering to it—a feature sometimes seen with digestive disorders such as inflammatory bowel diseases. These findings highlight the protective zone between the intestine and bacteria and its importance for maintaining intestinal health, with the antimicrobial protein RegIIIγ playing a critical role in maintaining this zone.

In a related study, the research group focused on the role of intestinal immune cells called γδ intraepithelial lymphocytes (IELs) in selectively defending the intestine against potentially harmful bacteria. IELs are found in large numbers between epithelial cells in the intestinal mucosa and are an important part of the mucosal immune response to intestinal bacteria. Researchers purified γδ IELs from the small intestines of mice lacking intestinal microbes (raised under sterile conditions) or mice with intestinal microbes (raised under standard conditions), and compared the genes turned on in these cells using microarray technology. They found that the IELs turned on antimicrobial proteins such as RegIIIγ, but only when intestinal microbes were present, specifically harmful bacteria that invade epithelial cells such as *Escherichia coli* and *Salmonella typhimurium*. To investigate how the IELs might receive the signal to defend against these harmful bacteria, the scientists next investigated communications with neighboring epithelial cells equipped with bacteria-sensing receptors. Using mice that had been genetically altered so that some of their epithelial

cells lacked the protein MyD88, which helps the cells sense bacteria, the researchers showed that this protein is needed for the epithelial cells to alert neighboring IELs to the presence of harmful bacteria, prompting their antimicrobial protein production. They also showed that these IELs act early in the course of bacterial exposure to limit epithelial invasion of harmful bacteria, using mice genetically altered to lack γδ IELs compared to wild-type mice. This research has shown how the immune cell γδ IEL plays an essential role, in collaboration with nearby epithelial cells, in maintaining "homeostasis," or balance between the intestinal immune system and the intestinal bacterial community, by acting quickly and discriminately to limit invasion by harmful bacteria.

These findings offer insights into unique aspects of the intestine's built-in defense system against pathogens, which is set up to "screen" microbes and react to harmful bacteria, but peacefully co-exist with beneficial species. This system involves good communication among intestinal cell types to coordinate responses and also a physical barrier or "demilitarized zone" between the intestine and bacteria. The key elements of this system identified in these studies, including antimicrobial proteins such as RegIIIγ and the bacteria-sensing protein MyD88, as well as IEL immune cells, offer clues to how some "system failures" may come about in certain digestive disorders and represent potential new targets for treating or preventing these disorders.

Vaishnava S, Yamamoto M, Severson KM, et al. The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. Science 334: 255-258, 2011. Information on this reference adapted from NIH Research Matters; original article by Ms. Vicki Contie, published on October 24, 2011.

Ismail AS, Severson KM, Vaishnava S, et al. γδ intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. Proc Natl Acad Sci USA 108: 8743-8748, 2011.

GENETICS OF INFLAMMATORY BOWEL DISEASES

Study Doubles Number of Genetic Variants Associated with Crohn's Disease: An international group of researchers, including those from the NIDDK's IBD Genetics Consortium, have combined data from six genome-wide association studies to uncover additional genetic variants associated with Crohn's disease, effectively doubling the number of known genetic associations in this disease. Crohn's disease is a form of inflammatory bowel disease that can occur in the small intestine and colon. It is thought to result from a complex interplay between genetic factors, which set up the host immune system to respond inappropriately, and the environmental factors they sometimes respond to, such as benign microbes living in the intestine. Research groups from around the world, including the NIDDK IBD Genetics Consortium, have been extremely successful in identifying 32 genetic regions associated with Crohn's disease that have led to new insights into disease processes. Still, they estimated that these factors accounted for only about 20 percent of the genetic contribution to this disease.

To improve their chances of finding the additional genetic variants that play a role in the development of Crohn's disease, a research team called the International IBD Genetics Consortium, which includes the NIDDK IBD Genetics Consortium, utilized data from six genome-wide association studies of samples from patients with Crohn's disease compared to people without this disease. They conducted a "meta-analysis" of genetic data from all of these studies, combining their findings to analyze a larger number of people together, which could enable them to bring to light the effects of additional genetic variants. With this approach, the team identified 30 new genetic regions associated with Crohn's disease and replicated results with additional genetic variants that had been identified previously, bringing the total up to 71. Some of the genes located in these genetic regions are associated with other types of chronic inflammatory disorders and perform functions that would explain some of the underlying disease mechanisms. However, identifying the specific roles of these genes in Crohn's disease, their therapeutic potential, and the vast remainder of genetic factors contributing to the disease will require further research.

Franke A, McGovern DPB, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 42: 1118-1125, 2010.

Study Greatly Increases Number of Genetic Variants Associated with Ulcerative Colitis: Research scientists have taken a significant step toward understanding the genetic basis of ulcerative colitis (UC) with the identification of 29 new genetic variants that increase the risk for UC, more than doubling the number of previously known variants. UC and Crohn's disease are related complex genetic diseases that are both major forms of IBD. Risk for these diseases is caused by many genetic variants with contributions ranging from strong to subtle impacts. However, genetic risk factors identified in past studies explain only 11 percent of the inherited component of UC. Genetic studies have shown that risk variants for IBD can be common to both UC and Crohn's disease or unique to either disease. The current study identifies additional variants that are unique to UC and some that are shared by both diseases, pointing the way to understanding biological processes underlying IBD.

The first phase of this study began with an analysis of data from six genome-wide association studies, conducted by the NIDDK IBD Genetics Consortium and five other international research groups, which had previously identified a combined total of 18 genetic variants associated with UC. This "meta-analysis" of data collected from multiple studies had the statistical advantage of a much larger number of study participants, enabling the scientists to identify an additional 29 variants, bringing the total number of genetic risk factors for UC to 47. Next, the researchers turned their attention from the identification of these new risk associations in unique chromosomal locations, or loci, to identification of genes within the loci that confer disease risk. Most of the loci identified in the meta-analysis contain multiple genes, and the scientists used various analytical tools to determine which of the genes were likely to confer disease risk and to identify key biological pathways involving these genes. One new analytical tool that was employed is a statistical method known as "GRAIL" (Gene Relationships Among Implicated Loci), which mines the scientific literature to assess genes from various loci for whether they have related biological functions and may thus be involved as a group in a disease pathway. Another of the research methods identified risk variants that changed the structure of the protein coded for by the candidate gene. With these analyses, the scientists identified several candidate genes with roles in intestinal barrier integrity, immune response, autophagy (a cellular mechanism causing cell death, previously implicated in Crohn's disease), and other biological pathways.

The researchers compared the UC risk loci from this study to a set of Crohn's disease risk loci that were identified in a previous study (also described in this chapter). Several loci were found that were common to both UC and Crohn's disease. Based on this analysis, the total number of identified IBD risk loci has increased to 99, with 28 genetic variants in common between UC and Crohn's disease. These studies identified biological mechanisms and pathways that may be associated with UC. However, further research is required to confirm which of the genes within these loci are causing the effects. Although these results represent only a small percentage of predicted UC genes and associated pathways, they are part of a critical foundation on which important advances in UC research and treatments can be built.

Anderson CA, Boucher G, Lees CW, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 43: 246-252, 2011.

INSIGHT INTO CELIAC DISEASE DEVELOPMENT

A Surprising Role for Vitamin A in Model of Celiac Disease Development: In research on the mechanisms underlying the development of celiac disease, scientists have uncovered an unexpected role for a form of vitamin A called retinoic acid, while simultaneously filling in details of the larger immunological and cellular machinery at work and creating a new animal model of the disease. The human intestine contains a resident immune system tasked with distinguishing between helpful substances, such as nutrients and health-promoting microbes, and harmful ones, such as unhealthy bacteria. In celiac disease, this system goes awry, with immune cells inappropriately reacting to the dietary protein gluten, which is found in grains such as wheat. The precise mechanisms by which this loss of immune "tolerance" of a dietary antigen occurs have remained mysterious, beyond the knowledge that some individuals have a genetic susceptibility to celiac disease.

Researchers set out to fill in some of the details behind celiac disease development by identifying some of the key molecules and cells involved in this process. They focused their attention on intestinal immune cells and the molecules they produce, called cytokines, as well as a form of vitamin A called retinoic acid. Retinoic acid plays a role in the development (differentiation) of a type of immune cell called the regulatory T cell, which typically suppresses the development of inflammation-promoting immune cells and helps to maintain tolerance of ingested nutrients. The scientists created mice with a genetic mutation boosting levels in their intestinal immune tissues of the cytokine IL-15, which is known to be elevated in the intestines of patients with celiac disease. In these mice and in cultures of cells taken from them, high IL-15 levels suppressed differentiation of the regulatory T cells—the first clue as to how this cytokine takes the brakes off of the intestine's natural tolerance of dietary substances. When they added retinoic acid to the mix in mice with high intestinal IL-15, they found a surprising result—rather than countering IL-15's effects on suppressing regulatory T cell differentiation, retinoic acid enhanced it. These synergistic effects of IL-15 and retinoic acid led to a cascade of events associated with an inappropriate immune response, including turning on signaling pathways, increasing release of other cytokines, and activating inflammation-promoting immune cells. Next, the researchers created a new animal model of early-stage celiac disease. They engineered mice to have high intestinal IL-15 levels and also to have on their cells certain proteins that are associated with human celiac disease, called HLA-DQ8, which interact particularly well with gluten. They then fed the mice gliadin, a component of gluten. In these animals, an inappropriate intestinal immune reaction was mounted towards the ingested gliadin, which was further promoted by also feeding them retinoic acid.

This study has improved understanding of how celiac disease develops, including the immune mediators involved, and also yielded a new animal model of early-stage celiac disease to enable future research. It also highlights a surprising role for retinoic acid, which, in the context of underlying inflammation and elevated IL-15, further enhances inappropriate immune responses, rather than protecting against them as it does in other immunological settings. These findings warn against using vitamin A or retinoic acid as a treatment for patients with celiac disease, and potentially other diseases with inappropriate immune responses marked by high IL-15 levels. Lastly, this research identifies molecules such as IL-15 as potential targets for new drug development to treat those with celiac disease who are not completely responsive to a gluten-free diet.

DePaolo RW, Abadie V, Tang F, et al. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. Nature 471: 220-224, 2011.

VISUALIZING THE UNDERLYING DISEASE PROCESS OF GASTROPARESIS

Changes in Stomach Cells Associated with Two Forms of Gastroparesis: Researchers have found evidence of changes at the cellular level in the stomachs of individuals with gastroparesis, yielding new insights into this digestive disorder. Gastroparesis is a chronic condition characterized by impaired "motility"—the muscular contractions that move food along the gastrointestinal tract. This limited motility results in delayed food emptying from the stomach into the intestines, as well as many symptoms that compromise quality of life, including nausea, vomiting, bloating, weight loss, and abdominal pain. Gastroparesis is most commonly associated with diabetes, which is thought to damage nerves connecting to the stomach that control muscular contractions. However, often the cause of the disorder is unknown or "idiopathic." Clinical studies on this disorder have been limited.

Scientists in the NIDDK's Gastroparesis Clinical Research Consortium are now conducting research at sites across the nation to improve understanding of disease processes and develop effective treatments for this disorder. For this study, they collected stomach tissue samples from individuals with diabetic and idiopathic forms of gastroparesis, as well as control samples from patients undergoing gastric bypass surgery who did not have gastroparesis, in order to compare their cellular structures. To identify the cells under a microscope, including cells of the nervous system, interstitial cells of Cajal (ICCs), smooth muscle cells, and immune cells, the scientists used visible molecular tags in combination with antibodies that react to unique proteins on the surface of these different cell types. A stain was also used to estimate the amount

of scarring in the tissue—a sign of cellular damage. Researchers noticed cellular abnormalities in the majority of samples from patients with either diabetic or idiopathic gastroparesis. The most frequent type of abnormality seen in stomach tissue from gastroparesis patients was a reduction in the number of ICCs, which play an important role as "pacemakers," controlling muscular contractions in the stomach. Another common alteration was seen in the shape and increased number of immune cells present in the muscle layer. Some alterations in the gut nervous system were also observed.

Using transmission electron microscopy, the researchers were able to peer further inside these cell layers. They again saw reduced ICCs and some altered nerve structures, as well as an abnormal connective tissue layer with some scarring. Many of the ICCs present in both forms of gastroparesis looked unusual, with large holes called vacuoles, swollen mitochondria (energy-generating structures within the cell), and no visible contact with neighboring nerves, muscle cells, or other ICCs. Some unique cellular features were also noted between diabetic and idiopathic gastroparesis—in diabetic gastroparesis, ICCs also featured a thickened but broken outer membrane, and some scarring was noted around muscle cells, while idiopathic gastroparesis showed scarring in connective tissue around nerve structures.

This Consortium study represents the most comprehensive, clinical study of diabetic and idiopathic gastroparesis to date. The finding of cellular abnormalities in the stomachs of most individuals with this disorder—including changes in the structure and number of ICCs, nerve cells, and immune cells—sheds light on the underlying disease processes at work in both forms of gastroparesis, and paves the way for future therapeutic development. Future research by the Consortium members will continue to explore these abnormalities, such as how loss of contact amongst these cells might translate into the limited gastrointestinal motility seen in patients with gastroparesis.

Grover M, Farrugia G, Lurken MS, et al. Cellular changes in diabetic and idiopathic gastroparesis. Gastroenterology 140: 1575-1585, 2011.

BIOENGINEERED APPROACHES TO TREATING FECAL INCONTINENCE

Functioning Bioengineered Anal Sphincter Implants in Mice: Building on research that may have implications for future treatment for fecal incontinence, scientists have successfully implanted a physiologically functional bioengineered internal anal sphincter (IAS) in mice. The IAS is a ring-like muscle located just inside the rectum; along with the external anal sphincter, these two muscles keep the anus closed and maintain fecal continence. Loss of IAS muscle tone is a primary cause for the uncontrolled release of stool that occurs in people with fecal incontinence, a condition that places devastating emotional, social, physical, and economic burdens on people who are affected by it.

Scientists used smooth muscle cells obtained from human IAS to bioengineer three-dimensional IAS rings. The researchers grew the cells using special plates that contained a mold around which the cells could create the appropriate three-dimensional ring structure. The plates were first coated with mouse nerve cells that were then overlaid with the human IAS cells. Once the IAS ring had formed, it was surgically implanted into a small pocket under the skin of a mouse. After allowing the bioengineered sphincters to develop for nearly 1 month, the researchers removed and examined them. They found that the IAS rings had developed an ample blood supply and nerve connections. They exhibited appropriate muscle tone, and relaxed and contracted in response to various chemical stimuli. All of these observations suggest that the bioengineered IAS is physiologically functional.

Previous studies demonstrated that it was possible to grow bioengineered IAS from isolated human IAS circular smooth muscle and to successfully implant physiologically functional bioengineered mouse IAS constructs in mice. The current study is the first to demonstrate implantation of a bioengineered human IAS in a mouse where both the muscular and nerve components are viable and responsive to stimuli. This study may be translated into bioengineered IAS for people suffering from fecal incontinence. This would be of enormous benefit, greatly improving the daily lives of these individuals and alleviating the social and financial burdens associated with this disorder.

Raghavan S, Gilmont RR, Miyasaka EA, et al. Successful implantation of bioengineered, intrinsically innervated, human internal anal sphincter. Gastroenterology 141: 310-319, 2011.

RESEARCH ON NONALCOHOLIC FATTY LIVER DISEASE

Vitamin E Helps Diminish a Type of Fatty Liver Disease in Children: In a recent clinical trial, the Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC), a form of vitamin E improved the most severe type of fatty liver disease in some children. Nonalcoholic fatty liver disease is the most common chronic liver disease among U.S. children. It ranges in severity from steatosis, defined as fat in the liver without injury, to nonalcoholic steatohepatitis or NASH, which is characterized by fat, inflammation, and liver damage. Fatty liver increases a child's risk of developing heart disease and liver cirrhosis. Most children with fatty liver disease are overweight and have insulin resistance, a hallmark of both prediabetes and type 2 diabetes. Boys are more likely affected by fatty liver disease than girls, as are Hispanic children compared to African Americans and whites. Weight loss may reverse the disease in some children, but other than advice about diet and exercise, there are no specific treatments. Excess fat in the liver is believed to cause injury by increasing levels of oxidants, compounds that can damage cells.

