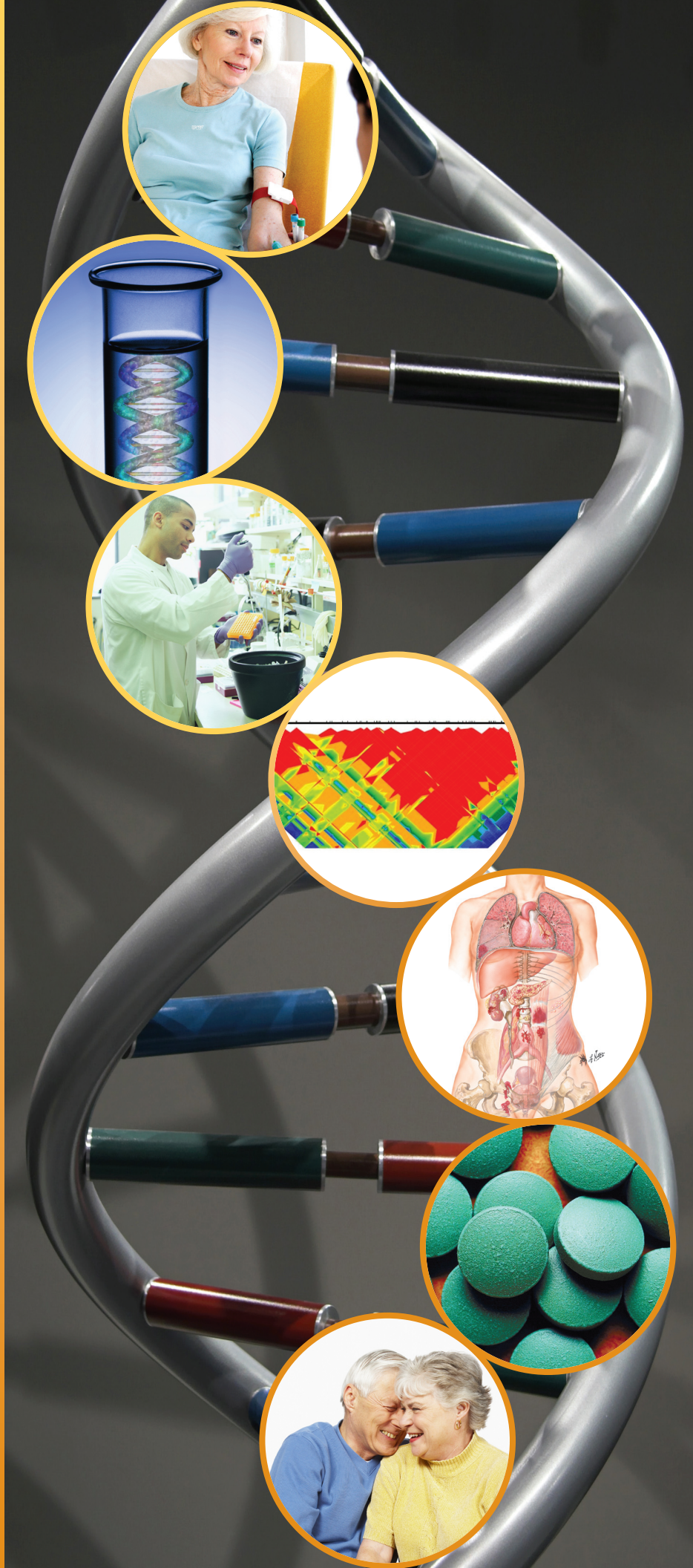
# NIDDK

## Recent Advances & Emerging Opportunities

February 2009



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

**The cover images illustrate the "bedside to bench to bedside" approach NIDDK is taking to build on recent breakthroughs in genetics research toward a goal of improving human health. Flowing from top to bottom, against the backdrop of a DNA double helix, the inset images (circles) represent specific steps or stages in this approach: The NIDDK supports clinical, or "bedside," research to study the genes of people with diseases and disorders within the Institute's mission (represented by patient in top circle). New research tools (second circle) are making it easier for scientists at the laboratory "bench" (third circle) to identify genes that influence a person's likelihood of developing common diseases. As described in this compendium, using genome-wide association studies (fourth circle) and other genetic approaches, new genes have been discovered that are associated with a multitude of diseases within the NIDDK mission, including type 1 diabetes, type 2 diabetes, kidney disease, and Crohn's disease. The NIDDK is building on this exciting new knowledge and is supporting research to understand how genes function in the body under normal conditions, and what goes awry in disease (fifth circle). This greater understanding could translate into new opportunities to return to the patient's bedside to test new prevention and treatment approaches (sixth circle), with the ultimate goal of improving health and quality of life (bottom circle).**

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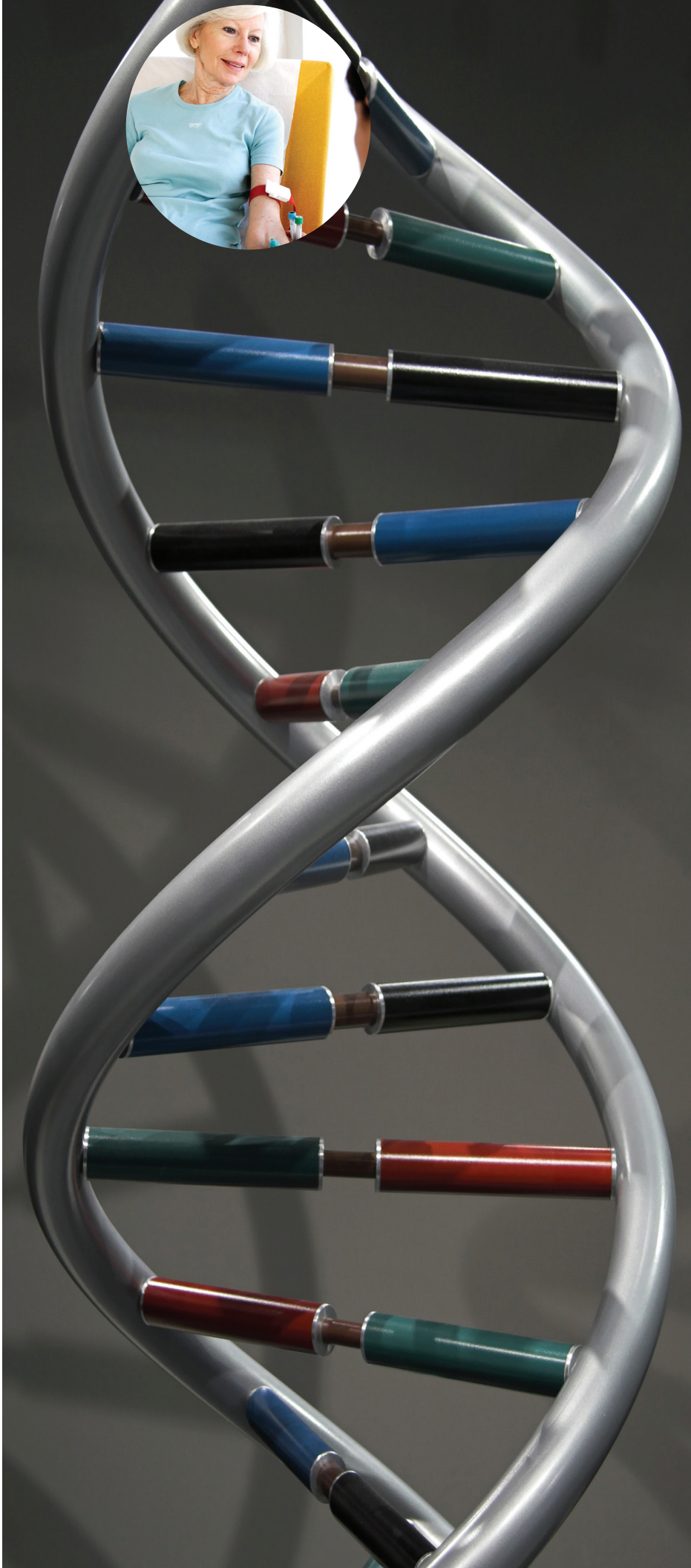

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Digestive & Kidney Diseases

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# Message from the Director

As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual 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, such as interstitial cystitis and prostate disease; and hematologic diseases, such as Cooley's anemia.

Now in its ninth year, this compendium illustrates recent NIDDK-supported scientific advances, such as the:

- Discovery of a novel group of adult pancreatic progenitor cells that generate insulin-producing beta cells.
- Revelation that gut bacteria may protect against development of type 1 diabetes.
- Identification of additional genetic variants associated with risk of developing type 2 diabetes.
- Demonstration that a protein, menin, may be involved in the development of gestational diabetes mellitus.
- Finding of a defective biological pathway that may contribute to IgA nephropathy, a relatively common form of kidney disease.
- Discovery of how *H. pylori* interacts with stomach stem cells to influence disease progression that can lead to stomach cancer.
- Demonstration that more intensive renal dialysis in patients with acute kidney injury does not improve outcomes.
- Revelation that a protein, BMP-7, contributes to the formation of "brown fat," which may lead to a novel strategy for counteracting obesity.
- Discovery of genetic variation near the *MHY9* locus as conferring greatly increased risk in African Americans for both non-diabetic end-stage renal disease and focal segmental glomerulosclerosis.

This compendium also includes stories of patients who are participating in—and thus helping to advance—NIDDK-supported research. An adolescent girl is participating in a clinical trial to determine whether an experimental drug may slow the progression of type 1 diabetes. A woman is enrolled in a YMCA-based diabetes prevention program designed to help people with risk factors for type 2 diabetes lose weight and increase physical activity. Another adolescent girl is participating in an observational clinical study to help determine whether bariatric surgery is an appropriate treatment option for extremely overweight teens. A man is enrolled in a clinical trial that tested the impact of anti-clotting reagents in preventing early failure in "vascular access," which is required for dialysis.