In the clinical trial, conducted by the NIDDK-supported NASH Clinical Research Network, researchers studied whether fatty liver disease in children could be improved by either vitamin E, which is an antioxidant, or the diabetes drug metformin. A total of 173 children ages 8 to 17 participated in the trial. The majority of participants were white and Hispanic boys. The children were divided into three groups and given vitamin E, metformin, or placebo daily. The researchers then analyzed blood samples from the participants to determine whether there was a sustained reduction in levels of the liver enzyme alanine aminotransferase (ALT) to be closer to normal. They also looked for improvements in the liver as shown by biopsy, a more rigorous test. Although a sustained reduction in ALT was not achieved, after treatment for almost 2 years, researchers found that 58 percent of the children on vitamin E no longer had NASH, compared to 41 percent of the children on metformin, and 28 percent on placebo, as assessed with liver biopsies. Vitamin E was better than placebo because it significantly reduced enlargement and death of liver cells. The results of this study in children are similar to a previous study, also conducted by the NASH Clinical Research Network, that had shown vitamin E to be effective in some adults with the disease. However in the TONIC trial, neither vitamin E nor metformin was significantly better than placebo in improving the children's ALT levels. From other observations, the researchers concluded that the children benefited from the frequent diet and exercise advice provided throughout the study. While the vitamin E results based on liver biopsy are encouraging, patients should be under a doctor's care if vitamin E is used. Researchers now hope to build on these results by looking for other therapies for fatty liver disease and reliable, non-invasive ways to monitor the disease and response to therapy.

Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA 305: 1659-1668, 2011.

Possible New Therapeutic Target for Treating Fatty Liver Disease Associated with Diabetes: Researchers have discovered that a protein thought to be involved in protecting liver cells from damage may actually be involved in regulating glucose and lipid (fat) metabolism in the liver. Scientists were looking for genes in mice that are turned on by a protein called FXR, which is one of a group of proteins called nuclear receptors that are involved in varied aspects of metabolism. They focused on one gene identified as being turned on by FXR in mice, called *AKR1B7*. Previous research suggested that the AKR1B7 protein, an enzyme capable of catalyzing biochemical reactions in cells, may protect against the by-products of a process called lipid peroxidation, which can result in cellular damage. To investigate this possibility, the researchers experimentally introduced high levels of AKR1B7 protein into the livers of mice. Surprisingly, the protein did not have an effect on lipid peroxidation in the liver. Rather, it caused the animals' blood glucose levels to drop, in association with reduced glucose production by the liver. The scientists did the

same experiment in a diabetic mouse model and again observed a decrease in the animals' blood glucose levels; the treated diabetic mice also had lower levels of fat in their livers. These findings identify a novel role for AKR1B7 in regulating liver and glucose metabolism. The scientists note that a human protein (AKR1B10) may be structurally similar to mouse AKR1B7. If future research shows that the human protein has similar functions, it may be a potential therapeutic target for treating fatty liver disease associated with diabetes.

Ge X, Yin L, Ma H, Li T, Chiang JYL, and Zhang Y. Aldo-keto reductase 1B7 is a target gene of FXR and regulates lipid and glucose homeostasis. J Lipid Res 52: 1561-1568, 2011.

TREATING CHRONIC HEPATITIS C

Combination Antiviral Therapy More Effective for Pediatric Chronic Hepatitis C: A clinical trial conducted at 11 sites throughout the United States has shown that combination therapy with peginterferon and ribavirin is more effective than therapy with peginterferon and placebo in treating chronic hepatitis C in children. Children can contract the hepatitis C virus at birth from mothers who are infected or through transfusion with infected blood, though the U.S. blood supply is screened to prevent this. Although chronic hepatitis C in children is less common and generally less damaging than in adults, it can lead to cirrhosis requiring liver transplantation, or liver cancer, later in life. Treatment of this disease in children with a combination therapy using the antiviral drugs peginterferon and ribavirin has been based on extensive adult studies, but only a single, uncontrolled clinical trial in children, who may respond differently to the drugs. For example, children show a higher response to peginterferon than adults, and ribavirin has been shown to be potentially harmful to young animals. Therefore, a well-controlled clinical trial was needed to test the true safety and efficacy of combination antiviral therapy compared to peginterferon treatment alone for maximizing care of children with chronic hepatitis C.

Researchers in the Peds-C Clinical Research Network conducted a prospective, randomized controlled clinical trial at 11 U.S. medical centers in which they treated people ages 5 to 18 years who have chronic hepatitis C. The Network was funded by the NIDDK with other Federal and non-Federal support. The children and adolescents in this trial were given either a combination of peginterferon and ribavirin, the standard combination therapy used in adults, or a combination of peginterferon and placebo for 1 year, and then followed for 1 to 2 years after going off the treatment. During this time, they were monitored for whether they had hepatitis C virus in their blood (as measured by the viral genetic material, or RNA). Sustained response to treatment was defined as undetectable hepatitis C viral RNA in the blood 6 months after treatment. The group receiving the combination therapy with both antiviral drugs had a 53 percent sustained response rate compared to 21 percent in those receiving only the antiviral peginterferon and placebo. Notably, some participants who did not respond early to the treatment nevertheless went on to have a sustained response. Additionally, those who responded to treatment in either group still had undetectable viral levels 2 years after stopping treatment. Some adverse effects were observed in both treatment groups, including flu-like symptoms, headache, GI symptoms, and low levels of certain immune system cells (neutrophils) in the blood, requiring a reduction in the treatment dose in some cases.

The addition of ribavirin to peginterferon as part of combination therapy greatly increased response rate in children and adolescents with chronic hepatitis C with minimal change in side effects, providing much-needed evidence for designing optimal therapy for this group. This study also shows the benefits of giving the full course of combination therapy to young people who do not show early signs of responding, as well as the importance of monitoring for common side effects such as low neutrophils.

Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. Gastroenterology 140: 450-458, 2011.

Dietary Supplement Improves Response to Antiviral Therapy for Hepatitis C: Scientists have shown that a dietary supplement, S-adenosyl methionine (SAMe), safely and effectively boosts response to standard antiviral therapy in people infected with a type of hepatitis C virus that typically does not respond well to such therapy. The current standard of therapy

for hepatitis C—a combination of the antiviral drugs pegylated interferon and ribavirin—is effective in less than half of those with this disease. Interferon is also produced naturally by the body in the early phase of viral infection to mobilize the immune response and clear the virus. A type of hepatitis C virus called genotype 1, which is the most prevalent type of virus infecting people in the United States, is particularly unresponsive to antiviral treatment. Therefore, scientists have been seeking ways to improve response to therapy.

In this study, the research team tested SAMe, which is a naturally occurring compound found in living cells. The clinical portion of the research included people with chronic hepatitis C who were infected with the genotype 1 virus and, thus, did not respond well when treated in the past with standard antiviral therapy. In the study, they were given standard antiviral therapy for a few weeks to establish a baseline response and serve as their own controls in the trial, then taken off antiviral drugs and given SAMe for a few weeks prior to treatment with the combination of antivirals and SAMe for 1 year. After each course of antiviral therapy, with or without SAMe, the researchers measured viral response (the amount of virus still detectable in the blood) and also the genes activated in response to interferon in the patients' blood cells that help the body fight off viral infection. Both viral response and molecular indicators of response to interferon improved when the antiviral therapy was given together with SAMe, compared to when the same patients were previously given antiviral drugs alone. In cultured liver cells infected with hepatitis C virus, a similar boost in response to interferon treatment was observed when the cells were pretreated with SAMe. Additional cell culture studies showed that SAMe likely works through countering viral inhibition of a molecule that transmits the response to interferon.

The dietary supplement SAMe improved response to antiviral therapy in people with hepatitis C who had not responded to therapy in the past, both in terms of viral response and host immune response. Thus, SAMe could be a helpful addition to antiviral therapy in such people with hepatitis C, particularly those infected with the genotype 1 virus. Future research will also determine whether people with hepatitis C who have never received antiviral treatment can benefit from SAMe taken during therapy.

Feld JJ, Modi AA, El-Diwany R, et al. S-adenosyl methionine improves early viral responses and interferon-stimulated gene induction in hepatitis C nonresponders. Gastroenterology 140: 830-839, 2011.

LIVER TRANSPLANTATION RESEARCH

Living Donor Liver Transplantation Improves Survival Compared to Other Options:

Adult-to-adult living donor liver transplantation—removal of part of a living adult's healthy liver for transplantation into another adult whose organ has been damaged—has been performed in the United States since the late 1990s. Recipients of living donor livers include people with end-stage liver disease due to causes such as chronic hepatitis C, or in those with liver cancer or acute liver failure. However, as with other organs, the demand for livers for transplant far exceeds the supply of available living donor or deceased donor organs. Moreover, the living donor procedure carries some risks for the donor.

The need to distribute available organs in a way that is fair and also maximizes survival benefits led to the development of a numerical scale, based on laboratory tests, that is used to determine how urgently a patient needs a liver transplant. It is called the Model for End-stage Liver Disease (MELD). Yet, even with this system in place, information is lacking to assist the decision-making process of candidates for deceased or living donor liver transplantation, their health care providers, people who might consider being living donors, and those who manage organ allocation.

The Adult-to-Adult Living Donor Liver Transplantation (A2ALL) Study, conducted from 2002 to 2009 and currently supporting ongoing ancillary studies, investigates outcomes for candidates for living donor liver transplantation, as well as donors, compared to outcomes for those who receive organs from deceased donors. As part of the A2ALL study, researchers collected data at nine liver transplant centers around the country on 4.5-year outcomes for candidates for liver transplantation who received a living donor transplant, compared to a group of those who remained on the waiting list or received a transplant from a deceased donor. By tracking mortality data on these groups, the researchers found that those who received living donor liver transplants had significantly higher survival rates

compared to the other group. Interestingly, this benefit was seen across MELD "scores" in patients with liver disease of varying severity.

Previous studies had suggested that there was little or no benefit to performing transplants in patients with lower MELD scores who were less ill. In contrast, this study demonstrates that there is a greater chance of survival for patients who receive a living donor liver transplant, although this benefit was not seen in individuals with liver cancer, likely due to the priority they are given to receive deceased donor transplants relatively quickly, so that they spend less time on the organ waitlist. Although potential risks to liver donors must continue to be weighed, living donor liver transplantation shows clear benefits for transplant recipients. This information will be useful for patients, their caregivers, and physicians who are advising transplant candidates about the benefits of living donor liver transplantation.

Berg CL, Merion RM, Shearon TH, et al. Liver transplant recipient survival benefit with living donation in the model for endstage liver disease allocation era. Hepatology 54: 1313-1321, 2011.

Dining In with a Few Trillion Fascinating Friends—How Gut Bacteria Affect Health and Disease

What we eat, or do not eat, affects our health in surprising ways—by sustaining not only us, but also the trillions of bacteria that reside in our gut. Over the past decade, a researcher supported by the NIDDK, Dr. Jeffrey Gordon has illuminated the extraordinary and diverse roles our gut microbes play in health and disease.

Dr. Gordon has described the complex interactions between us and our gut microbes as "dining in with a few trillion fascinating friends." Building on knowledge that bacteria are able to digest some dietary components that our own intestinal cells cannot, Dr. Gordon speculated that some bacteria may be better at this than others—and thus might contribute to obesity in their human or animal "host." His research team found that, in fact, the relative abundances of different types of gut bacteria differ between lean and obese mice and people, and the bacteria more prevalent in obesity are better able to extract nutrients, and associated calories, from food. These microbes also influence whether their hosts burn calories or store them as body fat. Dr. Gordon and his colleagues have discovered that these microbes play other surprising roles in the health of their hosts: they help direct normal development of blood vessels in the digestive tract, and may even modulate risk for type 1 diabetes. Additionally, humans' indigenous bacteria communicate with their surrounding host cells in ways that maintain a peaceful and productive relationship; at the same time, they fortify the intestines against unwanted invaders by inducing host cells to produce anti-bacterial proteins. Other researchers have implicated aberrant immune reactions to our resident gut microbes as contributing to inflammatory bowel diseases, and have found differences in gut bacteria associated with irritable bowel syndrome.

Dr. Gordon has suggested that a strategy for preventing or treating disease may be to alter the types of bacteria in our gut—through probiotics or other means. Such an approach would require deeper understanding of what the different types of bacteria have to offer—that is, what functions may be encoded by their collective genomes, referred to as the gut "microbiome." And, scientists would need to know how to shape these communities of microbes effectively.

Because the local cuisine may attract different types of bacteria, in the past year Dr. Gordon and his team have investigated whether the kinds of foods we eat may affect the mix of microbes in our gut. In an innovative study, published this past year, Dr. Gordon and his team explored whether very different diets are associated with distinct collections of gut bacteria.[1] With cutting-edge technology and computational methods, the scientists canvassed the gut bacterial communities from a wide range of animals, sequencing and analyzing the bacterial genomes. They found that the sets of bacterial species within each of the animals differed depending on whether the animals were meat eaters, plant eaters, or ate both forms of food. Regardless of diet, however, the microbial communities within the guts of all of the animals shared in common a core set of bacterial genes, perhaps required for living in an intestinal neighborhood. Yet, other aspects of the bacterial genomes did vary according to diet. For example, the meat-eaters' bacteria harbored more genes for breaking down proteins, while the plant-eaters' bacteria were enriched for genes involved in synthesizing the building blocks of proteins. In subsequent analyses of gut microbiomes from people who kept strict records of the types of foods they ate, the researchers found that different diets correlated with different bacterial species and different sets of bacterial genes within humans.

In another study, also published this past year, Dr. Gordon and other scientists in his laboratory tested the effects of different foods on the relative abundance of the different bacterial species with experiments in mice.[2] To focus on the types of microbes that live in our gut, the researchers first raised mice under sterile (germ-free) conditions, and then gave them intestinal bacteria taken from humans. With an initial set of microbial residents in place, the researchers then fed the mice a series of defined diets with simple ingredients: pure sugar, cornstarch, corn oil, and

casein (protein). Based on changes in the gut microbial communities, the scientists developed a mathematical model to predict how the relative abundance of different bacterial species would vary in response to different dietary components. To test their model, they served the mice meals more similar to what people eat, in the form of various combinations of pureed baby foods: apples, peaches, peas, sweet potatoes, beef, chicken, oats, and rice. Largely as predicted, they observed that the community of gut bacterial species fluctuated in response to changes in diet.

By further defining the genomes and food preferences of humans' intestinal dining companions, these new studies from Dr. Gordon's laboratory may help guide dietary, probiotic, or other strategies to modify the composition of our gut bacteria. More broadly, the NIDDK and other NIH Institutes currently support research by Dr. Gordon and a number of other investigators on the multitude of microbes that inhabit not only our gut, but also niches elsewhere in the body, through the NIH Human Microbiome Project (*https://commonfund.nih.gov/hmp/*) and other studies. With a better understanding of our bacterial partners, scientists may be able to develop novel interventions to enhance nutrition, prevent or treat disease, and improve public health.

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[1] *Muegge BD, Kuczynski J, Knights D, et al. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970-974, 2011.*

[2] *Faith JJ, McNulty NP, Rey FE, and Gordon JI. Predicting a human gut microbiota's response to diet in gnotobiotic mice. Science 333: 101-104, 2011.*

Launching the New NIDDK Bowel Control Awareness Campaign

On June 2, 2011, the NIDDK launched the Bowel Control Awareness Campaign, "Let's Talk About Bowel Control." Fecal incontinence, commonly known as bowel control problems, is the inability to hold a bowel movement until reaching a bathroom. Fecal incontinence also refers to the accidental leakage of solid or liquid stool. Many feel upset or embarrassed by incontinence, but it can be caused by various medical conditions, and treatments are available. Thus, talking about fecal incontinence with a health care provider can help patients get successful treatment.