The NIDDK continues its efforts to ensure that knowledge gained from its major research advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's educational programs, such as the National Diabetes Education Program and the National Kidney Disease Education Program. The

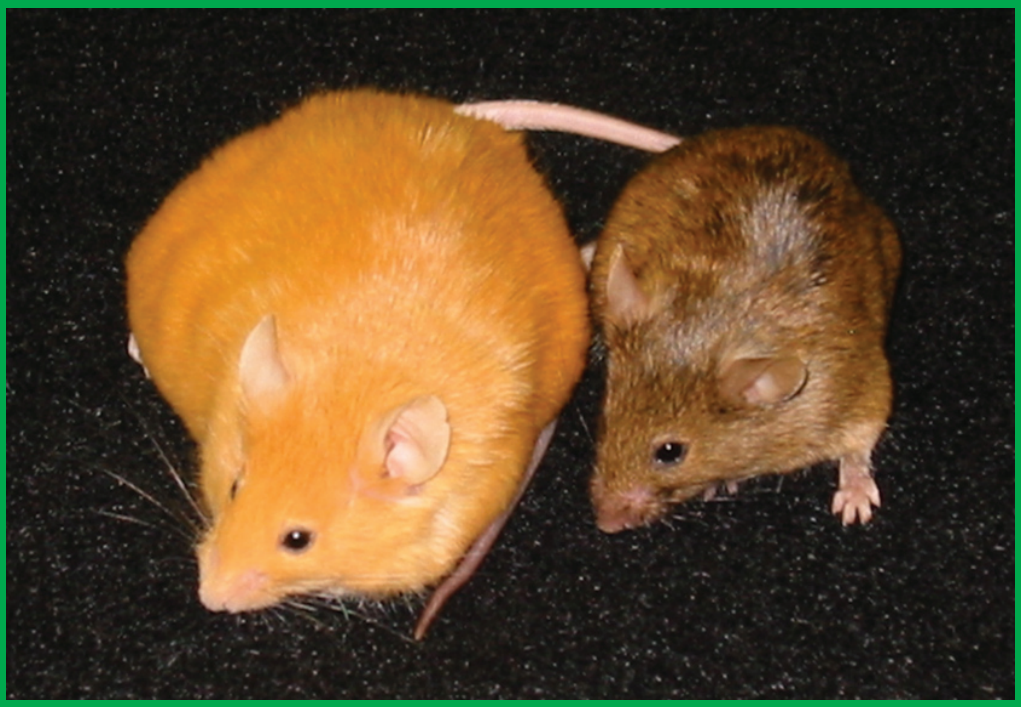
Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available in printed copy and on the NIDDK website so that they are readily available for patients, health care providers, and the public. I invite you to visit the website at: *www.niddk.nih.gov*

This compendium reflects only a fraction of the immense body of work performed by basic scientists, clinical researchers, 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
Department of Health and Human Services

The materials featured in this publication reflect the core mission of the NIDDK, including the Director's following 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; and
- Ensure knowledge dissemination through outreach and communications.



**“Epigenetics” is the study of heritable changes in the regulation of gene activity and expression (whether genes are turned on or off) that are not dependent on the sequence of DNA. These changes may occur in several ways, including chemically marking the DNA itself. This image shows mice that have an identical DNA sequence in a gene that both determines the color of their fur and, when not properly regulated, also promotes obesity. Their different coat colors and body size arise from variation in the chemical modification of this gene. Therefore, even though the mice have the same DNA sequence, the epigenetic marks have a dramatic effect on their physical appearance. Regulation of gene activity by epigenetics plays a crucial role in human development, health, and disease. As described in this chapter, the NIDDK is spearheading new research in this emerging field of science.**

*Image provided by Dr. Robert A. Waterland and reprinted from Journal of Pediatrics, 149, Waterland RA, Epigenetic mechanisms and gastrointestinal development, S137-S142, Copyright 2006, with permission from Elsevier.*

# Cross-Cutting Science

*Advances in medicine are largely dependent upon the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism—its genes, the proteins they encode, and the workings of cells. Major strides in fighting disease can be traced back 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 new discoveries and advances are arising ever more rapidly with the development of new technologies, new approaches, and even new scientific disciplines. Described here are some recent studies that each span multiple areas within the NIDDK mission. These include research on fundamental biologic processes as well as development of new technologies that make such studies possible. The insights gained through this type of research can be expected to propel disease-oriented research, not only within the NIDDK mission, but also in many other fields. Today's cross-cutting advances may lead to tomorrow's health care strides.*

## BUILDING ON RECENT GENETICS STUDIES: PAVING THE WAY TOWARD IMPROVING PEOPLE'S HEALTH

New research tools, including the Human Genome Project and the International HapMap project, are making it easier for scientists to identify genes that influence a person's likelihood of developing common diseases. Taking advantage of these new research tools and technologies, scientists are conducting "genome-wide association studies" to identify genetic differences between people with specific illnesses and healthy individuals. Through this comparison, it becomes possible to identify even subtle genetic differences that may affect whether an individual develops a particular disease.

Recently, genome-wide association studies and other genetics studies have led to an explosion in the identification of genes important in diseases within the NIDDK mission. For example, genes associated with type 1 diabetes, type 2 diabetes, diabetic kidney disease, Crohn's disease, and focal segmental glomerulosclerosis have been identified. Often, the associated genetic region is unexpected—the function of the gene may be completely unknown or it may be involved in cellular processes that were not thought to be important in the particular disease. For example, as described elsewhere in this edition of *NIDDK Recent Advances & Emerging Opportunities*, a genetic region strongly associated with type 2 diabetes was also recently implicated in a very different condition: prostate cancer. In other cases, a gene may be involved in cellular processes already thought to be important in the disease. However, even if a gene's function has been previously studied, it is usually not precisely known how it plays a role in disease.

Although these genetic findings are extremely exciting in and of themselves, there is still much work to be done to understand how the genes function in healthy conditions and what goes wrong in disease. Research in this area sets the stage for even more scientific breakthroughs. For example, a newly-associated gene may produce a protein that interacts with numerous other proteins. Therefore, discovering the disease association not only implicates that protein in the disease, but also the proteins with which it interacts. This knowledge could illuminate several new therapeutic targets for disease prevention or treatment. Studying genes that were not thought to be involved in a disease can lead to brand new avenues for research that would likely not have been pursued otherwise. Identifying the functions of genes may not only enhance understanding of molecular mechanisms that underlie disease, but may also reveal new targets for therapy.

To propel research progress, the NIDDK is spearheading new efforts to elucidate the functions of genes associated with diseases within its mission. For example, the NIDDK sponsored a scientific conference in April 2008, entitled "Diabetes Genes and Beta Cell Function: How Can We Assemble the Puzzle?" The

workshop included presentations on genes newly-discovered to be associated with type 2 diabetes, and specific beta cell functions and pathways potentially influenced by those genes. These presentations served as a springboard for trying to put together the pieces of the type 2 diabetes genetics puzzle and to understand how the genes may influence beta cell function and contribute to disease. To foster collaboration and new research directions, the workshop brought together scientists from multiple disciplines, including investigators researching the genetics of diabetes and scientists studying the development and function of the insulin-producing beta cell. Furthermore, the NIDDK announced a new initiative to support research toward understanding the functions of genes newly-discovered to be associated with type 1 diabetes.

In addition to fostering research to understand the function of new genes, the NIDDK is spearheading research on translating genetics findings to the clinic and examining the social and ethical implications of this research. In March 2008, the NIDDK sponsored a meeting on behalf of the NIH-wide Genes, Environment, and Health Initiative (GEI) entitled, "Translating Whole Genome Association Data into Clinical Research and Practice." The meeting included presentations from scientists on important new genetic findings on certain diseases, such as inflammatory bowel diseases and type 2 diabetes. It also included discussion about approaches to use those genetic findings for therapeutic or diagnostic purposes, and the ethical and social issues pertinent to this type of research. The meeting was an opportunity for participants to discuss emerging themes and questions about translating genetic data from genome-wide association studies into clinical research and applications and to identify key questions for future research.

The NIDDK leads two GEI initiatives related to topics discussed at the March 2008 meeting. One initiative focuses on the translation of significant genetic findings into clinical or public health use. The other initiative will support planning grants for major clinical studies to increase understanding of how the public responds to genetic findings. This research will measure the responses of patients and providers to information about genetic determinants of common diseases and to determine how to effectively educate the public to use the information appropriately for clinical care and disease prevention.

These new and emerging research areas have been made possible by breakthroughs in the fields of genetics and biotechnology during the last few years. The NIDDK will continue to build on these exciting genetics discoveries, paving the way toward capitalizing on new knowledge and improving people's health.

## NIH ROADMAP FOR MEDICAL RESEARCH

The NIH Roadmap for Medical Research is an "incubator space" for nascent programs that cut across the NIH in terms of relevance or complexity. In order to be considered for Roadmap funding, initiatives must be truly transforming, outcomes should synergize with the missions of individual NIH Institutes and Centers to promote health, and trans-NIH participation must be required to address a scientific area in which no single NIH entity alone is likely to engage. The initiatives are developed under the auspices of the Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), a trans-NIH coordinating and planning structure within the NIH.

Roadmap initiatives emerge from input gleaned from a wide range of stakeholders in the intramural and extramural scientific community, the patient advocacy community, and the general public. DPCPSI coordinates a review of the submitted scientific concepts and the relevant NIH portfolios—applying criteria that potential Roadmap initiatives should meet. Directors of the NIH Institutes and Centers then select the general areas and the specific initiative concepts that could be pursued. NIDDK representatives have participated in several of the Working Groups charged with developing these concepts. Two concepts approved as major Roadmap initiatives that began in Fiscal Year 2008 as 5-year programs were the Epigenomics Program and the Human Microbiome Project.