In December 2007, the NIH held the "State-of-the-Science Conference: Prevention of Fecal and Urinary Incontinence in Adults," sponsored by the NIDDK and the Office of Medical Applications of Research, to review current knowledge and develop recommendations for addressing fecal incontinence. A Conference Statement prepared by the State-of-the-Science Panel recommended that efforts be made to raise public awareness of incontinence and the benefits of prevention and management. The new Awareness Campaign was developed in response to this recommendation.

"Our findings indicate that fecal incontinence is a significant public health burden in the United States—affecting close to 10 percent of the adult population over 40 years old. The Bowel Control Awareness Campaign's main objective is raising public awareness of fecal incontinence to aid in prevention of incontinence and to improve the lives of men and women living with the condition," said Dr. Griffin Rodgers, NIDDK Director.

Developed by the NIDDK, along with professional and patient-advocacy organizations that focus on fecal incontinence, the Bowel Control Awareness Campaign, "Let's Talk about Bowel Control," is located at *www.bowelcontrol.nih.gov* The web-site features:

- A fact sheet on fecal incontinence
- An easy-to-read booklet on bowel control
- NIH bowel control research information
- Links to professional and patient-advocacy organizations
- A link to the National Digestive Diseases Information Clearinghouse

"The lack of communication between health care professionals and patients appears to be one of the main challenges with bowel control problems. Being able to talk about the problem is the first step in both prevention and treatment," said Dr. Stephen P. James, Director of the NIDDK's Division of Digestive Diseases and Nutrition. "People experiencing bowel control problems need to know that they are not alone and that the condition can be managed. The Bowel Control Awareness Campaign will inform health care professionals and the public that bowel incontinence is a common condition and that effective treatments are available."

STORY OF DISCOVERY

Celiac Disease

Although celiac disease had been described and named by the Greek physician Aretaeus of Cappadocia in the Second Century A.D., nearly 2 millennia passed before the cause of this disease was identified and a reliable treatment was discovered. Celiac disease is now known to be driven by an aberrant inflammatory immune response to foods that contain gluten, a component of wheat, rye, and barley. People with this disease may have symptoms such as vomiting, diarrhea, weight loss, and, for children, failure to grow and thrive. However, great individual variation in the intensity and spectrum of symptoms makes celiac disease difficult to diagnose. The critical identification of gluten as the trigger of celiac disease took place in Europe in the mid-20th century. Since then, the NIDDK has also been a major supporter of scientific advances that are now revealing the mysteries of this complex disease.

During what was known in the Netherlands as the "Winter of Starvation" during World War II, the Dutch pediatrician, Willem-Karel Dicke, substantiated his belief that cereals were responsible for the symptoms of celiac disease. Although the pressures of the war had brought unprecedented food shortages, Dr. Dicke, Medical Director of the Juliana Children's Hospital in The Hague, witnessed improvement in his young celiac patients who had been suffering from abdominal pain and diarrhea before basic foods such as cereal became scarce, only to relapse when bread was dropped into the Netherlands by Allied planes. Watching this scenario unfold convinced Dr. Dicke that grain was the cause of his young patients' illnesses and he began a series of experiments that would validate his theory. Dr. Dicke published two articles in 1953, the first showing that wheat flour (not wheat starch) caused celiac disease, and the second demonstrating that the gluten component of wheat flour was the primary cause of the disease. Within 4 years, two additional medical breakthroughs significantly changed the methods for diagnosis of celiac disease. One was the description of a deterioration of the intestine's fingerlike projections, called "villi," that resulted in a characteristic flattening of the surface of the intestinal lining in celiac patients. The other was the development of a procedure to perform biopsies of the small intestine to detect and monitor this villi atrophy. The biopsy was soon improved with the introduction of a more flexible device called the "Crosby Capsule." In 1969, the "Interlaken criteria" was developed as the gold standard for diagnosing celiac disease. In 1990, the Interlaken criteria was modified to take advantage of a research study published the previous year that determined accurate diagnosis could be accomplished by screening with celiac-specific antibodies that had been identified, followed by a single biopsy.

Celiac disease at one time was thought to be primarily a European disease. However, a study in 2003 showed the prevalence of celiac disease in the United States was approximately 1 percent of the population—100 times greater than previously thought. In another NIDDK-supported study, researchers sought to determine the incidence of celiac disease and how it may be affected by genetic variation within *HLA* genes, a set of immune system genes. They screened over 22,000 newborns for a particular *HLA* gene known to be associated with celiac disease and followed a subset of the infants for 5 years. Comparisons of celiac disease onset in children having zero, one, or two copies of the celiac disease-associated *HLA* gene found that, overall, about 1 percent of the children at age five were estimated to have celiac disease. Children with one or two copies of the celiac-associated *HLA* gene were shown to be at increased risk of celiac disease compared to children without the gene.

The *HLA* genes *DQ2* and *DQ8* are the strongest genetic contributors to celiac disease. Coincidentally, *HLA-DQ2* and *-DQ8* are also type 1 diabetes susceptibility genes, and having one of these diseases is associated with

increased risk for the other disease. In celiac disease, the HLA proteins (encoded by the *HLA* genes) present gluten fragments to immune T cells via "receptors" on the surface of the cells, and this interaction initiates an aberrant immune response against the gluten protein. To better understand how HLA-DQ8, in particular, interacts with gluten, NIDDK-supported researchers examined the molecular properties of the DQ8 protein. The researchers found that the DQ8 variant contains an unusual positive electrical charge that enables it to interact not only with a negatively charged, modified gluten fragment but also with the unmodified native gluten fragment to elicit an immune response. However, gluten fragments with different electrical charges interact with different sets of T cell receptors. Negatively charged fragments interact with a large repertoire of receptors. In contrast, the unmodified gluten fragments interact with a very limited set of receptors. The ability of DQ8 to elicit immune responses to both forms of gluten fragments may lead to the excessive immune reactivity to gluten in celiac disease.

Although HLA-DQ8 is linked to celiac disease, not everyone who has this form of HLA develops the disease. Researchers thus sought to gain additional insights into the immune response to gluten by generating a new mouse model of the disease. These mice not only had the *HLA-DQ8* gene, but they also produced high levels of another immune molecule, IL-15, similar to humans with celiac disease. IL-15 can alter a pathway that normally allows tolerance to food; this pathway also involves retinoic acid, a vitamin A metabolite. The researchers fed a component of gluten to these and other mice. They found that mice that harbored HLA-DQ8 but not elevated IL-15 were tolerant of the gluten protein. By contrast, in the mice with both HLA-DQ8 and elevated IL-15, the retinoic pathway was altered by the presence of excessive IL-15, triggering an inflammatory response to gluten characteristic of celiac disease.

Through genome-wide association studies, researchers supported by the NIDDK have successfully identified additional celiac disease susceptibility genes and are gaining insight into their disease consequences. One study identified two gene variants that are required for celiac disease and 12 chromosome regions associated with celiac disease risk. A subsequent study looked for variants that could have smaller, yet critical, effects on disease risk. This study included DNA samples from a larger number of celiac disease patients and healthy volunteers that were analyzed using a denser concentration of probes. This approach was successful in uncovering 13 new chromosome regions associated with celiac disease and 13 additional chromosome regions with suggestive celiac disease associations. Many of the regions were found to contain genes with functions related to the immune system. In addition, analysis of the genetic variants led to the identification of four specific immunological pathways that are relevant to the pathogenesis of celiac disease.

Research on the effects of undiagnosed celiac disease has had mixed results. One study compared undiagnosed celiac disease in three groups of men by screening samples of their blood for celiac disease-specific antibodies, which would indicate presence of the disease. Samples from U.S. Air Force personnel that were taken and stored frozen 45 years earlier were compared to current samples from men the same age as the Air Force personnel, and younger men, the age the Air Force personnel were when their blood samples were taken. Death rates from all causes were found to be nearly 4-fold higher for men with undiagnosed celiac disease than for those without the disease. Analyses comparing the older and younger men found that the prevalence of undiagnosed celiac disease increased more than 4-fold over 50 years. Another study screened almost 17,000 American men and women 50 years of age and older, and found that over a 10-year period, undiagnosed celiac disease did not increase their risk of death, although other health consequences were observed. Those who had undiagnosed celiac disease had increased risk of osteoporosis and hypothyroidism, but they also had lower BMIs (body mass index) and cholesterol levels.

STORY OF DISCOVERY

Because these two studies differ in epidemiological criteria, such as the participants' ages and periods of observation, direct comparisons cannot be made between them. New studies will be needed to better understand the effects of undiagnosed celiac disease.

Research has also led to a new strategy for detection of celiac disease. A study compared tests for two different types of antibodies made by the immune system of celiac patients: antibodies to a small modified gluten protein called DGP, and those to a protein called transglutaminase (TGAA). The scientists followed 50 children, 6 months to 17 years of age, who were participants in the NIDDK-sponsored Celiac Disease Autoimmunity Research (CEDAR) study. The children were at high risk for developing celiac disease because of their genetic predisposition to celiac disease and/or type 1 diabetes. Antibody levels to DGP and TGAA were tracked over time, beginning prior to their disease diagnosis until they were detected. The study found that detection by the newly developed DGP antibody test was earlier in certain individuals and provided a more sensitive tool for evaluating the success of a gluten-free diet intervention.

Through NIDDK-supported studies of the cells lining the intestine, scientists have made additional discoveries about what goes awry in celiac disease. These intestinal cells possess fingerlike villi that protrude into the intestine, creating a large surface area through which small nutrient molecules are absorbed. Aligned closely together, these cells are joined by "tight junctions" that normally form a barrier to large molecules such as gluten. In people with celiac disease, the intestinal cells secrete a protein called zonulin which causes the tight junctions to loosen, allowing gluten to breach the intestinal barrier. Once gluten breaches this barrier, it precipitates an inflammatory response. Of note, small intestinal exposures to bacteria and gluten have been identified as two of the more powerful stimuli of zonulin secretion. The knowledge that gluten-triggered zonulin release led to the hypothesis that inhibiting zonulin might prevent gluten from entering and damaging intestinal tissue. Early clinical studies with a zonulin inhibitor have shown promise, but additional studies are needed to determine if this approach will be a successful treatment.

Although research studies had shown that the prevalence of celiac disease in the United States was approximately 1 percent, it was considered by many to be a rare disease and, therefore, at risk of being undiagnosed. In 2004, the NIDDK and the NIH Office of Medical Applications of Research sponsored the Consensus Development Conference on celiac disease, focusing on awareness, diagnosis, and management of celiac disease. In the Consensus Statement following the meeting, a Conference panel concluded that heightening awareness of celiac disease was imperative and recommended that the NIDDK lead an educational campaign for physicians, dietitians, nurses, and the public, informing them about celiac disease. In response to this recommendation, the NIDDK's National Digestive Diseases Information Clearinghouse launched the Celiac Disease Awareness Campaign. The Campaign remains a vital resource for the celiac disease community, offering fact sheets, booklets, practice tools for health professionals, NIH research information, and resources from professional and voluntary organizations that focus on celiac disease. These science-based Campaign materials can be accessed at *www.celiac.nih.gov/*

The NIDDK and the many celiac disease research scientists it supports continue the quest to gain insight into the complex underpinnings of celiac disease in pursuit of improved diagnostics, treatments, and a cure. Until the time this is accomplished, a gluten-free diet provides a treatment that relieves the symptoms of most celiac disease patients, and the Awareness Campaign continues its mission as an important resource for the celiac disease community.

New Clues to Liver Cell Death by "Toxic Fat"

Dr. Gregory J. Gores

Dr. Gregory J. Gores is the Reuben R. Eisenberg Endowed Professor in Gastroenterology and Hepatology, Professor of Medicine, and Chair of the Division of Gastroenterology and Hepatology at the Mayo Clinic in Rochester, Minnesota. Dr. Gores earned his M.D. from the University of North Dakota and completed his residency in internal medicine and fellowship training in gastroenterology and hepatology at the Mayo Clinic, followed by a post-doctoral fellowship in the Department of Cell Biology at the University of North Carolina at Chapel Hill. His research is focused on the mechanisms of liver cell death in models of relevance to human disease. Dr. Gores has received support from the NIDDK since 1989, and he has received a MERIT award from the Institute for his high research productivity. He has published over 400 original articles, book chapters, reviews, and editorials. Dr. Gores presented research findings from work conducted in his laboratory at the February 2011 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council. The following are highlights from his presentation.

Dr. Gores set the stage by describing the problem of nonalcoholic fatty liver disease, a condition in which excess fat is stored in the liver. Usually associated with other metabolic conditions, such as obesity and type 2 diabetes, this condition and its more severe form, called nonalcoholic steatohepatitis (NASH), have increased in prevalence in American adults and children in concert with the obesity epidemic. In those who develop NASH, their livers not only store excess fat, but also show signs of cell injury, inflammation, scarring (fibrosis), and cell death that are similar to changes found in the livers of individuals with alcoholic liver disease. But, the case of exactly how liver injury and cell death develop as nonalcoholic fatty liver disease progresses is far from closed, as researchers track down the cellular pathways and molecular partners responsible. If scientists could crack the case of how nonalcoholic liver disease and NASH develop, they would then be able to design better prevention and treatment strategies. Dr. Gores shared some of his lab's progress in solving the mystery of how fat in the liver turns "lipotoxic" (literally, toxic fat), injuring cells and contributing to the development of nonalcoholic fatty liver disease and NASH.

Exhibit A: Death by Apoptosis

Upon close inspection under a microscope, liver samples taken from people with NASH display a sobering scene, with many of the liver cells, called hepatocytes because they represent the major type of "hepatic" or liver-related cell, undergoing a type of cell death called apoptosis. When cells die by apoptosis, their DNA and other cellular components break down into small "apoptotic bodies." Because Dr. Gores and his team of experienced investigators had seen this before in other types of liver disease, they asked whether hepatocyte apoptosis was a truly unique hallmark of NASH compared to other conditions involving fatty liver, such as simple steatosis (fat in the liver without noticeable signs of injury) and alcoholic hepatitis. Using an assay that counts the number of cells undergoing apoptosis, they compared liver samples taken from people with these conditions. They found that hepatocyte apoptosis was indeed more dramatically elevated in the livers of people with NASH. Additional assays showed that the number of hepatocytes dying by apoptosis correlated with the level of inflammation and scarring present. From this evidence, they deduced that liver cell death by apoptosis plays an important part in NASH. These researchers went on to find that fragments of major structural liver cell proteins, broken down during apoptosis, ended up in the blood of people with

NASH, but not in those with only fat in the liver or no liver condition, offering a possible diagnostic alternative to liver biopsy.

Dr. Gores next turned to his group's investigations into the fate of the many apoptotic bodies that are formed in the livers of patients with NASH when hepatocytes undergo apoptosis. They knew that another cell type in the liver—the stellate cell—contributed to fibrosis (scar tissue formation) following liver injury in many forms of chronic liver disease. Could the liver's stellate cells be engulfing these apoptotic bodies and then developing "indigestion," promoting the development of scar tissue formation? To test their theory, they treated hepatocytes grown in the laboratory (in cell culture) with UV light to prompt damage and the formation of apoptotic bodies, which were then added to a culture of human hepatic stellate cells. Because the researchers also marked the apoptotic bodies and the cells with different fluorescent dyes, they could clearly see the stellate cells swallowing up the apoptotic bodies. Soon after engulfing the apoptotic bodies, the stellate cells increased their production of collagen, a material that accumulates during scar formation. These findings implicated stellate cells in the development of liver scarring.

Prime Suspects at the Molecular Level

Dr. Gores and the members of his lab knew that the stellate cells were not acting alone to cause nonalcoholic fatty liver disease. Other prime suspects in causing liver injury in this disease were "free fatty acids," which are released into the blood in large amounts by the body's fat tissue in individuals who are obese, insulin resistant, and/or have nonalcoholic fatty liver disease. These fatty acids were suspected of traveling to the liver, where they might behave like Dr. Jekyll or Mr. Hyde—either quietly storing energy as a form of fat called triglycerides, which are thought to be relatively harmless to the liver, or having a more sinister effect by causing hepatocytes to die by apoptosis. But, the researchers had to catch the fatty acids in the act.

They set up cell cultures with rodent hepatocytes and then added either of two types of free fatty acids: a saturated fatty acid called stearic acid, found in animal tissues and foods such as meat and dairy products, or a monounsaturated fatty acid called oleic acid found in olive oil. Then they watched as a large number of the cells cultured with stearic acid died by apoptosis—the saturated fatty acid had been caught red-handed. When they treated mouse and human liver cells in culture with another saturated fatty acid, palmitic acid, they observed the means by which these fatty acids can cause liver cell death. Palmitic acid stimulated liver cell production of a protein known to increase apoptosis called PUMA. Elevated PUMA levels were also detected in liver tissue samples taken from patients with NASH. The researchers inferred that saturated fatty acids acted through PUMA to cause liver cell apoptosis in NASH.

For further proof of PUMA's role in mediating the toxic effects of saturated fatty acid on liver cells, the researchers removed PUMA from the equation by genetically altering mice to be deficient in this protein. Without PUMA, hepatocytes were resistant to apoptosis caused by the saturated fatty acid palmitic acid, implicating PUMA as a go-between in carrying out the fatty acid's lipotoxic effects. Upon additional sleuthing, they identified another accomplice in the form of a signaling protein called JNK1. In mouse hepatocytes treated with palmitic acid, PUMA levels rose in mice with normal JNK1, but not in mice lacking this protein. A picture was beginning to emerge of this complex chain of events: in nonalcoholic fatty liver disease, saturated fatty acids released by the fat tissue traveled to the liver and activated the signaling molecule JNK1, which elevated PUMA levels, leading to liver cell death by apoptosis, followed by stellate cell activation and scar formation.

Tiny RNAs to the Rescue?

Now that the researchers had their line-up of molecular suspects involved in liver cell death and scarring due to toxic fat, they turned their attention

to looking for ways to limit this group's activities in the future and prevent nonalcoholic fatty liver disease progression. MicroRNAs were a natural choice as a possible disease-fighter because others had noticed that levels of these tiny molecules were altered in human NASH. MicroRNAs are capable of blocking protein production. In liver samples from people with NASH compared to obese people without NASH, levels of a particular type of microRNA known as miR-296 were reduced and accompanied by higher PUMA levels. By boosting levels of miR-296 in hepatocytes in culture, they were able to keep PUMA levels in check, suggesting new therapeutic possibilities for limiting hepatocyte apoptosis in human NASH. For example, by developing drugs or therapeutic microRNAs that target PUMA or other components of the cell death pathway, scientists may one day find a means to reduce liver damage in patients with NASH.

Conclusions

In presenting his lab's ongoing research, Dr. Gores made the case that free fatty acids, especially the saturated kind, are a major culprit underlying liver cell death by apoptosis in nonalcoholic fatty liver disease, and he showed that stellate cells play a key role in subsequent scar formation. These investigations also shed light on the cellular partners and pathways used by the free fatty acids to carry out this deed, as well as possible molecular targets for limiting disease. This research is improving understanding of the molecular pathways responsible for the liver injury that occurs in forms of nonalcoholic fatty liver disease such as NASH. Beyond getting to the bottom of this hepatic whodunit, these investigations could also lead to the development of new diagnostic and therapeutic approaches to nonalcoholic fatty liver disease in the future.

PATIENT PROFILE

Durga Dingari

One Woman's Journey Living with Chronic Pancreatitis

Durga Dingari

When 42-year-old Durga Dingari says she has her "good days and bad," for most of us that would be a gross understatement.

A native of India, now living in the United States, Durga experienced her first pancreatic attack in her home country in 1994, at the age of 26. "I didn't know what was happening to me. The pain was unbearable," she says. "I started vomiting." At first she thought it was acid reflux.

Shortly after the attack, Durga underwent a procedure called an endoscopic retrograde cholangiopancreatography, or ERCP, which enables physicians to diagnose problems in the liver, gallbladder, bile ducts, and pancreas. But the test revealed nothing.

The following year, Durga, her husband, and their then 4-year-old son immigrated to the United States for her husband's job as a software engineer. "At the time I arrived in the United States, I was 70 pounds," says Durga. However, within months she says she started feeling better, regained the weight she had lost, and had completely forgotten about her pancreatic attack. Everything seemed to be going fine.

Then, in 1998, while attending her mother-in-law's funeral in India, Durga suffered another major pancreatic attack. Again, she recovered. A CT scan showed some calcification (hardening due to calcium deposits) of her pancreas, but her gynecologist told the Dingaris that it would be safe to go ahead with their plans for having a second child.

"It was a hard pregnancy," Durga says. She suffered from the pancreatic attacks, as well as gestational (pregnancy-related) diabetes, and in the final stages of her pregnancy ended up on bed rest. Despite these difficulties, her daughter was born in January 2000, which "was the happiest day of our lives," after such a tense pregnancy. The Dingaris named their daughter Spoorthi, which means "inspiration."

Ever since then, Durga has needed all the inspiration she can muster to battle her pancreatic condition and the pain it brings.

"I didn't know what was happening to me. The pain was unbearable," says Durga of the first pancreatic attack that began her journey living with chronic pancreatitis.

Within 2 weeks of giving birth to her daughter, Durga again suffered severe pain that began a cycle of hospital admissions and a seemingly endless series of medical procedures, including the removal of calcified

stones from her pancreas. Her attacks continued, and Durga next underwent a pancreatic operation called a Puestow procedure. In this operation, used to treat pain associated with chronic pancreatitis, the pancreatic duct is connected to the small intestine so that pancreatic secretions can drain directly into the intestine. During the operation, Durga's surgeons realized that her gallbladder was also damaged and they removed it. Durga spent the next month in the hospital recovering from her surgeries.

Once again, she was fine for a few months before the pancreatic attacks started to recur. "Eating caused pain and lots of attacks. It's hard to describe what my family and I were going through," says Durga.

Her health continued to deteriorate. "But I was given new hope from another chronic pancreatitis patient who told me that she had gotten her life back" through a surgical intervention called a Whipple procedure. During this surgery, the head of the pancreas, a portion of the bile duct, the gallbladder, and part of the small intestine are removed. Occasionally, a portion of the stomach may also be removed. After these structures are removed, the remaining pancreas, bile duct, and intestine are sutured back together to direct gastrointestinal secretions back into the gut.

But before physicians could do the procedure, Durga first had to regain her weight and nutritional status. It was now 2001, and Durga's weight had dropped back down to 70 pounds. So surgeons implanted a type of feeding tube called a jejunostomy tube, or "J-tube" in the upper section of her small intestine, just below the stomach. The purpose of the J-tube is to provide elemental nutrients—including salts, glucose, amino acids, lipids, and added vitamins—to the patient while bypassing the usual process of eating and digestion.

Once Durga's weight and nutritional status were satisfactory, she underwent the Whipple procedure. Unfortunately, in a relatively short period of time, her pain returned. "I was totally devastated," says Durga. She was told then that she had a form of pancreatitis called "idiopathic" chronic pancreatitis.

About Chronic Pancreatitis

The pancreas is a small gland nestled near the small intestine. It is responsible for producing enzymes that, mixed with bile from the gallbladder, aid in the digestion of food. In a healthy pancreas, these enzymes are released in an inactive form, to become activated only when they reach the intestine. However, when the pancreas is inflamed, as in pancreatitis, these enzymes become activated while still within the pancreas, where they degrade the very tissue that produced them, causing episodes of pain that can range from mild to severe. Pancreatitis can be acute, with inflammation resolving within a few days, or chronic, involving long-term inflammation and tissue damage.

A variety of factors may contribute to chronic pancreatitis, including genetic and other factors, but the form of the disease that Durga has, "idiopathic" chronic pancreatitis, is the result of unknown causes. People who have a history of diabetes in their family also are at greater risk for contracting the disease, usually between the ages of 30 and 40. In Durga's case, many of her relatives have either type 1 or type 2 diabetes.

Over time, chronic pancreatitis leads to permanent damage to the pancreas. In addition, patients who, over a long period of time, suffer with the disease are at increased risk for pancreatic cancer, which is one of the most devastating of all malignancies.

Pancreatitis also can be excruciatingly painful. On a good day, medications keep Durga's pain bearable. "On a bad day I feel like the pain is going to kill me," says Durga. In the past, these painful episodes have often necessitated a trip to the nearest hospital emergency room.

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Currently, there are no cures or preventive therapies for chronic pancreatitis. Even when their appetite and eating habits are normal, people with chronic pancreatitis often lose weight. The weight loss occurs because the body does not secrete enough pancreatic enzymes to digest food, so nutrients are not absorbed normally. Patients' symptoms and complications from the disease are treated through a combination of rehydration and pain management therapy, nutritional support and pancreatic enzyme supplementation, and eating a nutritious, low-fat diet while also avoiding smoking and alcohol consumption.

Living with Chronic Pancreatitis

Over the years, Durga has undergone two pancreatic surgeries and numerous other procedures related to her condition. She often takes up to 10 painkillers a day. Even before the onset of her chronic pancreatitis, Durga was a small woman weighing approximately 100 pounds. Today, she takes pancreatic enzyme supplements when she eats to make up for her limited pancreatic function and aid her digestion. However, Durga says she struggles to maintain her weight at 94 or 95 pounds.

"My diet consists mainly of toast with honey, coconut water, and yogurt," she says, but adds that even these mild foods often don't agree with her. Currently, Durga is getting much of her nutrition from a combination of the J-tube and a treatment called total parenteral nutrition, or "TPN," a nutritional support intervention in which a nutrition-laden solution runs through a line connected to one of her veins, and like the J-tube, bypasses the usual process of eating and digestion.

In the meantime, Durga courageously struggles on a daily basis with her pain, and strives to lead as productive and normal a life as she can with her family, including her now 21-year-old son and 11-year-old daughter.

"I try to keep myself as busy as possible so I don't think about my condition," says Durga, and adds with great emotion, "I don't want my kids to worry about me too much. They are good, smart kids and I want them to have a good and normal life."

Despite her pain, Durga hosts her own Internet radio show in her mother tongue, Telugu, in which she selects Indian songs and comments on their style and lyrics, "especially if they relate to my life and family. I share everything with my listeners—things regarding my health, my children, my family, everything." She also authors her own blog, and of late has taken an interest in beadwork, which she does to pass the time when she's taking in nutrients through her J-tube. "I give most of my beadwork away as gifts," she says with a sense of joy and satisfaction, which helps to make the bad days a little brighter.

"I try to keep myself as busy as possible so I don't think about my condition," says Durga, a wife and mother-of-two who does beadwork, authors a blog, and even hosts her own Internet radio show. "I share everything with my listeners—things regarding my health, my children, my family, everything," she says.

Hope Through Research

The NIDDK actively supports research on many forms of pancreatitis to help people like Durga, who suffer from this disease. NIDDK-sponsored research has led to such advances as the discovery of genetic factors associated with hereditary and chronic forms of pancreatitis, as well as understanding the mechanisms by which pancreatic enzymes are formed and lead to damage in acute pancreatitis. The NIDDK also supports an initiative to encourage clinical and epidemiological research studies to facilitate the translation of promising new developments into the clinical setting for diseases such as pancreatitis.

With NIDDK support, scientists are currently conducting research to identify biomarkers to

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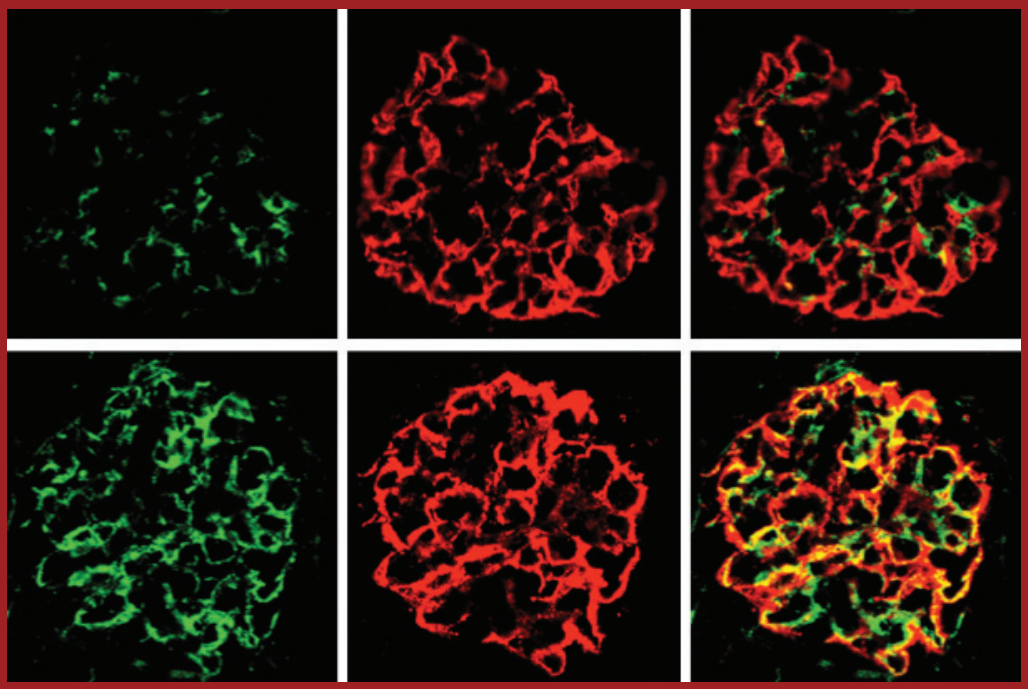
facilitate diagnosis, additional genetic triggers to improve understanding of risk factors and disease processes, mechanisms underlying debilitating pancreatitis symptoms such as pain, and nutritional support and novel treatments. For example, the North American Pancreatic Study 2 is a multi-center clinical study building on past research to try to uncover additional genetic markers that may help to identify individuals susceptible to pancreatitis. Researchers hope to discover new approaches to prevent and/or treat pancreatitis and its disease progression. The study is also investigating whether there are any racial or ethnic differences that put some groups at higher risk than others for developing pancreatitis in response to genetic and environmental factors. An ongoing clinical trial, the Study of Nutrition in Acute Pancreatitis (SNAP), is designed to test the effectiveness of different "enteral" feeding methods—which deliver nutrients through a tube placed either through the nose to the stomach or in the small intestine—in providing nutrition to patients with severe acute pancreatitis who cannot eat by mouth.

For more information:
http://digestive.niddk.nih.gov/ddiseases/pubs/pancreatitis/index.aspx



Elevated levels of the circulating protein suPAR have been identified as a potential mediator of the kidney disease focal segmental glomerulosclerosis. This figure shows sections of mouse kidney following injection of either a low dose (top row) or a 10-fold higher dose (bottom row) of suPAR. In the left column, green dye identifies cells that have responded to suPAR by activating beta-3 integrin; the higher dose (bottom left) elicits a stronger response than the lower dose (upper left). In the middle column, red dye identifies specialized cells called podocytes that help filter waste in the kidney. When the two sets of images (left and center columns) are superimposed (right column), yellow identifies cells that stained positively with both dyes, indicating that podocytes respond to elevated suPAR levels by activating beta-3 integrin. Activation of this protein in podocytes has been shown to disrupt cell-cell contact and compromise the kidney's filtering function.

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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, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

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, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Loss of kidney function, whether sudden or slow, represents an important health challenge.