Epigenetics is an emerging frontier of science that involves the study of heritable changes in gene function that cannot be explained by changes in DNA sequence. The Epigenomics Program aims to accelerate the promise of this field into applications that affect human health and a wide range of common complex human diseases by fostering the development of novel resources for research in this field. Research supported by the Epigenomics Program will characterize the

"epigenome" (a catalog of the stable epigenetic modifications or "marks" that occur in the genome) and its impact on health and disease. Initiatives include the creation of mapping centers to develop reference epigenomes, the establishment of a publicly available epigenetic database, as well as other research resources. In addition, support will be provided for fundamental discoveries of novel epigenetic marks and of the roles of epigenetic marks in specific diseases, conditions of development or aging, and responses to environmental exposures. Epigenetics may provide a mechanism by which environmental factors, such as diet, contribute to diseases such as type 2 diabetes and gastrointestinal cancers. As such, the NIDDK has taken an active role in the Epigenomics Working Group, and serves as the lead Institute for the novel marks discovery initiative. Awards for the Epigenomics initiatives were made in summer 2008, with the exception of the initiative focused on the Epigenomics of Human Health and Disease. The first Request for Applications under this initiative was released in summer 2008; it is expected that additional requests will be released annually for 5 years.

The Human Microbiome Project is focused on defining the variability in the microbiome across the population as a first step to assessing its role in both health and disease. The NIDDK Director serves as a co-leader of this Project, which will develop new tools and reference sequence data needed to study the human microbiome. Because the gastrointestinal tract is the site of the body's microbial population, the Human Microbiome Project could greatly aid efforts to understand microbial effects on digestive health and disease.

Another new NIH Roadmap initiative is the Transformative R01 (T-R01) Program. This program is designed to stimulate research to challenge existing paradigms or create new paradigms where none currently exist. It will support highly creative, "outside-the-box" projects. This program also is a High Risk/High Reward Demonstration Project in which novel approaches to peer review and program management will be piloted. Several "highlighted needs" topics are articulated as being in particular need of transformative research. However, the program is open to transformative ideas from any field relevant to biomedical or behavioral research. The first T-R01 awards are expected to be announced in Fiscal Year 2009. As with all Roadmap initiatives, investigators with proposals relevant to the NIDDK research mission are strongly encouraged to apply.

While these new Roadmap initiatives and pilot studies go forward, Roadmap Coordination Working Groups will continue to assess current efforts and future opportunities for cross-cutting collaborations and will continue to seek ideas for Roadmap initiatives from NIH stakeholders. More information on the NIH Roadmap programs can be found at: *http://nihroadmap.nih.gov/*

## STEM CELLS, PROGENITOR CELLS, AND DISEASE APPROACHES

Stem cells have the potential to develop into many different cell types in the body. To better understand these cells and "progenitor cells," which have a more limited developmental potential, scientists continue to characterize their properties and seek potential new ways of using them to benefit patients.

**Reprogramming Human Skin Cells to Embryonic Stem Cell-like Pluripotency:** In a groundbreaking study, scientists have shown that, by introducing just four genes into adult human skin cells, cells resembling embryonic stem cells could be produced. This approach also worked in human cells from various developmental stages.

A key characteristic of embryonic stem cells is that, unlike most cells in the body, they remain "pluripotent." That is, a single embryonic stem cell is able not only to multiply and produce more stem cells, but, by definition, can develop, or differentiate, into a wide variety of mature cell types and tissues. However, the use of cultured stem cells derived from human embryos poses ethical dilemmas, and any potential cell-based therapies derived from stem cells would need to address issues related to patient compatibility. Scientists are therefore seeking ways to "reprogram" differentiated cells, such as skin cells, so they revert back to a pluripotent state.

The genes used in these studies—*Oct4*, *Sox2*, *Klf4*, and *Myc*—encode transcription factors, cellular components that regulate whether other genes are turned on or off. When introduced into certain types of human cells, such

as those derived in the laboratory from differentiated embryonic stem cells, these four factors caused the cells to regain characteristics very closely resembling those of the original embryonic stem cells. The scientists refer to these as induced pluripotent stem (iPS) cells. Remarkably, researchers were also able to generate iPS cells from more developmentally advanced cells—human neonatal and adult skin cells—by introducing these genes, together with two additional genes, *hTERT* and *SV40 large T antigen*. These two additional factors enhanced the efficiency of reprogramming in these more mature cells.

These findings show that it is possible to reprogram differentiated human cells into pluripotent stem cells. This approach may facilitate the establishment of human iPS cell lines from patients with specific diseases that could be used as research tools. Such cells would provide a rich source of pluripotent embryonic stem cell-like material. Further studies of these cells will thus complement ongoing research on other types of stem cells. This technique, or variations of it, may also, one day, allow patient-specific stem cells to be generated for use in stem cell-based therapies. One caution: the genes used for reprogramming were introduced into the cells with a method involving viruses, which could have adverse health effects. If, however, safe alternate methods based on this research were developed for reprogramming cells, then iPS cells may lead to novel, personalized therapies.

*Park I-H, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, and Daley GQ: Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451: 141-146, 2008.*

**New Stem Cell Resource To Advance Understanding of Human Diseases and Disorders:** Scientists have generated stem cell lines from patients with 10 different genetic diseases and disorders, providing a valuable resource for the scientific research community. Significant understanding of human biology, including healthy and diseased states, has come from studies of human cell lines grown in the laboratory. Since human tissue has a limited life span in the laboratory, scientists most commonly study cells that have been "immortalized" by their tumoral origin or by techniques that modify the cells in the laboratory. These and other limitations, until now, have resulted in a lack of models for normal and pathogenic tissue growth and formation.

To generate the new disorder-specific cell lines, investigators first obtained cells from patients with different genetic (inherited) conditions. These included Parkinson's disease, type 1 diabetes, Huntington's disease, Down syndrome, "bubble boy disease" (severe combined immunodeficiency), Gaucher's disease, forms of muscular dystrophy, and others. The scientists had previously discovered a small set of genes that, when introduced into adult cells, could reprogram the cells to turn them into embryonic-like cells with the capacity to give rise to a variety of different cell types (pluripotency). By introducing these reprogramming factors into the cells obtained from patients, the scientists have now been able to transform them into cells that looked like embryonic stem cells and had many of the markers of stem cells. These cells are known as induced pluripotent stem (iPS) cells—cells that originate from adult body cells, but upon introduction of reprogramming factors, change into cells that have the properties of stem cells.

To ensure that the resulting iPS cells truly resembled disorder-specific stem cells, several tests were conducted of the iPS cell lines. First, for disorders with a clearly defined genetic basis, it was confirmed that the iPS cells still maintained the genetic defect that caused the human disorder. Next, it was confirmed that the observed stem cell markers came from the genome of the iPS cells, not from the technique the scientists used to introduce the reprogramming factors into the cells. Also, by analyzing the DNA of the cell lines, it was determined that the iPS cell lines were pure—that the cells had not been contaminated by other stem cell lines the scientists were studying in the laboratory. Finally, the investigators showed that the iPS cells were able to become several different types of tissue—one of the hallmarks of stem cells. The results of these tests confirmed that the investigators had generated disorder-specific iPS cell lines.

The scientists noted that the disorder-specific iPS cell lines produced in this study will be maintained and made available to the scientific community. These cell lines can be used in numerous ways to advance the understanding of these disorders. Comparing the disorder-specific cell lines to normal cell lines

may provide insights into the development and progression of these disorders. The cell lines may also be used in the laboratory to screen new therapies. Finally, research on these cell lines could inform the development of cell therapies. With modifications of the technique used to introduce the reprogramming factors, patient-specific iPS cells could one day be generated for cell replacement therapies that would avoid immune rejection and gene therapies to correct genetic defects. This exciting new resource has the potential to transform the study, understanding, and treatment of human disease.

*Park I-H, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, and Daley GQ: Disease-specific induced pluripotent stem cells. Cell 134: 877-886, 2008.*

**Identification of Progenitor Cells in the Adult Pancreas that Form Insulin-Producing Beta Cells:** Scientists have discovered a novel group of adult pancreatic progenitor cells that generate insulin-producing beta cells. Researchers previously demonstrated that new beta cells could be regenerated from existing beta cells in the pancreas. However, it remained unknown as to whether embryonic-like progenitor cells exist in the adult pancreas that have the ability to form beta cells. To gain further understanding about pancreatic progenitor cells, scientists, including members of the NIDDK-supported Beta Cell Biology Consortium, surgically induced a specific type of wound to the adult mouse pancreas, which caused the number of beta cells to double. The scientists took advantage of this doubling in number to test for the presence of a well-established marker of embryonic pancreatic progenitor cells, called Ngn3. Ngn3 is essential for development of the endocrine pancreas, but is not normally found in the adult pancreas. They observed that levels of *Ngn3* increased in the pancreas in response to the injury. When the scientists first inhibited the production of Ngn3 and then damaged the pancreas, the beta cells did not double in number like before. This observation indicated that Ngn3 plays a role in increasing beta cell numbers following the injury.

The scientists needed to determine, however, whether the cells expressing *Ngn3* produced the new beta cells seen in response to the pancreatic injury, or if the new beta cells were arising from different cells. To do this, they first purified the *Ngn3*-expressing cells from an adult mouse pancreas that had been damaged in the way that would increase beta cell numbers. They then placed the cells into an embryonic pancreas that had been isolated from a mouse that did not have its own *Ngn3*, due to a genetic mutation, and would thus not normally produce beta cells. However, when the purified *Ngn3*-expressing cells were added, beta cells developed within this embryonic pancreas. In addition, these newly-formed beta cells responded to glucose by releasing insulin. These results demonstrated that the cells expressing *Ngn3* in response to the injury were truly progenitor cells capable of producing beta cells. By further examining the *Ngn3*-expressing cells within the injured mouse pancreas, the scientists demonstrated that these cells showed many of the same characteristics as embryonic progenitor cells.

This research suggests that the adult mouse pancreas contains progenitor cells that are able to regenerate beta cells. If, with further research, these embryonic-like progenitor cells are identified in the human pancreas, then this discovery may foster the development of therapies for type 1 and type 2 diabetes—diseases in which the number and/or function of beta cells has been adversely affected. These progenitor cells could potentially be isolated from human pancreata to grow new beta cells in the laboratory that could be transplanted into a patient with diabetes. It may also be possible to develop new therapies to stimulate these cells within the patient to form new beta cells.

*Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, and Heimberg H: Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132: 197-207, 2008.*

**Purified Stem Cell Transplants Capable of Repopulating the Liver:** Researchers have demonstrated that purified rat fetal stem cells transplanted into animals missing two-thirds of their livers are capable of fully repopulating these organs, lending support for the consideration of stem cell transplantation as an alternative to whole or partial liver transplantation. Currently, liver transplantation

is the only successful treatment option available to patients with end-stage liver disease. But the number of livers available for transplant is limited, and many patients die while awaiting a transplant. Although the adult liver contains its own stem cells capable of some degree of regeneration, they are unable to fully replace the functional liver as is possible with liver transplantation. However, stem cells from fetal livers possess unique properties that could enable them to repopulate the organ and restore its function, provided that these cells could be delivered in sufficient quantity and purity to the recipient. Researchers developed a strategy to purify fetal liver stem cells by separating them from other cell types that might have unwanted effects. To accomplish this purification of the stem cells, they mixed cells isolated from rat fetal liver with special beads under conditions that allowed the beads to attach to the cells via a protein on the cells' surface called Dlk-1. This protein is found on stem cells in the fetal liver of rodents and humans, but not on other liver cell types present. They subsequently retrieved the stem cells that had attached to the beads. The purified stem cells were then tested in culture to ensure that they displayed all the hallmarks of liver stem cells, including production of unique proteins and robust proliferation. After a few days in culture, they also started to produce proteins associated with functions of mature liver and bile duct cells. The researchers then tested the "behavior" of a transplanted population of purified fetal stem cells in an animal model of liver loss—adult rats in which two-thirds of the liver had been surgically removed. Transplantation of the purified fetal stem cells into these rats led to an impressive repopulation of their liver and bile duct cells after 6 months. The scientists were able to visualize where the transplanted cells had repopulated the liver using a marker for an enzyme unique to these cells as they matured. The purified stem cells specifically homed in on the liver and did not lodge in other organs where their robust proliferation could have adverse effects. This series of groundbreaking experiments provides the methods necessary to prepare purified liver stem cells suitable for transplantation, laying the foundation for possible therapeutic applications in the future.

*Oertel M, Menthena A, Chen Y-Q, Teisner B, Jensen CH, and Shafritz DA: Purification of fetal liver stem/progenitor cells containing all the repopulation potential for normal adult rat liver. Gastroenterology 134: 823-832, 2008.*

### Bacterial Interaction with Gastric Stem Cells Related to Stomach Cancer Development:

NIDDK-sponsored research has yielded insights into how the bacterial species *Helicobacter pylori* evolves and interacts with stem cells in the human stomach, contributing to disease progression to conditions such as cancer. *H. pylori* is the major cause of peptic ulcers, which affect a large number of individuals in the U.S. Most of those who become infected with this bacterium develop an inflammation of the stomach known as gastritis. However, in a small subset of individuals, gastritis progresses to a more severe form called chronic atrophic gastritis, in which some stomach cell types are destroyed. This condition may progress further to a type of stomach cancer known as gastric adenocarcinoma. Little is known about the role that *H. pylori* plays in these disease progressions; however, the bacteria are known to interact with the outer surface of epithelial cells that line the stomach and to establish themselves within gastric stem cells.

To explore the impact of interactions between *H. pylori* and gastric cells on disease progression, researchers engaged in a multi-species series of studies based largely on genomic techniques. Starting in the clinical realm, they analyzed samples taken originally for a population-based endoscopy study in Sweden. They focused their attention on samples collected from the stomach of one participant who developed gastric adenocarcinoma over the course of 4 years after an initial diagnosis of chronic atrophic gastritis. From these samples, they isolated *H. pylori* present before and after cancer development, and compared their genomes. They found that a single strain of *H. pylori* persisted throughout disease progression, but that several characteristics of the bacteria had changed over time. To study this disease-related *H. pylori* strain further, they switched to two experimental models: a mouse model of chronic atrophic gastritis, and a gastric stem cell culture model in which they could compare the direct effects of infection by the *H. pylori* specimens. Using these models, they were able to identify unique characteristics of the *H. pylori* present before and after stomach cancer development, including a gene turned on only in the cancer-associated bacteria that could serve as a biomarker for bacterial adaptation and stomach cancer. The cancer-associated *H. pylori* was also found to be better adapted to life inside the gastric stem cells, where it could influence cancer development. At the same time, the experiments also revealed changes in the

mouse stomach stem cells. For example, the cancer-associated form of the bacteria affected mouse genes related to tumor development, among other genes. This study provides insights into how *H. pylori* interacts with stomach stem cells to influence disease progression that can culminate in stomach cancer. This new knowledge enhances understanding of the host-microbe interactions that contribute to these gastric diseases, and how this information might be used to predict risk of disease development and progression.

*Giannakis M, Chen SL, Karam SM, Engstrand L, and Gordon JI: Helicobacter pylori evolution during progression from chronic atrophic gastritis to gastric cancer and its impact on gastric stem cells. Proc Natl Acad Sci USA 105: 4358-4363, 2008.*

**Stem Cell Gene Therapy with a HOXB4-expressing Retroviral Vector Causes Leukemia in Large Animals:** Researchers have recently reported the development of leukemia in large animals approximately 2 years after transplantation with retroviral-infected stem cells that overexpressed a gene called *HOXB4*. The researchers sought to investigate the effects of retroviral gene transfer into stem cells because of earlier reports of adverse effects in humans. Several patients with severe combined immunodeficiency disease (SCID) who were enrolled in French- and British-supported gene therapy clinical trials developed leukemia 2 to 3 years after transplantation of stem cells containing a retrovirus vector. The researchers suspected that some cells with the retrovirus may have had a growth advantage compared to other cells, which could lead to excess proliferation and cancer. For their experiments, they studied the effects of retroviruses that carried the *HOXB4* gene, because overexpression of this gene (excess production of the protein it encodes) was previously shown to confer a growth advantage in blood cells. Although blood cell abnormalities have not been detected in mouse studies using transplanted stem cells overexpressing *HOXB4*, experimental studies in mice do not always translate to outcomes in humans. Thus, the scientists evaluated the safety of transplanting retroviral-infected stem cells expressing *HOXB4* in larger animals, such as monkeys and dogs. Three of the four animals studied developed leukemia approximately 2 years after transplantation with retroviral-infected stem cells overexpressing *HOXB4*. In contrast, retroviral-infected stem cells not expressing *HOXB4* did not drive the development of leukemia. The researchers also suggest that in the clinical trials with patients with SCID, the gene used in the gene therapy may also have conferred a growth advantage to the transplanted cells. This study underscores the value of including large animal models in risk assessment analyses when developing gene therapies, and that for such therapies, overexpression of a gene in stem cells that confers a particular growth advantage to the cells, such as *HOXB4*, may carry an unacceptably high risk of cancer or have other serious unintended consequences.

*Zhang X-B, Beard BC, Trobridge GD, Wood BL, Sale GE, Sud R, Humphries RK, and Kiem H-P: High incidence of leukemia in large animals after stem cell gene therapy with a HOXB4-expressing retroviral vector. J Clin Invest 118: 1502-1510, 2008.*

## OMEGA-3 FATTY ACIDS AND INFLAMMATION

**Understanding How Fish Oil Reduces Inflammation:** Findings from a recent study of the anti-inflammatory properties of omega-3 fatty acids may help increase the therapeutic potential of these essential nutrients. Chronic inflammation damages body tissues and plays a critical role in many diseases and conditions, including diabetes, obesity, inflammatory bowel disease, heart disease, and rheumatoid arthritis. Omega-3 fatty acids have emerged as one therapeutic option for reducing inflammation, but the mechanism(s) by which they exert this effect is not well understood. The present study focused on an omega-3 fatty acid, docosahexaenoic acid, or DHA, found in fish oil and other foods. DHA is oxidized in the body to form a variety of bioactive molecules. One class of molecules produced by DHA oxidation, called $A_4$-NP, resembles a group of anti-inflammatory agents found naturally in the body. Researchers used this knowledge to determine whether $A_4$-NP acts on the same molecular pathway to suppress inflammation as the natural agents. They found that $A_4$-NP does indeed suppress activation of the same pro-inflammatory molecular pathway—the NF-kappaB pathway—in cells of the immune system called macrophages. When the researchers pre-treated laboratory-grown macrophages from mice with $A_4$-NP and then added bacterial compounds or other stimulants, inflammation was potently inhibited. Delving deeper, they discovered

that $A_4$-NP prevents inflammation by short-circuiting the pro-inflammatory molecular pathway, keeping the NF-kappaB protein from moving into the cell's nucleus, where it would promote production of cyclooxygenase-2 (COX-2) and other pro-inflammatory molecules. Migration of NF-kappaB normally depends upon the activity of a cellular enzyme that is activated by inflammatory signals. The investigators found evidence that at least part of the mechanism underlying $A_4$-NP's effect is through interference with this enzyme. Finally, to determine how much of DHA's anti-inflammatory properties are actually due to the activity of $A_4$-NP, the team tested whether DHA needed to be oxidized to inhibit inflammation. They found that only oxidized DHA inhibited inflammation and that the anti-inflammatory properties of oxidized DHA were strongly linked to the amount of $A_4$-NP and to a similar oxidation product, $J_4$-NP. While these experiments were performed using laboratory-grown mouse cells, the results suggest that DHA's anti-inflammatory effects in humans may be due, at least in part, to its oxidation and subsequent inhibition of a key pro-inflammatory pathway. A fundamental understanding of the anti-inflammatory properties of DHA and other omega-3 fatty acids may lead the way to new, targeted therapeutic approaches for a wide range of inflammatory conditions.