Chronic kidney disease has two main causes: high blood pressure and diabetes. The latest estimates put the number of Americans with chronic kidney disease at more than 23 million.[1] If unchecked, the recent increases in obesity and type 2 diabetes in the United States—especially among children and adolescents—have grave implications, as individuals are likely to face any secondary health consequences at an earlier age than people who develop these conditions as middle-aged adults. In fact, roughly half of the people with kidney disease will die from cardiovascular disease before they progress to kidney failure.[2]

In mid-2011, NIDDK-supported researchers identified a new biomarker involved in phosphate metabolism that appears to predict progressive kidney disease, kidney failure, and death. This biomarker was later shown to be a "mediator" of cardiovascular disease in rats with chronic kidney disease. This knowledge could help to identify individuals whose kidney function is likely to be stable over time as compared to those whose disease is likely to progress and who may require more intensive therapy. More about this research can be found later in this chapter. Also in mid-2011, the Institute hosted a meeting titled "Reducing the Impact of Chronic Kidney Disease: Opportunities for Randomized Clinical Trials." It included a discussion about ways to optimize the conduct and impact of Phase III clinical trials in patients with chronic kidney disease.

Chronic kidney disease, 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 2009, over 550,000 patients received treatment for ESRD: nearly 390,000 received dialysis and over 165,000 were living with a kidney transplant. Minority populations, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease and ESRD. African Americans are over three times more likely to develop kidney failure as non-Hispanic whites are.[3] American Indians and Hispanics have twice the risk for kidney failure as do non-Hispanic whites. NIDDK-supported research has led to important insights about how to improve the health and well-being of people with kidney failure. The Frequent Hemodialysis Network Daily Trial showed that frequent, in-center daily hemodialysis improved heart health and self-reported physical wellness compared to thrice-weekly hemodialysis in patients with kidney failure. More about this research advance can be found later in this chapter.

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute'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

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[1] *Levey AS, et al. Ann Intern Med 150: 604-612, 2009.*

[2] *Kundhal K and Lok CE. Nephron Clin Pract 101: c47-c52, 2005.*

[3] *U.S. Renal Data System, USRDS 2011 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, 2011.*

to progression of kidney disease to ESRD, and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. In late 2011, the Institute provided funding for several planning grants for translating chronic kidney disease research into improved clinical outcomes. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute'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 chronic kidney disease and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public.

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 particular interest include the causes of and treatments for major adult urological diseases and disorders, such as benign prostatic hyperplasia, urinary incontinence and urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS) in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent) U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS.[4] Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with painful bladder syndrome.[5] NIDDK-supported basic and clinical research is focused on elucidating the causes of IC/PBS, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. The NIDDK's Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network supports studies designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. The goals and approaches of the MAPP Research Network reflect the most current thinking on IC/PBS pathology and involve significant new advancements in how IC/PBS is studied. All efforts are designed to provide insights that can be translated to improve the clinical care of patients with IC/PBS. A prospective epidemiological study in a racially and ethnically diverse sample of men and women, the Boston Area Community Health Survey (BACH), seeks to identify patterns and risk factors for those bothersome symptoms. A similar study, the Olmsted County (Minnesota) Study, is studying lower urinary tract symptoms in men.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[6] Many suffer in silence due to embarrassment and lack of knowledge about 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 the effect of different diagnostic tools and interventions on patient outcomes. For example, the utility of a common pre-surgical urinary test, called urodynamic testing, has not been proven to be helpful to women or their doctors. The NIDDK's Urinary Incontinence Treatment Network recently completed the Value of Urodynamic Evaluation clinical trial to clarify the necessity of urodynamic testing for a woman with stress urinary incontinence who is planning surgical treatment, and results are expected in 2012.

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[4] Berry SH, et al. J Urol 186: 540-544, 2011.

[5] Link CL, et al. J Urol 180: 599-606, 2008.

[6] Urological Diseases in America. NIDDK, NIH Publication Number 07-5512, 2007.

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 chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron-chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

The NIDDK supports an extensive array of research centers that bring together diverse teams of investigators to address critical research questions. George M. O'Brien Kidney and Urology Research Centers conduct interdisciplinary investigations that address basic, clinical, and applied aspects of biomedical research in kidney and genitourinary physiology and disease. Kidney diseases of hypertension and diabetes, renal and urinary tract dysfunction in obstructive diseases of these organs, immune- and nonimmune-related mechanisms of glomerular injury and kidney disease, nephrotoxins and cell injury, and benign prostatic hyperplasia are among the research areas emphasized. Research Centers of Excellence in Pediatric Nephrology conduct coordinated, interdisciplinary, and multi-institutional studies on mechanisms regulating the development of the kidney and urinary tract and on childhood nephrotic syndrome. Polycystic Kidney Disease (PKD) Research and Translation Centers are part of an integrated program of research established to promote multidisciplinary interactions and to provide shared resources needed to address complex biomedical problems in this area, such as therapy of PKD. Centers of Excellence in Molecular Hematology have integrated teams of investigators from a wide range of specialties; share specialized, often expensive equipment and staff; and serve as regional or national resources for other researchers. The Centers provide a focus for multidisciplinary investigations into gene structure and function; the cellular and molecular mechanisms involved in the generation, maturation, and function of blood cells; and the development of strategies for the correction of inherited diseases.

RESEARCH TOWARD IMPROVED TREATMENT OF KIDNEY DISEASE

Daily Hemodialysis Provides Additional Benefit to Kidney Patients; Overnight Dialysis Is as Effective as Standard Therapy: More frequent, in-center daily hemodialysis improved heart health and self-reported physical wellness compared to standard, thrice-weekly in-center hemodialysis in patients with kidney failure, according to the Frequent Hemodialysis Network (FHN) Daily Trial. A related trial, the FHN Nocturnal Trial, found that more frequent sessions of overnight, in-home dialysis did not improve patient outcomes compared to standard hemodialysis.

The FHN Daily Trial involved 245 patients who were randomly assigned to receive either conventional (three times a week) dialysis, or six shorter treatments a week; the treatments were given in a dialysis center. The FHN Nocturnal Trial involved 87 patients who were randomly assigned to receive either conventional dialysis or six treatments a week delivered overnight; most treatments in both arms were done at home. Both studies compared two co-primary outcomes in the two groups of patients: death or change in left ventricular mass (the size of the heart's left ventricle—a sign of heart health); and death or change in patient responses to a questionnaire that is widely used in clinical medicine to determine how well a person feels and functions.

In addition to the benefits observed in the FHN Daily Trial, both the FHN Daily and Nocturnal Trials found that patients undergoing more frequent hemodialysis had improved blood pressure and phosphate levels. However, patients receiving more frequent dialysis in both trials were more likely to have complications related to problems with the site on their bodies where the blood was removed and returned during dialysis (known as a vascular access site). While neither study was designed to detect differences in death rates between treatment groups, the Daily Trial showed that more frequent dialysis improved patients' heart health and self-reported physical wellness, which suggests that it could be of benefit to some people.

Previous observational data suggested that the dose of hemodialysis correlated with patient survival. However, results from the NIDDK-funded HEMO Study in 2002 showed no added benefit of

increasing the per-treatment dose of hemodialysis in the conventional, three times per week method. Additionally, smaller studies have shown benefits of nocturnal hemodialysis. By undergoing dialysis more often, patients in the daily dose or overnights arms of the two trials effectively received a substantially higher weekly dialysis dose overall. This was found to be beneficial in the Daily Trial. The Nocturnal Trial did not demonstrate a definitive benefit compared to conventional hemodialysis, perhaps because of the smaller number of patients enrolled. These findings have important implications for patient care. The benefit of daily hemodialysis must be weighed against the added burden to patients as well as increased cost.

Chertow GM, Levin NW, Beck GJ, et al. In-center hemodialysis six times per week versus three times per week. N Engl J Med 363: 2287-2300, 2010.

Rocco MV, Lockridge Jr RS, Beck GJ, et al. The effects of frequent nocturnal home hemodialysis: the Frequent Hemodialysis Network Nocturnal Trial. Kidney Int 80: 1080-1091, 2011.

Focal Segmental Glomerulosclerosis Proves Resistant to Two Second-line Therapies: A recent study found no difference between two different drug regimens to treat a form of kidney disease that is stubbornly resistant to standard therapy. Both treatment approaches were effective in only a few of the patients, leaving researchers to search for more options.

Focal segmental glomerulosclerosis (FSGS) is one of the leading causes of kidney failure. It is most often diagnosed in children and young adults. The disease is characterized by scarring in scattered regions of the kidney. Kidney damage resulting from this disease can allow protein in the blood to leak into the urine, a condition termed proteinuria. Initial treatment of FSGS generally involves corticosteroids, a class of hormones that reduces inflammation. This therapy results in reversal of proteinuria in approximately one-fourth of patients. For the majority of patients, whose FSGS does not respond to this treatment, there is no agreed-upon next step.

The NIDDK-supported FSGS Clinical Trial was designed to compare two different approaches to treating steroid-resistant FSGS in children and young adults. The researchers randomized 138 patients—two-thirds of whom were under 18 years of age—to receive either cyclosporine, an immunosuppressant drug, or a combination of mycophenolate mofetil and dexamethasone, an immunosuppressant drug and a synthetic steroid, respectively. After 1 year, more patients receiving cyclosporine showed reversal of proteinuria (46 percent, 33 of 72 individuals) than those receiving mycophenolate/dexamethasone (33 percent, 22 of 66 individuals). However, the difference in response rates between the two groups was not statistically significant; that is, the difference was so small that it may not indicate a real benefit of one therapy over the other. There were also no differences in response rates among the patients regardless of their age or race.

The FSGS Clinical Trial was the largest clinical trial of pediatric and adult patients with steroid-resistant FSGS. Nevertheless, the absolute number of patients enrolled was relatively small, and the treatments did not have large effects; thus it is difficult to interpret the response rates definitively. Additionally, the relatively low rate of responsiveness to either therapy raises the question of whether approaches that target the immune system are likely to be generally effective in patients with FSGS. The results of this trial underscore the importance of continued research to identify new markers of disease progression (biomarkers) and other factors that contribute to this disease, which may provide new targets for therapy and allow physicians to more closely monitor a patient's response to treatment.

Gipson DS, Trachtman H, Kaskel FJ, et al. Clinical trial of focal segmental glomerulosclerosis in children and young adults. Kidney Int 80: 868-878, 2011.

Key Link Discovered Between Kidney Disease and Heart Disease: High levels of a hormone that regulates phosphate metabolism are associated with an increased risk of kidney failure, cardiovascular disease (CVD), and death among people with chronic kidney disease (CKD).

In a previous study of patients who were beginning hemodialysis for treatment of kidney failure, those with elevated blood levels of the hormone fibroblast growth factor 23 (FGF-23) were found to be at nearly six times greater risk of death compared to those with lower levels. However, this factor had not been studied in the

much larger population of patients with the full range of CKD of varying severity who had not progressed to kidney failure. Researchers now report that all CKD patients with elevated FGF-23 levels are at three times higher risk of death compared to patients with lower levels of the hormone. Furthermore, CKD patients with only mild to moderately impaired kidney function and elevated FGF-23 levels are at nearly two times higher risk of progressing to kidney failure.

Several months later, investigators reported that elevated levels of FGF-23 are independently associated with increased risk of CVD in patients with CKD. Elevated FGF-23 levels were shown to be associated with increased risk of an adverse heart condition—change in left ventricular mass (the size of the heart's left ventricle). Experiments conducted in animal models supported the findings found in patients with CKD. For example, mice developed enlarged left ventricles following injection of FGF-23; this and other experiments suggest that FGF-23 may actually cause CVD. Future studies will develop strategies to interrupt FGF-23 action and prevent or lessen damage to the heart in patients with CKD.

These findings come, in part, from the NIDDK-supported, multi-center, observational Chronic Renal Insufficiency Cohort (CRIC) Study, which enrolled nearly 3,900 racially diverse participants with CKD. The major goal of CRIC is to determine which factors might predict loss of kidney function and the development and worsening of heart disease in patients with CKD. This study is part of a broader effort by the NIDDK to identify biological "markers" that can allow physicians to better predict how various diseases are likely to progress in different patients and thereby personalize treatments to improve their health.

Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA 305: 2432-2439, 2011.

Faul C, Amaral AP, Oskouei B, et al. FGF23 induces left ventricular hypertrophy. J Clin Invest 121: 4393-4408, 2011.

Identification of a Circulating Factor That May Cause the Kidney Disease Focal Segmental Glomerulosclerosis: Scientists have identified a factor circulating in the blood of some patients with focal segmental glomerulosclerosis (FSGS) that may play an important role in the disease's initiation, progression, and recurrence. This discovery may have important implications both for research and for decisions regarding patient care.

Blood enters the kidneys through arteries that branch into tiny clusters of looping vessels that allow the removal of waste products, salts, and excess fluids. Each of these clusters is termed a "glomerulus," from the Greek word meaning "filter." FSGS is characterized by scarring and changes in cell structure in scattered glomeruli throughout the kidney. It may arise from another disorder or it may develop without a known cause. For many years, researchers have speculated that some factor outside of the kidney may play a role in the development of some cases of FSGS. This hypothesis was based on the observation that the disease can recur, sometimes quite rapidly, in FSGS patients who have received kidney transplants. Evidence that such a factor might circulate in the blood comes from the observation that plasmapheresis—the removal, purification, and replacement of blood—has been used with some success to treat recurrent FSGS. Previous research has shown that activation of the urokinase receptor (uPAR), which is found on the surface of specialized kidney cells that wrap around the glomerulus termed podocytes, can cause abnormal changes in podocyte structure and the leakage of protein into the urine, two features characteristic of FSGS. A cleaved form of this cell surface receptor—a serum-soluble urokinase receptor, or suPAR—had been detected in small amounts in the bloodstream, but whether it played any role in kidney disease was unknown.

To investigate whether suPAR might play a role in FSGS, researchers examined 78 patients with FSGS and measured the levels of suPAR in their blood. They compared these readings with blood samples taken both from people without kidney disease and from patients with glomerular diseases other than FSGS (minimal change disease, membranous nephropathy, and pre-eclampsia, a pregnancy-related condition). Two-thirds of patients with FSGS had elevated suPAR levels compared to healthy volunteers or people who had other kidney diseases, with the exception of a minority of patients with membranous nephropathy, whose levels were only slightly higher than normal. The highest suPAR levels were seen in samples taken

from FSGS patients who later developed recurrent disease after receiving a kidney transplant. In patients who underwent plasmapheresis, circulating suPAR levels were significantly lower after the procedure.

The researchers also conducted experiments in animals to determine the biological effect of suPAR in podocytes. Using three different mouse models, they demonstrated that increased levels of suPAR caused changes in podocyte structure and leakage of protein into the urine. Specifically, suPAR was shown to activate beta-3 integrin, one of the major proteins that helps anchor the podocyte to surrounding tissue. This activation leads to podocyte detachment and subsequent dysfunction.

These findings add substantially to the growing understanding of the causes and mechanisms of glomerular disease, and they identify a novel biological pathway that may play a role in FSGS. Further study of suPAR and its biological function could lead to the development of therapies that target suPAR, beta-3 integrin, or other mediators of suPAR's effects. These findings may also have implications for the clinical management of this kidney disease, such as allowing physicians to identify patients at risk of recurrent disease following transplantation and to tailor treatment regimens accordingly.

Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med 17: 952-960, 2011.

INSIGHTS INTO THE CELLULAR MECHANISM OF KIDNEY REPAIR

Origin of Cells Involved in Kidney Repair: Acute kidney injury is a serious medical condition characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. Even though a significant fraction of patients with acute kidney injury will regain kidney function, many do not. Following acute kidney injury, the repair and repopulation of the damaged areas of the organ are primarily a result of cell division by remaining, non-lethally injured cells, according to a recent study of the condition in a mouse model. This report is the latest piece of information in an ongoing effort by researchers to better understand kidney recovery following injury and identify the cellular and molecular actors in this process.

In adults, the rate of cell division in the kidney is normally quite low. Following an injury, cell proliferation increases dramatically as the kidney initiates a repair process to replace damaged cells. However, there has been vigorous debate over the mechanisms governing the repair process and the origin of these new cells. Potential explanations have included the "de-differentiation" of mature cells into more generalized cell types, which then begin to divide; activation of hypothetical adult kidney stem cells following injury; or the re-activation of proliferation in the remaining, previously static kidney cells, which might be limited to uninjured cells or also include injured, but surviving cells.

In the current study, researchers devised a way to identify cells in mice that were actively proliferating following kidney injury. Because cells that are preparing to divide must first make another copy of their genetic material, the researchers used specially modified nucleic acids—the building blocks of DNA—that could be readily detected, as a way to identify cells that had recently synthesized new chromosomes. They injected the mice with the modified nucleic acids and, a short time later, induced kidney injury by restricting the blood flow to the organ for a brief time. Subsequently, the kidneys were removed and carefully studied. The scientists found that the increase in cell proliferation in the kidney following injury occurred as a result of self-duplication of existing mature kidney cells, not from stem cells. They also noted that the cells most likely to proliferate were those that had been injured, but survived, rather than neighboring, uninjured cells.

Currently, there is no effective drug therapy to reverse acute kidney injury. A better understanding of how kidneys recover from injury, from this research and other studies, could have important implications for future treatment strategies to address this serious condition.

Humphreys BD, Czerniak S, DiRocco DP, Hasnain W, Cheema R, and Bonventre JV. Repair of injured proximal tubule does not involve specialized progenitors. Proc Natl Acad Sci USA 108: 9226-9231, 2011.

UNDERSTANDING AND TREATING UROLOGICAL DISORDERS

Tissue-engineered Urethras To Treat Urination Problems in Boys with Urethral Defects: Research clinicians have recently reported success in engineering urethras for boys who needed urethral reconstruction. The urethra is a tube that carries urine from the bladder to the outside of the body. Non-functional urethras can arise from injury, disease, or genetic mutation. Patients with defective urethras often face difficulties urinating, and catheters may need to be inserted to facilitate bladder emptying. Although surgical repair of short defects are routinely performed, larger urethral defects are not amenable to this approach and other strategies to address this situation, such as tissue engineering, have been under development.

Building on previous clinical research and research conducted in animal models, researchers have successfully engineered urethras for five boys between the ages of 10 and 14 who had defective urethras as a result of injury. A bladder biopsy was obtained from each patient and the tissue used to isolate the different types of cells—muscle and epithelial cells—needed to generate a new urethra. The cells were grown in laboratory culture for 3 to 6 weeks. Once grown to sufficient numbers, the epithelial cells were used to seed the inside surface and the muscle cells were used to seed the outer surface of a tubular-shaped biodegradable scaffold. Approximately 6 days later, each engineered urethra was surgically implanted back into the patient who initially provided the biopsy sample (*i.e.*, autologous engraftment). Patients underwent regular clinical assessments for 6 years after surgery to monitor the function of the newly-reconstructed urethra. Post-surgical biopsies showed that the engineered urethral tissue grafts developed a normal appearing architecture by 3 months post-surgery. The study further showed that the engineered urethras showed functional characteristics similar to native urethras—including the maintenance of an adequate urine flow during the 6-year study follow-up. This example of regenerative medicine is the first to demonstrate the use of a patient's own cells combined with a biodegradable scaffold to successfully replace defective urethral tissue.

Raya-Rivera A, Esquiliano DR, Yoo JJ, Lopez-Bayghen E, Soker S, and Atala A. Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. Lancet 377: 1175-1182, 2011.

Herbal Compound Does Not Relieve Urinary Symptoms Attributable to Benign Prostatic Hyperplasia: A large clinical trial has found that a commonly used herbal therapy, saw palmetto, is no more effective than placebo for improving urinary symptoms in men with prostate enlargement. Non-cancerous growth of the prostate, or benign prostatic hyperplasia (BPH), is a common cause of bothersome lower urinary tract symptoms (LUTS) in men, such as weak or intermittent urine stream, an inability to empty the bladder completely, and having to urinate frequently, especially at night. Men with these symptoms seek relief in a variety of ways, including not only surgery or medication, but also the use of plant extracts such as saw palmetto; however, the clinical benefit of herbal dietary supplements for LUTS symptoms had not been rigorously tested.

Following up on previous clinical trials that found no benefit of saw palmetto compared to placebo when used at a standard dose of 320 milligrams per day, researchers conducted a larger, randomized, multi-center trial to determine whether doses up to three times that amount would improve LUTS. Men 45 years old and older with LUTS were randomly assigned to receive either saw palmetto extract or placebo. Over the course of the trial, men in the saw palmetto group received a standard dose for 24 weeks, then a double dose for 24 weeks, and finally a triple dose for 24 weeks, while men in the other, control group received a placebo the entire 72 weeks. Because commercially available herbal supplements can vary in composition from batch to batch, the saw palmetto used in the trial was carefully standardized to ensure consistency across all doses through the duration of the trial. At the end of the trial, the researchers observed no significant difference in frequency of LUTS or in any other measures of urinary symptoms (*e.g.*, peak urinary flow) between the men who had received the saw palmetto and those who had taken the placebo. These trial results demonstrate that saw palmetto, even at high doses, does not improve LUTS—information that men with these symptoms and their health care providers can use in discussing and making choices about conventional and alternative therapies for symptom relief.

Barry MJ, Meleth S, Lee JY, et al. Effect of increasing doses of saw palmetto extract on lower urinary tract symptoms: a randomized trial. JAMA 306: 1344-1351, 2011.

Brain Function and Anatomy in Chronic Prostatitis/Chronic Pelvic Pain Syndrome: Urologic chronic pelvic pain encompasses two major pain syndromes—interstitial cystitis/painful bladder syndrome, which primarily affects women, and chronic prostatitis/chronic pelvic pain syndrome, which only affects men. Both syndromes, however, share the characteristics of severe pain below the abdomen, often with urinary frequency and urgency, and in both cases their cause remains unknown. Research is revealing that millions of people worldwide may have symptoms of urologic chronic pelvic pain syndromes, with attendant suffering akin to patients with serious chronic illness. As fully effective treatments are elusive and there is no cure, people with these syndromes suffer and can also incur high medical costs for themselves and the health care system.

Using an imaging technology called functional magnetic resonance imaging (fMRI) to look at chronic pain states, scientists compared brain imaging data from adult male volunteers with chronic prostatitis/chronic pelvic pain syndrome and from healthy adult males. The study showed functional activation within the right anterior insula—a region of the brain that is associated with sensing signals from within the body, including pain—that correlated with the intensity of pain as reported by the patient. In addition to changes in brain activity, researchers sought to determine whether there were changes in the brain structure of people with urologic chronic pelvic pain syndromes compared to people who do not have these syndromes. No differences were found in regional grey matter volume; however grey matter density in pain-relevant regions (right anterior insula and anterior cingulate cortices) positively correlated with pain intensity and duration of pain. These imaging studies reveal an apparent correlation between the intensity and duration of pain and the density in the brain's gray matter in different brain regions. Interestingly, some of the brain areas that appear to be affected by urologic chronic pelvic pain are important in human function and behavior, particularly in emotional decision making.

This study shows an association between functional and anatomical changes in the brains of patients with chronic prostatitis/chronic pelvic pain syndrome compared to individuals without this condition. Future studies will determine whether the observed brain changes are a result of chronic pelvic pain or represent risk factors for the development of chronic prostatitis/chronic pelvic pain syndrome.

Farmer MA, Chanda ML, Parks EL, Baliki MN, Apkarian AV, and Schaeffer AJ. Brain functional and anatomical changes in chronic prostatitis/chronic pelvic pain syndrome. J Urol 186: 117-124, 2011.

Prevalence of Interstitial Cystitis/Painful Bladder Syndrome Among Women in the United States: A large national study has provided new estimates of the burden of interstitial cystitis/painful bladder syndrome (IC/PBS) among U.S. women. IC/PBS is challenging to diagnose, and no fully effective treatment exists. While this condition appears much more prevalent in women than in men, robust estimates of prevalence and impact in the U.S. population have been elusive. This information would be very helpful to the design of clinical research studies and for disseminating information about this condition to health care providers and the public.

To obtain this information, researchers in the Rand Interstitial Cystitis Epidemiology (RICE) study conducted a two-phase phone interview survey that involved contacting over 146,000 randomly chosen households across the United States. From an initial phone screening, they identified households with an adult female member reporting bladder symptoms or an actual diagnosis. They then followed up with a phone questionnaire to determine if women identified this way met study criteria for IC/PBS, and, if so, to collect demographic information and determine the severity and personal impact of this condition. The criteria included both a "high-sensitivity" definition and a "high-specificity" definition of IC/PBS. The "high-sensitivity" definition enabled the researchers to capture the largest number of possible cases in the survey, but it was less effective at excluding cases of pelvic pain not due to IC/PBS, whereas the "high-specificity" definition could better distinguish IC/PBS from other bladder and pelvic pain conditions, but was more likely to miss some cases of IC/PBS. When the researchers applied the high-specificity definition of IC/PBS to the group initially identified with the high-sensitivity definition, they estimated from their results that about 2.7 percent of adult U.S. women have symptoms consistent with IC/PBS;

calculations based on the high-sensitivity definition alone increased the estimate to about 6.5 percent. Moreover, the researchers noted that the severity of IC/PBS among the women who met the high-specificity definition is similar to that seen in women selected from urology practices to participate in clinical studies, yet only about one in ten of the former reported having been diagnosed with IC/PBS—suggesting that the condition may be underdiagnosed. This information provides new insight into the burden of IC/PBS in the United States and will help researchers in the design of future studies to better understand and improve treatment options for people suffering with this condition.

Berry SH, Elliott MN, Suttorp M, et al. Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States. J Urol 186: 540-544, 2011.

DEVELOPING NEW THERAPIES FOR GENETIC BLOOD DISORDERS

Potential Therapy To Limit Iron Overload and Improve Anemia in Beta-thalassemia: By increasing the level of hepcidin in a mouse model of beta (β)-thalessemia, researchers were able to reduce the build-up of iron in tissues and organs and improve anemia. β-thalassemia is a blood disorder that reduces the production of hemoglobin. Hemoglobin is the iron-containing protein in red blood cells that carries oxygen to cells throughout the body. In people with β-thalassemia, low levels of hemoglobin lead to a lack of oxygen in many parts of the body. Many people with β-thalassemia have such severe symptoms that they need frequent blood transfusions to replenish their red blood cell supply. Over time, an influx of iron-containing hemoglobin from chronic blood transfusions can lead to iron overload—a condition that can threaten health by damaging tissues and organs and is the primary cause of death in patients with this condition. In addition, iron overload can also occur in patients with β-thalassemia who are not receiving regular blood transfusions. Unfortunately, the human body does not have a natural way to rid itself of excess iron. Therefore, strategies are needed to reduce excessive iron absorption and tissue iron overload in patients with β-thalassemia.

Building on research findings over the last decade that have clearly established the role of the protein hepcidin in the regulation of iron absorption in the intestine, researchers sought to determine whether a moderate increase in the level of hepcidin would prove beneficial in a mouse model of β-thalassemia. Hepcidin, a hormone produced by the liver, is the master regulator of iron balance in humans and other mammals. Hepcidin inhibits iron transport by binding to the iron channel ferroportin, thereby functionally reducing iron absorption. Indeed, when mice with β-thalassemia were genetically altered to make more hepcidin than usual, they exhibited not only reduced organ iron overload, but also a remarkable improvement of their anemia. These findings led the scientists to suggest that the development of therapeutic interventions that could increase hepcidin levels or act similarly to hepcidin might help reduce excess iron absorption in individuals with β-thalassemia.

Gardenghi S, Ramos P, Marongiu MF, et al. Hepcidin as a therapeutic tool to limit iron overload and improve anemia in β-thalassemic mice. J Clin Invest 120: 4466-4477, 2010.

Modulation of Fetal Hemoglobin Levels—Implications for Red Blood Cell Diseases: Research teams have identified DNA regions on chromosomes 6 and 11 of the human genome that modulate fetal hemoglobin (HbF) levels in the red blood cells of human infants and adults, and have also demonstrated in a mouse model that regulation of this type of hemoglobin can be targeted to achieve a potential treatment for sickle cell disease and other hereditary hemoglobin disorders. People with sickle cell disease, a genetic disorder of hemoglobin (or hemoglobinopathy), suffer from chronic anemia and episodes of bone, joint, and muscle pain, as well as other complications, because their red blood cells form rigid, "sickle" shapes in small blood vessels leading to shortened red blood cell survival and impaired blood flow and oxygen delivery to tissues. Individuals with another genetic disorder of hemoglobin, β-thalassemia, also suffer from chronic anemia caused, in their case, by impaired adult hemoglobin synthesis, which results in reduced numbers and viability of red blood cells. If HbF expression is restored and increased to a sufficient degree, it can compensate for both the defective function of adult hemoglobin in sickle cell disease and the impaired synthesis of adult hemoglobin in thalassemia, thereby ameliorating these clinical

conditions. Although HbF is mostly undetectable in adults and children (after about 6 months of age) in the general population, increased levels persist to varying degrees in some people. Over the past decade genetic variants on chromosomes 2, 6, and 11 have been shown to be most responsible for determining adult levels of HbF, and recent research has advanced further understanding of how HbF levels are regulated in infants and adults.

Scientists recently reported that a small deletion in a region of chromosome 6 may be the most significant functional variant accounting for different levels of HbF in people of Chinese, European, or African American ancestry. This deletion removes a very short stretch of DNA from the chromosome. A DNA fragment surrounding this deletion site was shown to regulate expression of the gene for gamma globin—a component of HbF—when tested *in vitro*. In particular, gamma-globin gene activation was found to be stronger when this short stretch of DNA was deleted than when it was present. This DNA region was also found to serve as a binding site for at least four factors known to be involved in blood cell growth and development. Researchers hypothesize that changes in the normal binding configuration and spatial orientation of these factors could account for the increased enhancer-like activity in the variant associated with increased expression of HbF in red blood cells.

While assessing the distribution and frequency of thalassemia mutations in another population—people from Sri Lanka—a second group of scientists noted significant elevations of HbF in several individuals, and the families of two of these individuals were subsequently studied in detail. The family of a child of Kurdish origin living in the United States, who had been found to have elevated HbF was studied similarly. Hypothesizing that increased HbF levels in these individuals may have resulted from deletions in regions of DNA near the beta-globin gene, which encodes a subunit of the adult form of hemoglobin, the researchers performed an in-depth genetic analysis of DNA sequences in a region of chromosome 11 adjacent to the beta-globin gene in all three families. A novel deletion was detected in DNA near the beta-globin gene and it was determined that DNA sequences in the deletion are necessary for normal silencing of HbF production. Previous work had shown a specific DNA binding protein (BCL11A) binds to DNA sequences normally present in this region of chromosome 11 and acts to decrease HbF levels.

In a third study, scientists translated knowledge gained from research on HbF regulation into the design of a new approach for treating sickle cell disease and thalassemia. Building on previous research demonstrating that the protein BCL11A acts to repress the production of HbF shortly after birth, this same group of investigators used a genetic technique to inactivate *BCL11A* gene expression in mice with sickle cell disease, thereby blocking production of BCL11A protein. In the absence of the BCL11A protein, these mice were found to have persistent and increased levels of HbF after birth, preventing the development of the hematologic and pathologic abnormalities of sickle cell disease in the mice. Thus, interference with normal HbF silencing by genetic elimination of a single DNA binding protein (BCL11A) was found to be sufficient to prevent sickle cell disease.

These research studies have advanced understanding of how HbF levels are modulated after infancy and point the way to possible new genetically targeted approaches to treat children and adults with sickle cell disease or β-thalassemia by raising HbF levels.

Farrell JJ, Sherva RM, Chen ZY, et al. A 3-bp deletion in the HBS1L-MYB intergenic region on chromosome 6q23 is associated with HbF expression. Blood 117: 4935-4945, 2011.

Sankaran VG, Xu J, Byron R, et al. A functional element necessary for fetal hemoglobin silencing. N Engl J Med 365: 807-814, 2011.