*Musiek ES, Brooks JD, Joo M, Brunoldi E, Porta A, Zanoni G, Vidari G, Blackwell TS, Montine TJ, Milne GL, McLaughlin B, and Morrow JD: Electrophilic cyclopentenone neuroprostanes are anti-inflammatory mediators formed from the peroxidation of the omega-3 polyunsaturated fatty acid docosahexaenoic acid. J Biol Chem 283: 19927-19935, 2008.*

## HUMAN IMMUNODEFICIENCY VIRUS (HIV) RESEARCH

**NIDDK Scientists Identify Key Steps in Processing of HIV Proteins:** Researchers at the NIDDK's Laboratory of Chemical Physics have characterized a short-lived intermediary that appears during the maturation of a key protein involved in the replication of HIV. Following infection, one of the first proteins synthesized by the virus is "protease," which is capable of cleaving itself and other viral proteins to allow their assembly into new virus particles. Protease is essential for HIV infection—an entire class of drugs, protease inhibitors, is an important part of anti-AIDS therapy today. The NIDDK researchers were studying how HIV protease is processed from an inactive precursor into its final active form. This represents a seeming contradiction: if mature protease is responsible for activating molecules of inactive protease precursor, how is the first molecule of protease cleaved and activated? To study this question, the researchers used an inactive, shortened form of the precursor protein and a technique called paramagnetic relaxation enhancement to identify the structure of protein-protein complexes that form during protease processing. The scientists found that the mini-precursor formed a highly transient, very rare complex of two mini-precursor molecules. This was an important insight because mature protease acts as a dimer—two molecules of protease joined together to cleave other proteins. The fleeting mini-precursor complex had little enzymatic activity; however, it may be sufficient to allow self-processing of a few molecules of protease. The consequent mature, fully active protease enzymes can then go on to cleave other nascent proteins, including additional immature protease molecules. Because HIV protease plays an integral role in HIV infection, it is a prime target for the development of therapies aimed at preventing AIDS. This finding provides a detailed structural model for maturation of the largely inactive precursor into a fully active protein. An improved understanding of the steps involved in early HIV infection and progression may reveal new targets for therapy.

*Tang C, Louis JM, Aniana A, Suh J-Y, and Clore GM: Visualizing transient events in amino-terminal autoprocessing of HIV-1 protease. Nature 455: 693-696, 2008.*

# *Dr. Alexandra McPherron and Dr. Michelle Winn: NIDDK Scientists Receive Presidential Award*

Two scientists supported by NIDDK were honored at a White House ceremony in November 2007 for their outstanding scientific leadership in diabetes and kidney disease research.

The Presidential award, known as the Presidential Early Career Award for Scientists and Engineers (PECASE), was bestowed upon Alexandra C. McPherron, Ph.D., a scientist in NIDDK's Division of Intramural Research, and Michelle P. Winn, M.D., an NIDDK extramural grant recipient, along with 10 other grantees from the National Institutes of Health (NIH). PECASE is the most prestigious award given to young scientists in the U.S.

**Myostatin and Metabolism**

**Dr. Alexandra C. McPherron**

Dr. McPherron, a tenure-track investigator with the NIDDK's Genetics of Development and Disease Branch, was chosen for discovering myostatin, a secreted protein produced by skeletal muscle that inhibits muscle growth. Inhibiting myostatin might be therapeutically useful for treating muscle wasting diseases, diabetes, or obesity, according to Dr. McPherron. She is trying to understand the role of myostatin in adult metabolism. "The myostatin protein circulates in the bloodstream, so it might act on other tissues, such as adipose tissue, in addition to skeletal muscle," said Dr. McPherron. Her research in mice showed that loss of this protein results in improved glucose metabolism and reduces fat accumulation. The mechanisms for these effects are not yet clear. "We don't yet know whether the improvement in glucose metabolism is due purely to the increase in skeletal muscle mass, the loss of circulating myostatin acting on other tissues, or metabolic changes in skeletal muscle, such as becoming more sensitive to insulin. We are also trying to understand how myostatin regulates the proliferation and differentiation of muscle precursor cells and their incorporation into muscle fibers."

The Intramural Research Program of the NIDDK conducts basic, translational, and clinical biomedical research related to: diabetes mellitus, endocrine, bone, and metabolic diseases; digestive diseases, including liver diseases and nutritional disorders; kidney diseases; and hematologic diseases.

**Familial Kidney Disease**

**Dr. Michelle P. Winn**

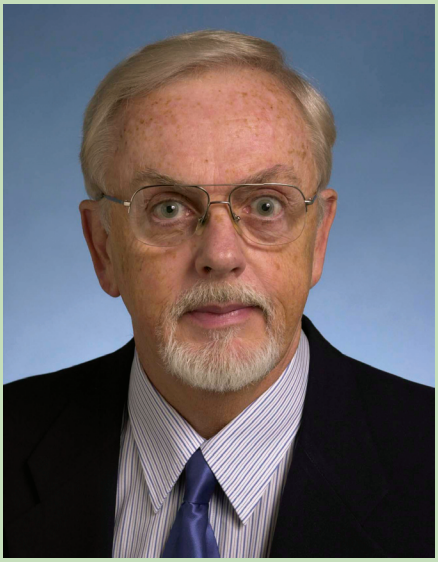
Dr. Winn, who is an Assistant Professor in the Division of Nephrology at the Duke University School of Medicine, was recognized for the discovery of the *TRPC6* gene as a cause of familial kidney disease. NIDDK-supported genetic studies aim to determine why focal segmental glomerulosclerosis (FSGS), which causes kidney failure, sometimes runs in families. FSGS is a common, irreversible process that can result in steroid-resistant

nephrotic syndrome, a condition marked by very high protein levels in the urine; low protein levels in the blood; swelling, especially around the eyes, feet, and hands; and high cholesterol.

Dr. Winn's research is important because it has opened a new angle to understanding a disease that is poorly understood and which disproportionately affects African Americans. Dr. Winn, who previously held an NIDDK K08 award, which provides physicians with up to 5 years of support to pursue research careers, is now in the third year of an R01 grant from the NIDDK. The R01 research project grant is awarded to eligible institutions on behalf of a principal investigator to support a discrete project related to the investigator's area of interest and competence.

The PECASE awards support the continued professional development of awardees, promote careers and foster innovation in science and technology, and recognize the scientific missions of participating agencies. A list of previous NIH recipients of this prestigious award is available at *www.grants.nih.gov/grants/policy/pecase.htm*

# *Dr. Bert O'Malley Wins National Medal of Science: Long-term NIDDK Grantee Pioneered Molecular Endocrinology*



**Dr. Bert W. O'Malley**

Dr. Bert W. O'Malley has won a National Medal of Science for his outstanding contributions to knowledge in the biological sciences. Dr. O'Malley is a long-term grantee of the NIDDK and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and former section chief for the National Cancer Institute (NCI). On September 29, 2008, Dr. O'Malley was presented the prestigious award in recognition of "his pioneering work on the molecular mechanisms of steroid hormone action and hormone receptors and coactivators, which has had a profound impact on our knowledge of steroid hormones in normal development and in diseases, including cancer." Dr. O'Malley, chair of Baylor College of Medicine's Department of Molecular and Cellular Biology, is the first scientist in the field of molecular endocrinology to receive the Medal, considered the highest national honor in biological sciences.

"Dr. O'Malley's prodigious career is a tribute to the importance of basic research," says Dr. Griffin P. Rodgers, NIDDK Director. "His research revolutionized the understanding of hormone action and the molecular regulation of processes as basic as metabolism and reproduction. By studying the mechanisms of hormone action, he unexpectedly found molecular pathways that lead to a number of diseases. Some therapies that capitalize on his findings are already in clinical trials."

Much of Dr. O'Malley's early work focused on the steroid hormones—glucocorticoids, mineralocorticoids, androgens, estrogens, and progestagens—which regulate reproduction and basic metabolism. He used the tools of physiology and biochemistry to study the hormones' role in reproduction and developmental diseases and was one of the first to apply new methods as they were introduced. The challenges in those days were immense, because scientists had not yet found the receptors for these hormones nor had they yet discovered that they all belonged to a common family.

In the 1980s, evidence was growing that receptors for steroid hormones had unique structural properties and belonged to a common family of receptors. Instead of attaching to receptors on the cell surface, these hormones linked up with receptors in the cell and its nucleus and acted as transcription factors to change the expression of genes. After the first nuclear receptor was cloned, scientists went on to find 49 such receptors, including those for steroid hormones, thyroid hormones, certain vitamins, and receptors for hormones that were still unknown. These "orphan receptors" also turned out to have profound effects on cells.

Dr. O'Malley was one of the first to create an *in vitro* transcription assay, or a test tube system, that could recapitulate what happened inside a cell to study the changes in gene expression. His assay stimulated much research that led to an even greater understanding of hormone action because scientists could use the method to study their favorite hormone and receptor.

Dr. O'Malley's discoveries led to the development of selective steroid receptor modulators. This class of new drugs selectively targets one tissue while leaving other tissues unaffected. His findings also laid the groundwork

for the development of two important drugs: a progestin compound that prevents preterm birth in certain cases and raloxifene, which prevents osteoporosis.

In 1995, Dr. O'Malley and others discovered a group of nuclear receptor coregulators, molecules in the nucleus that control how nuclear receptors work. Coregulators, he found, helped turn on and off transcription factors, such as nuclear receptors, which in turn helped to orchestrate the expression of many other genes. Probing deeper, he identified a subset of coregulators, called coactivators, which are required for hormone action. Other researchers soon found corepressors, which silence transcription.

"Hormones control almost all cellular physiology," Dr. O'Malley explains. "Receptors for steroid hormones, the most important class of hormones, are activated by the hormone, then they go into the cell's DNA and search out and find the target genes to be turned on or off. In the final step, they recruit complexes of coregulators, including coactivators, that perform all the functions to turn the genes on. In a sense, these coactivators are master genes because they can activate different transcription factors at the same time, so you get a coordinated physiologic outcome."