Xu J, Peng C, Sankaran VG, et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. Science 334: 993-996, 2011.

Gene Variant Increases Risk of Kidney Disease in African Americans—Disease Tends To Strike Earlier, Progress More Quickly

African Americans with two copies of certain variants in the *APOL1* gene are at increased risk of developing kidney disease, particularly focal segmental glomerulosclerosis (FSGS) and kidney disease related to infection with the human immunodeficiency virus (HIV). This finding comes from collaborative research led by Dr. Jeffrey Kopp of the NIDDK Intramural Research Program and Dr. Cheryl Winkler of the National Cancer Institute (NCI); additional investigators in the United States and Europe were part of the research team. The scientists studied people with kidney disease who came to the NIH Clinical Center or other collaborating medical centers and provided blood samples for genetic studies. This discovery is an important step towards understanding why African Americans are four times more likely to develop kidney failure than people of European ancestry.

Human cells typically have two copies of each gene—one inherited from each parent. African Americans with no normal copies of the *APOL1* gene, but instead two variant copies, have about a 4 percent lifetime risk of developing FSGS. Those who develop this disease tend to do so at younger ages than other FSGS patients, with 70 percent diagnosed between the ages of 15 and 39, compared to 42 percent in that age group for people with one or no *APOL1* variants. FSGS patients with two *APOL1* variants respond as well to steroid treatments, the therapy with the best chance of inducing a partial or complete remission of the disease, as people without the variants. However, the scientists found that the disease progresses more rapidly to kidney failure in patients with two *APOL1* variants. Among African Americans who are HIV-positive, but not receiving anti-viral therapy, possessing two *APOL1* variants raises the risk of developing HIV-associated kidney disease to 50 percent. (Anti-viral therapy appears fairly effective at preventing HIV-associated kidney disease.)

"These findings explain nearly all of the excess risk of non-diabetic kidney failure in African Americans. African Americans with no variant or one variant have about the same risk of end-stage kidney disease as their white counterparts," Dr. Winkler said. "People with two *APOL1* variants have greatly increased risk of particular kidney diseases—by 17- to 30-fold."

The persistence of *APOL1* variants in people of African descent may be partly explained by the ability of the APOL1 protein (which is encoded by the *APOL1* gene) to destroy the parasite *Trypanosoma brucei bruce* (*T. b. bruce*), which causes disease in a broad range of mammals but is unable to infect humans because it is destroyed by the normal APOL1 protein. Two related parasites, *T. b. rhodensiense* and *T. b. gambiense*, have evolved independent mechanisms to avoid destruction by normal APOL1. These parasites cause African sleeping sickness, a hematologic and neurological disease spread by the tsetse fly that kills thousands of people in sub-Saharan Africa each year. However, people with at least one copy of a variant APOL1 protein are protected against infection because both are able to destroy *T. b. rhodensiense* and *T. b. gambiense*. These two APOL1 variants appear to have evolved relatively recently—in the past 10,000 years or so. Their relatively recent appearance and high frequency in individuals of African descent suggest that they provide significant protection against parasitic infection.

Drs. Kopp, Winkler, and other researchers had previously found that risk of some forms of kidney disease was due to variants in the *APOL1* gene. The most recent research builds on these earlier findings by further characterizing and quantifying the magnitude of the effect of these *APOL1* variants in kidney disease. Still, important research questions remain. The protein encoded by the *APOL1* gene, apolipoprotein L1, is a component of so-called "good" cholesterol that is also expressed in the kidney. The mechanism by which *APOL1* variants cause kidney disease remains unknown. It is unclear whether circulating APOL1, kidney-expressed APOL1, or both contribute to kidney injury. Further research will likely be required to determine conclusively whether the *APOL1* variants are a causative agent in kidney disease.

It should be noted that most people with two *APOL1* variants do not develop kidney disease. Indeed, the much higher risk of kidney disease in patients with HIV suggests that a second triggering event or "hit," either with a virus or other factor, contributes to kidney injury in people who have two *APOL1* variants. Nevertheless, the observed increased risks of FSGS and HIV-associated kidney disease are the strongest effects yet discovered for common variants in a complex disease.

These findings have important implications for understanding the differences in kidney disease risk across populations. "In the future, knowing that you have these gene variants and are at increased risk of developing kidney disease may tell you when to start screening for the disease and how to choose therapy," Dr. Kopp said. "However, more research is needed, including clinical trials that test whether early genetic testing in the African American population makes a difference, whether screening tests for young adults with the variant copies detects kidney disease at an early stage, and whether early treatment affects long-term outcome."

Kopp JB, Nelson GW, Sampath K, et al. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. J Am Soc Nephrol 22: 2129-2137, 2011.

Genovese G, Friedman DJ, Ross MD, et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science 329: 841-845, 2010.

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For more information about the role of *APOL1* variants in kidney disease, see the Scientific Presentation in this chapter.

National Kidney Disease Education Program's Chronic Kidney Disease Diet Initiative

An estimated 23 million Americans may have chronic kidney disease (CKD)[1] and, according to the NIDDK-supported U.S. Renal Data System, over 550,000 patients are either on kidney dialysis or living with a kidney transplant.[2] Patients with CKD are at increased risk for kidney failure. It is estimated that treating the number of people with kidney failure, also called end-stage renal disease (ESRD), through dialysis or kidney transplantation costs U.S. taxpayers approximately $29 billion each year. ESRD is an enormous public health problem; prevalence rates increase with age (median age of 59.4 years), and the disease disproportionately affects minority populations.

While generally considered a specialist's disease, early CKD can be managed in the primary care setting and integrated into existing care for patients with diabetes and hypertension. However, CKD remains poorly managed, in part because clinicians, including general practice dietitians, feel inadequately educated. Surprisingly, less than 1 percent of physicians prescribe Medicare-covered medical nutrition therapy provided by a general practice dietitian for individuals with diabetes or kidney disease.[3]

The National Kidney Disease Education Program (NKDEP) developed the CKD Diet Initiative to improve outcomes for people with CKD. The CKD Diet Initiative aims to provide simplified and accessible professional and patient education materials, to train general practice dietitians to counsel people with CKD, and to facilitate referrals from primary care physicians for CKD medical nutrition therapy. Free, full-text, downloadable, reproducible materials have been designed to provide key information about CKD and diet for registered dietitians (*www.nkdep.nih.gov/professionals/ckd-nutrition.htm*). The NKDEP has also developed training materials which the American Dietetic Association has adapted as an online Certificate of Training in CKD for registered dietitians. The comprehensive evidence-based online program was launched in November 2011 and includes five modules covering assessment, disease progression, complications, dietary counseling, and an introduction to dialysis including changes in the diet. The program incorporates NKDEP patient materials and advice on using them in actual clinical practice. Learning occurs through interactive activities, case studies, clear graphics, and assessments. All materials developed are in the public domain and will be available on the NKDEP web-site to health professionals to develop their own trainings. In the coming months, additional interactive case studies will be developed for use by educators of dietetic students and interns, and for use by other primary care providers. The Initiative will also educate primary care physicians on the importance of CKD medical nutrition therapy and making referrals to registered dietitians when appropriate.

More information about the NIDDK's National Kidney Disease Education Program can be found at *http://nkdep.nih.gov*

More information about medical nutrition therapy coverage under Medicare can be found at *www.medicare.gov/navigation/manage-your-health/preventive-services/medical-nutrition-therapy.aspx*

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STORY OF DISCOVERY

The Search for Immune Self-targets in a Form of Kidney Disease

Membranous nephropathy is the second-leading cause of a serious kidney condition known as the nephrotic syndrome. It is believed to be an autoimmune disease, arising when the body's immune system mistakenly attacks components of the body as opposed to foreign invaders, such as viruses or bacteria. The target of an autoimmune attack is termed an autoantigen, and finding the autoantigen is a key discovery in understanding the disease process. Researchers have recently identified a protein that may be the trigger for the autoimmune attack that results in membranous nephropathy. The identification of the protein that induces this immune response builds on knowledge accumulated over the past half-century, and may open new avenues of exploration in membranous nephropathy including new treatment approaches.

The Kidneys, Nephrotic Syndrome, and Membranous Nephropathy

The kidneys are two bean-shaped organs, each about the size of a fist, that are located near the middle of the back, just below the rib cage on each side of the spine. Blood enters the kidneys through arteries that branch and sub-branch into tiny clusters of looping blood vessels. Each cluster is called a "glomerulus," which is derived from the Greek word meaning "filter." Each glomerulus is contained within a capsule of kidney cells, and together they represent a single, tiny unit that filters the blood. There are approximately one million glomeruli in each kidney. As the heart pumps approximately 200 quarts of blood through the kidneys each day, these filtering units remove about two quarts of waste products, salts, and excess water that will eventually leave the body as urine.

"Nephrotic syndrome" is a general term used to describe a cluster of symptoms that includes an abnormal amount of protein in the urine (termed "proteinuria"), low blood protein levels, high cholesterol levels, high triglyceride levels, and swelling of the body due to fluid retention. Nephrotic syndrome is not a disease in and of itself; rather, it is a physical manifestation of an underlying kidney disease. Therefore, treatment of nephrotic syndrome relies on controlling the disease that is causing it.

Membranous nephropathy is the second most common cause of the nephrotic syndrome in American adults. (The most common cause is diabetic kidney disease.) Membranous nephropathy is associated with unusual deposits in the glomeruli of antibodies and other proteins that are part of the body's immune system. It is, therefore, generally considered an autoimmune disease. Seventy-five percent of cases are idiopathic, which means that the cause is unknown; the remaining 25 percent of cases are the result of other diseases such as lupus, hepatitis B or C infection, or some forms of cancer.

About 20 to 40 percent of patients with membranous nephropathy progress—slowly, usually over decades—to kidney failure; these patients require dialysis, which replaces their lost kidney function, to live. Most patients with membranous nephropathy, however, either endure continued symptoms without progressing to kidney failure or, in some cases, experience complete recovery. Because there is no way to predict a particular patient's prognosis, doctors disagree about how aggressively to treat patients with this condition. Drugs that target the renin-angiotensin system—which are generally used to treat elevated blood pressure—can reduce proteinuria. Some, but not all, patients with membranous nephropathy benefit from steroids, which can modulate the autoimmune response. Other drugs that can suppress the immune system are helpful for some patients with progressive disease, but not others. In the end, there is no one-size-fits-all treatment approach to patients with membranous nephropathy.

STORY OF DISCOVERY

Of Rats and Men: The Search for the Membranous Nephropathy Autoantigen

In 1959, NIH-funded[1] researcher Dr. Walter Heymann and his colleagues at the Western Reserve University School of Medicine in Cleveland, Ohio, created the first animal model of membranous nephropathy. Their experiments showed that injection of crude preparations of puréed whole rat kidneys—but not similarly prepared extracts from other tissues—along with an immune-boosting compound could produce severe nephrotic syndrome in rats that closely resembled membranous nephropathy.[2] The disease induced by this procedure was termed "Heymann nephritis." It was thought that some factor in these crude kidney extracts somehow triggered the rats' immune systems and provoked a response, although the identity of the specific molecules or factors responsible for this autoimmune response would remain unknown for many years. Nevertheless, this animal model became the basis for research into human membranous nephropathy for the next several decades, and much of what scientists know about the disease process in human membranous nephropathy has come from studies of Heymann nephritis in rats.

It was not until 1982 that researchers funded by the NIDDK (then known as the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases) isolated and identified a potential target for the autoimmune response in the rat model of Heymann nephritis. That year, scientists reported purifying a single protein from rat kidneys that, when injected back into rats, produced the same symptoms that Dr. Heymann had observed more than 20 years earlier.[3] This protein, which the scientists termed "megalin," is a relatively large, cell membrane-spanning molecule. This model has been used to elucidate many important aspects of the disease process that leads to membranous nephropathy. Studies have demonstrated that anti-megalin antibodies create aggregates of the protein in the glomeruli and that they activate a component of the immune response known as the complement system.

While the Heymann nephritis model of membranous nephropathy has been extremely useful in allowing scientists to investigate the process of immune deposit formation and the mechanism by which such deposits cause kidney injury in rats, it also highlights important limitations of this animal model. In this case, because megalin is not found in human glomeruli (although it is present in other cell types within the kidney), some have questioned the relevance of this model to human disease. The search for the autoimmune trigger in human membranous nephropathy would continue.

The Identification of the Human Autoimmune Antigen

In 2009—50 years after Dr. Heymann developed the rat model of membranous nephropathy—an international team of researchers, supported in part with NIDDK funds, identified a putative autoantigen responsible for this disease in humans. In 70 percent of blood samples from patients with membranous nephropathy (26 of 37), self-reactive antibodies bound to a single protein in kidney extracts that was ultimately determined to be the M-type phospholipase A2 receptor or PLA_2R. Unlike megalin, this protein is expressed by cells in human glomeruli. The subtype of antibody that reacted with PLA_2R in the assay is the same kind that is found in immune deposits within the glomeruli in patients with membranous nephropathy. Antibodies isolated from glomeruli of patients with idiopathic membranous nephropathy react with PLA_2R, whereas antibodies isolated from the glomeruli of patients with other forms of nephrotic syndrome do not. Furthermore, autoantibodies against PLA_2R can be detected in the blood in patients with clinically significant disease, and levels of these antibodies decline or disappear during remission.[4]

In a subsequent study, published in 2011, NIH- and industry-funded scientists reported that treatment of patients with the drug rituximab, which destroys a subclass of immune cells thought to be important in membranous nephropathy, resulted in a decrease

in anti-PLA_2R autoantibodies. These autoantibody changes also correlated with a decrease in proteinuria in response to the drug.[5] Using serum samples from a previous study of rituximab in the treatment of membranous nephropathy, the researchers measured levels of anti-PLA_2R antibodies. Of 35 samples that were evaluated, 25 had anti-PLA_2R antibodies. Treatment with rituximab resulted in a significant decline or disappearance of these autoantibodies in 68 percent of patients (17 of 25). Those patients in whom anti-PLA_2R antibodies fell following rituximab treatment had a better clinical response than those whose antibodies did not decrease: at 12 and 24 months after beginning therapy, 59 and 88 percent, respectively, showed partial or complete remission of protein in their urine. In those patients whose PLA_2R antibodies did not diminish in response to therapy, none showed a decrease in urine protein at 12 months, and only a third did after 24 months. In the subset of patients who did respond to rituximab, the decrease in anti-PLA_2R antibodies preceded the reduction of proteinuria. This study suggests that, going forward, measuring anti-PLA_2R antibodies may be a method to predict a particular patient's response to treatment with rituximab. It also illustrates that biological samples stored as part of one trial can yield further valuable information in future analyses.

Looking Forward

Over 50 years after the introduction of the first animal model of membranous nephropathy, researchers are still charting new courses in our understanding of this disease. The recent discovery of the likely human autoantigen responsible for the majority of membranous nephropathy cases is an important milestone. Moving ahead, the generation of animal models of membranous nephropathy that express human PLA_2R in their glomeruli should allow for an even better understanding of the details of the disease process. Much of this progress has been made possible by funding from the NIH.

By advancing understanding of the basic biology of membranous nephropathy, these new findings will likely have important implications for patient care. For example, they may permit the noninvasive diagnosis of membranous nephropathy, predict which patients are likely to respond to a particular therapy, and provide an easier way to follow the disease in response to treatment. Better understanding of the potential triggers of autoantibody production in patients with a susceptibility to idiopathic membranous nephropathy may also uncover possible new targets for preventing or treating this disease.

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Genetic Factors in Chronic Kidney Disease

Dr. John Sedor

Dr. John Sedor is a Professor of Medicine and Physiology at Case Western Reserve University in Cleveland, Ohio. He is also the Vice President for Research on the MetroHealth System Campus at Case Western. His research interests span basic and clinical studies of the kidney, with a particular focus on understanding genetic mechanisms and progressive kidney disease, including kidney disease arising from diabetes.