Forging ahead, Dr. O'Malley and colleagues came to a stunning conclusion: nuclear receptor coregulators control physiologic processes as basic as cell growth, metabolism, inflammation, and reproduction. And if defective, these "little molecules with big goals" can lead to disease. "When the activities of these master genes are compromised, cellular processes can quickly deteriorate," says Dr. O'Malley. In overdrive, some can spur the uncontrolled growth of cancer cells. For example, the steroid receptor coactivator SRC-3 fuels the growth of most prostate tumors and 65 percent of breast tumors. Another coactivator, SRC-2, controls glucose production by the liver. When it is defective, a form of glycogen storage disease develops. Other SRCs influence fat cells, energy balance, and carbohydrate metabolism.

"Basic research is our fountain of knowledge," says Dr. O'Malley. "I always felt that if I knew how things work in normal cells, I'd have much better insight how to fix them when they go wrong in disease. If you open the hood of a car, you don't know how to fix it if you don't know how the motor works."

Dr. O'Malley is principal investigator of the Nuclear Receptor Signaling Atlas (NURSA). NURSA is a trans-NIH effort, led by the NIDDK, designed to develop a comprehensive understanding of the structure, function, and role in disease of nuclear hormone receptors. NURSA has a particular focus on metabolism and the development of a number of metabolic disorders, including type 2 diabetes, obesity, lipid dysregulation, and others, as well as on processes of aging and hormone-dependent cancers. For more information on NURSA, see *http://www.nursa.org/*

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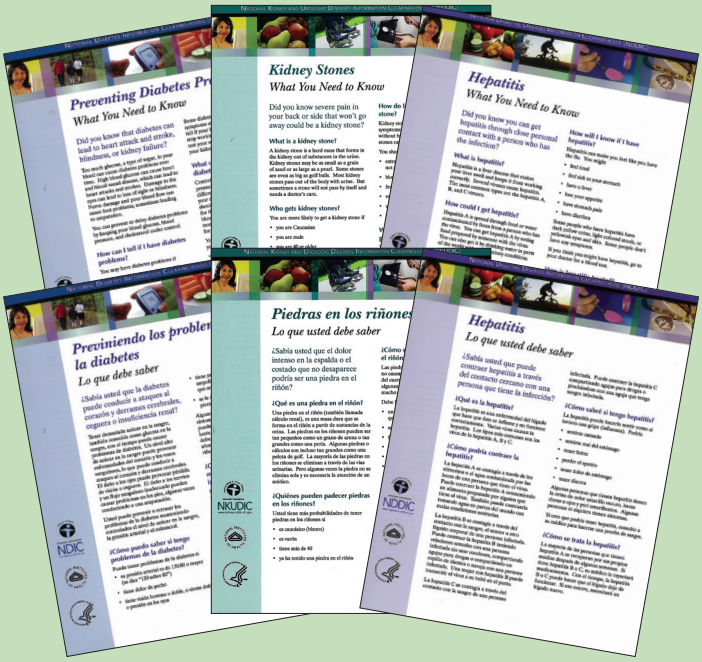
# *NIDDK Releases New Awareness and Prevention Series for Community Health Events*

The NIDDK has developed a new health information series to raise awareness about diabetes, digestive diseases, and kidney and urologic diseases among people not yet diagnosed with these illnesses. The NIDDK developed the Awareness and Prevention Series for community health fairs, workplace health forums, family reunions, and other similar events.

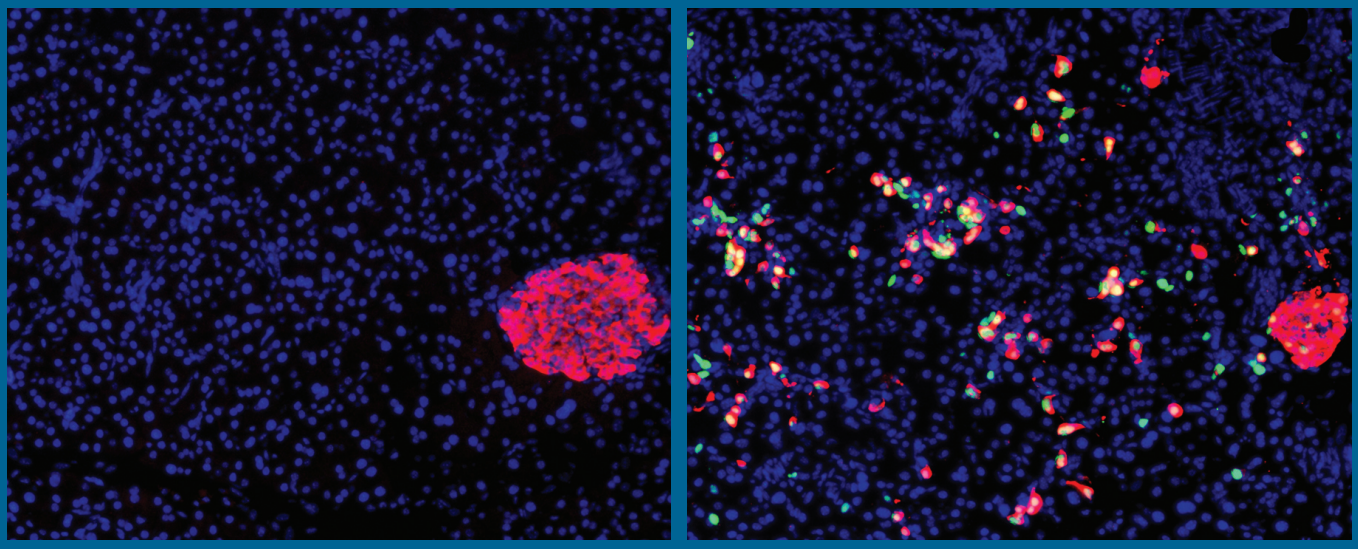
The Awareness and Prevention Series publications are each two-page fact sheets—one side in English and the other in Spanish—on a wide range of health topics, including bladder control, celiac disease, foodborne illness, irritable bowel syndrome, pre-diabetes, preventing diabetes complications, urinary tract infections, and many others. Each fact sheet gives readers a snapshot of an illness, highlighting risk factors, symptoms, prevention tips, and where to go for more information.

The new series is designed to encourage readers to consider whether these illnesses could be affecting them or a loved one. By raising awareness of these illnesses, their causes, and symptoms, the NIDDK is providing necessary information to the public to promote prevention and early diagnosis of many common conditions.

Copyright-free full texts of the Awareness and Prevention Series publications—and all other publications from the NIDDK Information Clearinghouses—are online at *www.niddk.nih.gov*. To order copies of the Awareness and Prevention Series fact sheets, click on "NIDDK Awareness and Prevention Series" and then on "*catalog.niddk.nih.gov*"




**The NIDDK's Awareness and Prevention Series was developed to raise awareness about diabetes, digestive diseases, and kidney and urologic diseases. The fact sheets are two-pages, with one side in English (top row) and the other side in Spanish (bottom row). In addition to the fact sheets shown here, the Series includes fact sheets on a wide range of other health topics within the NIDDK's mission.**



**Research highlighted in this chapter describes how adult pancreatic cells can be turned into insulin-producing beta cells. The image on the left shows an adult mouse pancreas, with insulin-producing beta cells (pink) in a little cluster called an islet. Other cells that do not produce insulin are shown in blue. The image on the right shows new beta cells that arise in the adult mouse pancreas after injection of a special, engineered virus containing a specific combination of three factors previously found to be key regulators of pancreatic development. The green color marks many of the cells infected by the virus. Some of the infected cells, although not originally beta cells, turned into insulin-producing cells as a result of the regulatory factors carried by the virus. These are seen as pink cells outside the islet cluster. Other cells are stained blue. Increasing beta cell mass is critically important for both type 1 and type 2 diabetes, and this exciting research identifies a way to generate new beta cells from pre-existing adult cells in a living organism.**

*Images provided by Dr. Douglas A. Melton and reprinted by permission from Macmillan Publishers Ltd: Nature, 455: 627-32, copyright 2008.*

# 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, they affect many millions of Americans and profoundly decrease their 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 23.6 million people in the U.S.—or 7.8 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 cardiovascular disease risk two- to four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult onset blindness.[1] In addition to these human costs, the estimated total financial cost for diabetes in the U.S. 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.[1]

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone which 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.

Type 1 diabetes affects approximately 5 to 10 percent of individuals 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 beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, produce the hormone insulin. If left untreated, type 1 diabetes results in death from starvation despite high levels of glucose in the bloodstream. 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 as well as they could if they had functional beta cells. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery, as well as working on new beta cell replacement therapies to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90-95 percent of diabetes cases in the U.S.[1] Type 2 diabetes is associated with several factors, including older age and a family history of diabetes. It is also strongly associated with obesity: more than 80 percent of adults with type 2 diabetes are overweight or obese.[3] Type 2 diabetes occurs more frequently among minority groups, including African Americans, Hispanic Americans, American Indians, and Native Hawaiians.[1]

In patients with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. Gradually, the pancreatic beta cells secrete less and

[1] *http://www.cdc.gov/diabetes/pubs/factsheet07.htm*

[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.*

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

less insulin, and the timing of insulin secretion becomes abnormal. Treatment approaches for controlling glucose levels include diet, exercise, orally administered medications, and, in some cases, injected insulin. There are also an estimated 57 million adults in the U.S. who have a condition called "pre-diabetes," in which blood glucose levels are higher than normal, but not as high as in diabetes.[4] This population is at high risk of developing diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with pre-diabetes can dramatically reduce their risk of developing full-blown diabetes with improvements in lifestyle or with drug treatment.

Type 2 diabetes was previously called "adult-onset" diabetes because it was 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. 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 diabetes in offspring. Thus, the rising rates of diabetes and pre-diabetes 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, health care providers may find it increasingly difficult to strictly control a patient's blood glucose level and thus prevent or delay the development of complications. Therefore, the advent of type 2 diabetes in youth has the potential to drastically worsen the enormous health burden that diabetes already places on the U.S.

The NIDDK is supporting research to better understand 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. In parallel, based on knowledge from past scientific research investments, the Institute is vigorously pursuing studies of prevention and treatment approaches for these diseases.