Dr. Sedor earned his M.D. from the University of Virginia in 1978. He went on to complete his residency in internal medicine and a fellowship focusing on kidney disease at University Hospitals, Case Western Reserve University, where he also began a research career that continues to this day. He was a participating investigator in the NIDDK's Family Investigation of Nephropathy of Diabetes (FIND) Consortium. From 1998 to 2003, Dr. Sedor was the Director of the NIDDK's George M. O'Brien Renal Research Center at Case Western. At the May 2011 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Sedor told his fellow Council members and other attendees of recent advances in understanding the genetics of chronic kidney disease.

Early-stage kidney disease often has no symptoms. Left unchecked, however, it can silently progress to kidney failure, a condition in which the kidneys are no longer able to filter waste and excess fluids from the blood. It is estimated that more than 20 million U.S. adults over the age of 20 have some degree of impaired kidney function,[1] and over a half million Americans were receiving life-sustaining kidney dialysis or were living with a kidney transplant at the end of 2009.[2] Despite recent advances in preserving kidney function in individuals with early-stage kidney disease, serious health complications are common. In fact, roughly half of the people with kidney disease will die from cardiovascular disease before their kidney function further deteriorates and they progress to kidney failure.[3]

Like many diseases, chronic kidney disease arises from both genetic and environmental factors. The two most common causes of kidney failure are diabetes and hypertension (high blood pressure), which together account for about 70 percent of all new cases.[2] Both conditions are seen more frequently in members of ethnic minorities, and African Americans bear an especially heavy burden of kidney disease. African Americans are over three times as likely as whites to develop kidney failure.[2] Not everyone with diabetes and/or hypertension will develop kidney disease, however, and researchers are only beginning to discover the factors that put some people at higher risk than others.

The Intersection of Genes and Environment

Physicians have long known that kidney disease tends to run in families and cluster in ethnic groups, meaning that it most likely has a genetic component. Of course, environmental factors play a role in disease susceptibility as well. To illustrate the complex ways in which these two factors can interact to produce different outcomes, Dr. Sedor shared the story of a former colleague. This woman developed type 1 diabetes as a child, but has been spared many of its complications during her adult life. In contrast, her brother, who developed type 1 diabetes at around the same age, has struggled with many of the disease's complications, including kidney, eye, and cardiovascular diseases. Another brother developed diabetes as an adult, in his 30s. A third brother remains free of diabetes to this day. This story of four siblings—all from the same family

(sharing much of their genetic background), growing up in the same home (sharing a similar environment), yet with four very different outcomes—illustrates the complex way in which interactions between genetic and environmental factors influences risk of diabetes and its complications. If there were a way to predict who among the four would develop diabetes, and among them who would develop complications, then treatment strategies could be tailored to each person much earlier, either ameliorating disease symptoms or possibly preventing the development of diabetes or its complications altogether. Such knowledge could spare some people from unnecessary treatment (because they were unlikely to either develop diabetes or experience complications) and facilitate more aggressive treatment of others (who were more likely to develop diabetes and its complications).

FINDing a Clue to the Genetics of Kidney Disease

The Family Investigation of Nephropathy and Diabetes (FIND) Consortium collected genetic material from participants with type 1 or type 2 diabetes. Initiated in 2000, the overall goal of FIND was to identify genetic pathways that may be critical for the development of kidney disease (nephropathy) and lead to potential therapeutic strategies to prevent the onset or progression of diabetic kidney disease. It was one of the largest family-based studies for diabetic nephropathy, with about 3,900 participants from 1,200 families involved. Researchers collected genetic samples from individuals of four different ancestries: African American, American Indian, Caucasian, and Mexican American. Most volunteers had severe kidney disease, and many were undergoing dialysis.

FIND investigators used a technique called "admixture mapping" to look for genetic variations that seemed linked to chronic kidney disease. Admixture mapping is particularly useful in examining the underlying genetic causes of complex diseases in which the frequency of disease is very different between two populations of different ancestries. This technique takes advantage of the fact that genetic variants that are not linked to one another tend to dissociate from one another rather rapidly—within a few generations—while those that are linked tend to stay together longer. Because of the striking difference in kidney disease and kidney failure rates between whites, who are largely of European ancestry, and African Americans, researchers had speculated that admixture mapping might be an effective way to try to identify which chromosomal regions are associated with the development of kidney disease.

In 2008, members of the FIND Consortium, along with scientists in the NIDDK's Intramural Research Program, reported that genetic variations on chromosome 22 were linked to greater incidence of non-diabetic kidney disease among African Americans.[4,5] Initially, attention focused on the region surrounding the *MYH9* gene. Further analyses revealed that much of the increased risk of kidney disease is actually due to two specific variations in the adjacent *APOL1* gene, which encodes the protein apolipoprotein L1, a minor component of so-called "good" cholesterol that is found circulating in the blood and in kidney cells. Two specific variants of this gene have been shown to account for nearly all of the excess risk of kidney failure arising from causes other than diabetes in African Americans. People with two copies of the variant *APOL1* genes are at greatly increased risk of developing the kidney disease focal segmental glomerulosclerosis (FSGS) and kidney disease associated with infection by the human immunodeficiency virus (HIV).[6]

The APOL1 Protein is Present in Different Cells in Normal and Diseased Kidneys

Dr. Sedor described some ongoing studies of the APOL1 protein that examined both the amount of APOL1 found in the kidneys as well as the types of cells that expressed the protein.[7] In the kidney, the basic structural and functional unit is the glomerulus—a collection of looped blood vessels surrounded by specialized cells—that filters waste products, salts, and excess fluid from the blood.

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Material filtered by the glomerulus drains into a proximal tubule, which is in turn connected to a system of collecting ducts that ultimately leads to the bladder. In many forms of kidney disease the glomerulus is damaged, leading to impaired filtering of waste and leakage of protein from the bloodstream into the urine.

Dr. Sedor described his research team's study of APOL1 protein levels in samples of normal kidney tissue and tissue from eight patients with FSGS and two patients with HIV-associated kidney disease. In normal kidneys, the APOL1 protein was found in specialized cells within the glomerulus called podocytes (literally, "cells with feet"), in cells of the proximal tubule, and in the arteries leading to the glomerulus. In samples from patients with FSGS or HIV-associated kidney disease, APOL1 was also observed in podocytes, proximal tubule cells, and arterial cells; however, fewer podocytes had APOL1 protein compared to normal kidneys. Additionally, in tissue from patients with kidney disease, APOL1 was detected in a subset of smooth muscle cells that surround the arteries leading to the glomerulus. This pattern of distribution of APOL1 protein was not found in normal tissue samples.

Little is known about the biological role of APOL1 in these various cell types in the kidney. The presence of APOL1 in samples of normal kidney tissue indicates that the protein may play some role in this organ. Its presence in arterial vessel walls in patients with kidney disease, but not in normal kidney tissue, suggests that a previously unrecognized problem with blood vessels may play an important role in FSGS and HIV-associated kidney disease. Dr. Sedor cautioned that these conclusions were tentative, given the relatively small number of tissue samples examined. Furthermore, it is not clear whether the changes in APOL1 presence and localization observed in the studies represent changes in whether the *APOL1* gene is turned on in the various cell types within the kidney or changes in uptake of cholesterol-associated APOL1 circulating in the blood. Nevertheless, the possibility that changes in blood vessels in the glomerulus might be involved in FSGS and HIV-associated kidney disease could provide important clues about the role of APOL1 in kidney function and disease.

Future Directions

Because there is no way to restore kidney function once it is lost, current approaches to therapy for chronic kidney disease are aimed at preserving existing kidney function and addressing the underlying health problem causing the kidney disease, not at addressing specific processes that damage the kidneys. The discovery that variations in *APOL1* confer susceptibility to kidney failure in African Americans provides an important clue in our understanding of disease mechanisms and may allow novel approaches to prevention and treatment of kidney disease in this population. The more recent data on the cellular distribution of APOL1 in the kidney and the changes in this pattern in diseased kidneys will help guide future studies. Given the high frequency of these *APOL1* variants in people of African descent and their strong effect on kidney disease risk, unraveling the molecular mechanisms by which they contribute to kidney injury could provide key insights into the causes of and possible treatments for kidney disease in African Americans, and further our understanding of the role of genetics in kidney disease in general.

The Family Investigation of Nephropathy and Diabetes (FIND) Consortium was led by the NIDDK with additional support from the National Eye Institute and the National Center on Minority Health and Health Disparities (now the National Institute on Minority Health and Health Disparities). The overall goal of FIND was to identify genetic pathways that may be critical for the development of nephropathy and thereby identify candidates that might be amenable to therapeutic strategies to prevent the onset or progression of kidney disease.

SCIENTIFIC PRESENTATION

NIDDK-supported George M. O'Brien Kidney Research Centers conduct interdisciplinary investigations that address basic, clinical, and applied aspects of biomedical research in renal and genitourinary structure, function, development, and disease. Areas of focus include kidney diseases arising from hypertension and diabetes, renal and urinary tract dysfunction, immune- and nonimmune-related mechanisms of kidney injury and kidney disease, kidney toxins, and cell injury.

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PATIENT PROFILE

John Saul

Acute Kidney Injury and One Person's Commitment to Helping Others

John Saul
Photo credit: Mary Jo Peairs

Sixty-three-year-old journalism professor John Saul is currently enrolled in an NIDDK-supported observational study called the Assessment, Serial Evaluation, and Subsequent Sequelae in Acute Kidney Injury, or ASSESS-AKI study. He was asked, and agreed, to participate in the study shortly after having had surgery to remove a benign tumor from behind his right eye.

In 2008, John discovered he had a benign tumor that was pushing on his right eyeball. As he describes it, "The tumor was more sight-threatening than life-threatening," and he decided to put off having the operation for as long as possible. But in 2010, John began experiencing double vision and decided it was time to act. On March 29, 2011, he had surgery to remove the tumor that had been growing out of the covering of his brain and affecting the vision in his right eye. After the surgery, he spent 2 weeks in the hospital and says the double vision went away within 2 months. John, at the time of his hospitalization, had a mild case of AKI. AKI (also called "acute renal failure") is a serious medical condition characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days, and more information about this condition can be found below under the "About AKI" heading.

Prior to his enrollment in the ASSESS-AKI study, John says he had no knowledge of AKI, which researchers say is not surprising. Most people do not know what AKI is. John recalls that, in 1990, he entered the hospital to remedy an old knee injury. "I went in to have surgery on my anterior cruciate ligament (ACL)," he says. But after the surgery, John experienced a pulmonary embolism (a blockage in a lung artery usually caused by a blood clot that travels to the lung from a vein in the leg) and he spent approximately 2 weeks on kidney dialysis, after which, his kidneys were able to resume functioning on their own. In 1990, the term "acute kidney injury" was not widely used by physicians; more common were phrases such as "your kidneys are not working well" or "your kidneys are not working normally." Although it is not known for sure, it would appear that John encountered his first AKI episode during his hospitalization for the ACL surgery.

Researchers are concerned by the fact that the long-term health consequences of AKI are not well understood for those who survive their episodes of the condition.

PATIENT PROFILE

About AKI

This medical condition can be repetitive (multiple episodes common) and can complicate or lead to chronic kidney disease (CKD). The resulting inability to excrete nitrogenous waste products and maintain fluid and electrolyte balance poses urgent health problems for patients and their physicians. AKI may arise from a number of causes, most commonly sepsis (a serious, whole-body inflammatory reaction caused by infection), decreased blood pressure, or kidney damage from certain drugs or other toxins. It is a relatively common complication among hospitalized patients, affecting between 1 and 15 percent of these patients. Even though a significant portion of patients with AKI will regain kidney function, many do not, and this medical condition is associated with high in-hospital mortality rates ranging from 50 to 80 percent among the critically ill. Equally concerning to researchers is the fact that the long-term health consequences of AKI are not well understood for those who survive their episodes of the condition. However, in one recent study, 20 percent of patients with AKI, who previously had normal kidney function, developed stage 4 CKD. Such patients can progress to higher CKD stages, which might eventually require treatment by dialysis or transplantation.

There is no effective drug therapy to reverse AKI. The current goal of treatment is to prevent fluid and waste from building up in the body while waiting for the kidneys to resume functioning. Treatment involves hemodialysis and other forms of life-sustaining therapy to replace lost kidney function. Dialysis removes waste products from the blood, and it also helps control blood pressure and maintains the proper electrolyte balance. Although dialysis has been used to treat AKI for over 60 years, it is still not clear when it is best to initiate therapy, which method of dialysis is best to use, and what dose of dialysis to deliver.

Experts who study kidney disease (nephrologists) now believe that the field of AKI research is poised for progress over the next decade, and observational study participants, like John, are integral to that progress.

There is no effective drug therapy to reverse AKI.

The ASSESS-AKI Study

ASSESS-AKI is a national research study that will follow John's health status, and that of 1,600 other people—with and without AKI—for the next 5 years. The purpose of the study is to determine what effect AKI has on a person's long-term health outcomes, including kidney function. Researchers are hoping to evaluate the utility of certain biological molecules found in urine (*e.g.*, IL-18) and blood (*e.g.*, serum cystatin C), to see whether these could serve as biomarkers—tools for detection or monitoring of health conditions—to assist with the early diagnosis of AKI and, after an episode of AKI, provide both short-term and long-term information on the health of the kidney and the patient. In addition, there is a need for new molecular and genetic markers of risk for poor outcomes in AKI to identify patients who might benefit from more aggressive care or new therapies. Therefore, using genome-wide association studies, researchers seek to identify genetic variants that confer susceptibility to the development of AKI, increase chances of recovery of kidney function after the development of AKI, or heighten long-term risk for development and progression of chronic kidney disease in survivors of AKI.

John's participation in the ASSESS-AKI study, and the participation of others like him, is extremely important in developing a prognostic risk score that will integrate a person's health characteristics and biomarkers to help inform providers and patients about the long-term risks after an episode of AKI.

"I'm all in favor of any kind of study that will help other people," John says. And he continues to maintain that commitment.

PATIENT PROFILE

"This study is very well worth my time. I feel like I'm making a contribution to science, and I'm glad my experience will help other people."

At the time of his enrollment in the ASSESS-AKI study, John was asked general background questions, including his age and medical history. Information about his surgery, laboratory results, medications, and daily urine output were collected from his hospital medical records.

Six months after being discharged from the hospital for his head surgery, John attended the ASSESS-AKI study clinic for the first of several follow-up visits. During the visit, blood samples were drawn, and John was asked to provide a urine sample to determine if any long-term kidney damage may have occurred since his discharge from the hospital. His blood pressure was measured, and his height and weight were recorded. He also was interviewed and asked to fill out questionnaires regarding his quality of life, family history, and list whatever medications he is taking.

"It was all very painless," says John of the first of what is expected to be a series of follow-up visits. Participants in this study attend follow-up visits every 3 months, with each visit lasting about 2 hours. But as John attests, "This study is very well worth my time. I feel like I'm making a contribution to science, and I'm glad my experience will help other people."

The ASSESS-AKI study is being conducted at four NIDDK-funded clinical centers: Kaiser Foundation Research Institute (Drs. Alan Go and Chi-yuan Hsu); Vanderbilt University Medical Center (Dr. Talat Ikizler); Yale University (Dr. Chirag Parikh); and University of Washington (Drs. Jonathan Himmelfarb and Mark Wurfel). The Data Coordinating Center is located at Pennsylvania State University (Dr. Vernon Chinchilli). Dr. John Stokes of the University of Iowa serves as the study chair. The trial is expected to report results near the end of 2013.

ACKNOWLEDGEMENTS

Printed February 2012

Research

The NIDDK gratefully acknowledges the contributions of the researchers whose studies are described in this report, and of the patients who participate in clinical research studies.

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The research advance “Communication and Protein ‘Fences’ Make Good Neighbors of Gut and Bacteria” includes information from *NIH Research Matters*. The original article by Vicki Conte was published October 24, 2011.

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NIH Publication Number: 12-7808