## GENETICS OF DIABETES

**Consortium Identifies Six More Genetic Variants Affecting Likelihood of Type 2 Diabetes:** Type 2 diabetes, by far the most common form of diabetes mellitus, is caused by a complex interaction of genes and the environment. Diabetes is much more common in some ethnic groups than in others, suggesting that genes may explain these health disparities. Type 2 diabetes is also strongly associated with obesity, but most of the genetic components identified so far are unrelated to factors influencing obesity. Indeed, most obese people do not develop diabetes, and some people with normal body weight do. Understanding the reasons why one person develops the disease and another does not is a critical research priority. However, until recently, there has been little definitive information about the genetic variants that predispose or protect a person from type 2 diabetes. In the last 2 years, three independent genome-wide association studies have employed powerful new genomic tools to identify 10 common genetic variants, each of which has a modest effect on the probability of a person developing type 2 diabetes.

Because a large sample size is required to uncover genetic variants that have relatively small effects, researchers from the three previous genome-wide association studies formed a consortium to combine their data to potentially identify additional type 2 diabetes genes. They also confirmed their results in samples from several other studies. The larger effective sample size—which combined genetic data from more than 70,000 people—provided the statistical power to identify 6 more common variants associated with an effect on the likelihood of developing type 2 diabetes, raising the known total to 16 genes. In the case of all of the new genes, and most of those previously identified, the precise genetic changes that influence the development of diabetes remain unknown—only their genetic neighborhood has been identified for certain. None of the new genetic variants were previously known to be associated with type 2 diabetes. Interestingly, the new gene that was most strongly associated with risk for type 2 diabetes was previously found also to be associated with prostate cancer.

[4] *http://diabetes.niddk.nih.gov/dm/pubs/statistics/index.htm*

When each genetic region found to influence development of type 2 diabetes has been carefully investigated, the precise genetic changes that exert these effects will be identified. Study of these genes should lead to greater biological understanding of the development of this serious disease, and may lead to new and better diagnostics and personalized treatments. Because the new genetic regions had not previously been associated with type 2 diabetes, this research opens up novel avenues for investigation of underlying causes of the disease. Furthermore, recent analysis of data from the NIDDK's landmark Diabetes Prevention Program clinical trial confirmed that a particular gene variant increases risk for type 2 diabetes in people participating in the trial. However, even the participants at highest genetic risk benefited from healthy lifestyle changes shown to prevent or delay development of type 2 diabetes. This result was very encouraging because, despite a person's genetic risk, lifestyle change could still reduce risk for developing type 2 diabetes.

*Zeggini E, Scott LJ, Saxena R, and Voight BF, for the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) Consortium: Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40: 638-645, 2008.*

**Genetic Variation Contributes to the Regulation of Glucose Levels in Humans:** Researchers have discovered variations in a region of DNA that are associated with differences in the level of blood glucose (sugar) in the human body. A recent boom of genetic information has enabled scientists to correlate markers in a person's genome—often a difference in a single letter in the DNA sequence—with the likelihood of developing particular diseases or conditions. In previous research, scientists compared the genomes of a large number of individuals—called genome-wide association studies—and identified several regions in the genome that associated with an increased risk for the development of type 2 diabetes. Because elevated glucose levels are associated with increased risk for diabetes, and untreated diabetes leads to a dramatic increase in glucose levels in a person's blood, investigators analyzed this genetic information to determine whether there are correlations between an individual's markers and his or her blood glucose levels. The scientists combined genomic data from two previous studies, the Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) and the SardiNIA Study of Aging, to scan a total of over 5,000 individual genomes. They looked only at people without diabetes to ensure that the measured blood glucose levels had not been artificially lowered by medications that individuals with diabetes may take. They also made statistical adjustments to account for increases in blood glucose associated with elevated body weight, because overweight and obesity are also associated with biologic changes that could increase glucose levels. By correlating genetic data with blood glucose levels measured after overnight fasting, it was determined that multiple markers in the DNA sequence were associated with variation in blood glucose levels. An additional 18,000 genomes of individuals who did not have diabetes were analyzed, using data from seven other studies, to verify the marker with the strongest association.

The marker with strongest association to blood glucose levels is near two different genes, either of which may be responsible for the effect. One of these genes plays an important role in glucose metabolism and thus seems likely to play a role in the regulation of blood glucose levels, while the other has not previously been demonstrated to participate in glucose level regulation. Further research is needed to determine whether and how either of these genes contributes to blood glucose level variation. This study is an important step toward better understanding of blood glucose regulation, and provides new insight into genetic contributions to elevated glucose levels, a precursor to type 2 diabetes. This exciting advance could inform the development of new therapies.

*Chen W-M, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orrù M, Grazia Piras M, Bonnycastle LL, Willer CJ, Lyssenko V, Shen H, Kuusisto J, Ebrahim S, Sestu N, Duren WL, Spada MC, Stringham HM, Scott LJ, Olla N, Swift AJ, Najjar S, Mitchell BD, Lawlor DA, Smith GD, Ben-Shlomo Y, Andersen G, Borch-Johnsen K, Jørgensen T, Saramies J, Valle TT, Buchanan TA, Shuldiner AR, Lakatta E, Bergman RN, Uda M, Tuomilehto J, Pedersen O, Cao A, Groop L, Mohlke KL, Laakso M, Schlessinger D, Collins FS, Altshuler D, Abecasis GR, Boehnke M, Scuteri A, and Watanabe RM: Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Invest 118: 2620-2628, 2008.*

## AUTOIMMUNITY IN TYPE 1 DIABETES

**Discovery of a New Marker for Pre-clinical Type 1 Diabetes:** Scientists have discovered a powerful new tool to help identify people likely to develop type 1 diabetes before symptoms occur. The discovery may facilitate prevention strategies currently in development. A general feature of the autoimmune destruction of insulin-producing pancreatic beta cells that occurs in type 1 diabetes is the body's development of antibodies to beta cell proteins. These antibodies are called autoantibodies because they attack proteins within the body, rather than invading pathogens. Importantly, these autoantibodies generally appear before overt symptoms of diabetes, and are therefore useful as clinical predictors of the disease. The utility of known autoantibodies is limited, however, because some are transient and do not persist until diagnosis, and some people with type 1 diabetes do not develop any of the autoantibodies used in existing screening tests.

To enhance understanding of the pathogenesis of type 1 diabetes and elucidate potential new therapeutic strategies, as well as to improve testing for autoimmunity, a group of researchers sought to identify additional beta cell proteins that generate autoantibodies. By examining a set of proteins made exclusively or almost exclusively in beta cells, and testing with antibodies taken from people with new-onset diabetes, scientists in the NIDDK-funded Beta Cell Biology Consortium (BCBC) have discovered that autoantibodies to a beta cell protein called ZnT8 are an excellent marker for pre-clinical diabetes. Taking advantage of samples collected through the NIDDK's Diabetes Autoimmunity Study in the Young (DAISY), BCBC scientists found that ZnT8 autoantibodies can substantially improve prediction of diabetes when used in combination with the previously discovered autoantibodies commonly used to monitor for pre-clinical type 1 diabetes in research studies. It appears that testing for this combination of autoantibodies can substantially improve the ability to predict risk for developing the disease. Improved risk prediction will facilitate the development, testing, and future administration of therapies to prevent or cure this disease. Once such therapies are available, it is likely that children will be monitored for pre-diabetic autoimmunity and the availability of this test will improve the accuracy of such monitoring. Studies such as DAISY have already shown that identifying individuals with pre-clinical type 1 diabetes can preempt dangerous diabetic episodes in undiagnosed children.

*Wenzlau JM, Juhl K, Yu L, Moua O, Sarkar SA, Gottlieb P, Rewers M, Eisenbarth GS, Jensen J, Davidson HW, and Hutton JC: The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. Proc Natl Acad Sci USA 104: 17040-17045, 2007.*

**New Clues to the Link Between Type 1 Diabetes Susceptibility and the Immune System—Effects of the *PTPN22* Gene:** By studying a genetic variant associated with an increase in risk for the development of type 1 diabetes and other autoimmune diseases, scientists discovered how this variant alters the body's immune system. The variant is a change in the DNA sequence of the gene *PTPN22*, which produces a protein that normally inhibits the activation of the T cells of the immune system. The scientists sought to understand how this gene variant could lead to a complicated disease.

In this study, investigators characterized T cells from people who had the *PTPN22* gene variant. To avoid confounding factors that may be associated with a complex disease, they chose volunteers who had not developed an autoimmune disease. T cells, like most cells, have two copies of every gene, and the T cells from these people had one copy with the change and one normal copy of the gene. The scientists found that the cells with the variant did not function normally based on several laboratory tests. For example, they had decreased levels of a signaling protein (IL-10) that T cells produce to affect other immune cells. These results indicated that the variant in *PTPN22* blocked the biologic steps required for activating T cells during an immune response. In addition, these changes were specific to a subset of T cells called "memory" T cells.

To further understand the effects of the *PTPN22* variant within the human body, the scientists looked at the overall composition of the T cell population in people with the variant, and found that they had more memory T cells than people without the variant. When the scientists explored potential effects on another type of immune cell, B cells (the cells that make antibodies), they noticed a reduction in the

number of a subset of B cells called memory B cells. These results suggested that the variant in *PTPN22* led to differences in the populations of both T and B cell types in the human body. The scientists propose that the many changes they observed in immune cells could create an environment that is susceptible to the development of autoimmunity. By uncovering how a particular gene variant that is associated with disease alters the immune system, scientists are gaining a better understanding of the development of autoimmune diseases, like type 1 diabetes, and identifying potential new targets for therapy.

*Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, and Buckner JH: Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. J Immunol 179: 4704-4710, 2007.*

**Targeting the Immune System To Combat Type 1 Diabetes:** Several recent studies suggest that manipulating dendritic cells of the immune system is a promising strategy to prevent, delay, or reverse type 1 diabetes. Type 1 diabetes is an autoimmune disease in which the patient's immune system destroys the insulin-producing beta cells of the pancreas. Central to the attack on the beta cells are immune system cells called T cells. T cells that recognize proteins from the patient's beta cells are normally eliminated during their maturation. However, in susceptible individuals, these T cells migrate to the pancreas and initiate an inflammatory process that destroys the beta cells and leads to the development of type 1 diabetes. Dendritic cells, also immune system cells, are involved in activating T cells. Recent studies have further developed approaches to modify dendritic cells to prompt the elimination of errant T cells to prevent the destructive immune attack.

In one study, scientists modified dendritic cells in culture to have high levels of a signaling protein called IL-4. Previous studies found that both humans and mice with type 1 diabetes have low levels of IL-4. Injection of these modified dendritic cells into a mouse model of type 1 diabetes significantly prevented or delayed onset of disease. By 30 weeks of age, 80 percent of non-treated mice developed the disease, compared to only 30 percent of the mice treated with the modified cells. This study used dendritic cells that were modified outside of the mouse. Other researchers are developing approaches to modify dendritic cells directly in the animal. For example, one study examined whether "microspheres" could be used to deliver molecules known to shut off CD40, CD80, and CD86, which are proteins found on cells of the immune system. These molecules modify dendritic cells in a way to make them suppress, rather than incite, T cell attacks on beta cells. A single injection of microspheres containing these suppressive molecules significantly delayed onset of diabetes in a mouse model of type 1 diabetes; several consecutive injections prevented the disease altogether. In mice that already had diabetes, the microsphere therapy reversed the disease. In another study using mice, researchers used an antibody to a protein found on dendritic cells to deliver specific molecules—ones that mimic a beta cell component and are recognized by diabetes-inducing T cells—to these dendritic cells. This approach led to the elimination of the destructive T cells. Together, these studies demonstrate that modifying dendritic cells is a possible therapeutic approach for preventing, delaying, or reversing type 1 diabetes. Additional studies to test dendritic cell therapy are needed to determine if it might have the same dramatic benefits in people.

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*Mukhopadhaya A, Hanafusa T, Jarchum I, Chen Y-G, Iwai Y, Serreze DV, Steinman RM, Tarbell KV, and DiLorenzo TP: Selective delivery of beta cell antigen to dendritic cells in vivo leads to deletion and tolerance of autoreactive $CD8^+$ T cells in NOD mice. Proc Natl Acad Sci USA 105: 6374-6379, 2008.*

*Phillips B, Nylander K, Harnaha J, Machen J, Lakomy R, Styche A, Gillis K, Brown L, Lafreniere D, Gallo M, Knox J, Hogeland K, Trucco M, and Giannoukakis N: A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. Diabetes 57: 1544-1555, 2008.*

## GUT BACTERIA AND TYPE 1 DIABETES

**Gut Microbes Protect Against Type 1 Diabetes in Mice:** Research in mice has found that the trillions of bacteria and other microbes that live in the gut can blunt the immune system attack that causes type 1

diabetes. The discovery may shed light on rising rates of type 1 diabetes in developed countries. Scientists don't know exactly what triggers the body's immune attack on beta cells in type 1 diabetes. During the past decades, researchers saw clues in the observed increased incidence of type 1 diabetes in developed countries. The scientists suspected that changes in the environment, including the microbes that live in our bodies, may be influencing the disease. Supporting this idea, previous studies found that the incidence of type 1 diabetes in mice susceptible to this disease can be affected by microbes in their environment. The researchers set out to further explore the possible connection between type 1 diabetes and microbes.

Receptors on certain immune cells recognize molecular patterns that mark the surface of microbes. These immune cells signal through a protein called MyD88 to launch an immune system response. When researchers disrupted the gene for MyD88 in a mouse model of type 1 diabetes, the mice no longer developed the disease. While the researchers confirmed that immune activation in the MyD88-deficient mice was suppressed in pancreatic lymph nodes, it was not eliminated. Thus, type 1 diabetes prevention was likely more than simply a matter of turning off part of the immune system. The researchers therefore raised the mice in a germ-free environment. These same mice developed type 1 diabetes when raised in this type of environment, showing that the disease is not dependent solely on the MyD88 pathway. The researchers next gave the germ-free mice a defined mix of "friendly" gut bacteria and found that the incidence of diabetes was significantly reduced. These experiments show that a complex interaction between the immune system and bacteria in the gut may help to lower the risk of developing type 1 diabetes. The widespread use of antibiotics and more aggressive cleanliness of modern society can alter the mix of microbes living in our body. This research suggests that an unintended consequence of this environmental change is an increased risk of autoimmune diseases like type 1 diabetes. The idea opens avenues for further exploration and hints at the possibility of developing bacteria-based treatments for people with autoimmune diseases.

*Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, and Chervonsky AV: Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature 455: 1109-1113, 2008.*

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## BETA CELLS AND DIABETES

**Adult Pancreas Cells Reprogrammed to Insulin-producing Beta Cells:** New research has taken a step closer to cell replacement therapy for diabetes. Scientists made an exciting discovery that some adult cells in the mouse pancreas, called exocrine cells, can be reprogrammed to become insulin-producing beta cells. Beta cells are at the center of the development of both type 1 and type 2 diabetes, and researchers are vigorously trying to find ways to replace damaged or destroyed beta cells in people with diabetes.

One way to approach this difficult task is to reprogram different adult cell types into beta cells. The pancreas is made up of many different cell types, of which exocrine cells are the most plentiful. To identify factors to reprogram exocrine cells, they focused on proteins called transcription factors, which regulate whether genes are turned on or off. Although over 1,100 transcription factors are known to be important in pancreatic development, the scientists limited their experiments to testing nine transcription factors of key importance, based on knowledge from earlier research on pancreatic development from embryonic to adult stages.

Using a genetically-engineered virus, they delivered a mix of the nine factors into pancreases of mice. By removing one factor at a time from the mix, the researchers identified a combination of just three transcription factors that reprogrammed some of the exocrine cells into beta cells. The newly-formed beta cells produced enough insulin to decrease high blood glucose levels in diabetic mice. This "reprogramming" appeared to occur directly from the exocrine cell type to the beta cell type, and the procedure did not require the addition of stem cells. If the same type of approach works in humans, this discovery could have a dramatic impact on the ability to increase beta cell mass in people with diabetes. While much work remains to be done before this becomes a safe and effective therapy, this adult cell reprogramming is a major step forward and serves as a model for other applications of regenerative medicine.

*Zhou Q, Brown J, Kanarek A, Rajagopal J, and Melton DA: In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455: 627-632, 2008.*

**New Insights into the Molecular Contributors to Gestational Diabetes Mellitus:** Researchers have identified a protein that may be involved in the development of gestational diabetes mellitus (GDM). GDM is a form of glucose intolerance diagnosed in some women during pregnancy. Pregnancy is a time when the body becomes slightly less sensitive to insulin and more insulin is needed to compensate. Studies suggest that, to meet this demand, insulin-producing beta cells proliferate. However, the molecular mechanisms underlying pregnancy-induced beta cell proliferation are poorly understood. Furthermore, it is unclear if impaired beta cell proliferation plays a role in the development of GDM.

To enhance understanding of the molecular underpinnings of beta cell proliferation during pregnancy, researchers studied a protein called menin. This protein had previously been found to play a role in cancer. Loss of menin promotes neuroendocrine tumors, including beta cell tumors. Because these observations suggest that this protein may regulate the growth of beta cells, researchers hypothesized that it may play a role in beta cell growth during pregnancy. They discovered that levels of menin, as well as levels of proteins controlled by menin, decreased during pregnancy in mouse islets, and then returned to prepartum levels after birth, corresponding to observed changes in maternal beta cell mass. To determine the effect of artificially increasing menin levels during pregnancy, the researchers generated a genetically-engineered mouse model in which they could induce higher levels of the protein in the animals' beta cells. They observed that increased menin levels during pregnancy caused the mice to have features of human GDM, including high blood glucose and insufficient insulin levels. In addition, pregnancy-induced beta cell proliferation was inhibited in these animals. The researchers also observed that administration of prolactin, a hormonal regulator of pregnancy, to non-pregnant mice reduced menin levels in islets and increased beta cell proliferation. These results suggest that menin is a key regulator of beta cell proliferation during pregnancy.

This study in mice sheds new light on the molecular mechanisms that underlie beta cell proliferation during pregnancy and the development of GDM. Menin was discovered by investigators in the NIDDK Intramural Research Program as the gene defective in a rare endocrine disorder. Further understanding of the role of this protein sheds light on GDM and mechanisms of beta cell proliferation. If it plays a similar role in regulating beta cell proliferation in humans, then menin—or other proteins in the menin pathway—may be potential therapeutic targets for treating or preempting GDM and other forms of diabetes.

*Karnik SK, Chen H, McLean GW, Heit JJ, Gu X, Zhang AY, Fontaine M, Yen MH, and Kim SK: Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. Science 318: 806-809, 2007.*

## DIABETES AND ITS COMPLICATIONS

**First Year of Look AHEAD (Action for Health in Diabetes) Trial Yields Encouraging Results:** The Look AHEAD trial has enrolled over 5,000 participants to determine the effects of an intensive lifestyle intervention on the long-term health of overweight and obese adults with type 2 diabetes. At enrollment, the study population had an average age of 59 years with 60 percent female and 37 percent minority. In addition, approximately 85 percent were obese and 87 percent were on diabetes medications. Few individuals were achieving optimal control of hemoglobin A1c (HbA1c, a measure reflective of blood glucose control over the preceding 2-3 months), blood pressure, and "bad" or low density lipoprotein (LDL)-cholesterol levels. High blood glucose, blood pressure, and cholesterol may all contribute to diabetes-related vascular complications, including cardiovascular, eye, and kidney disease; therefore, control of these three conditions is especially important in people with diabetes. At enrollment, only 10 percent of individuals in the Look AHEAD population were meeting all three goals established by the American Diabetes Association (ADA) of HbA1c less than 7.0 percent, blood pressure less than 130/80 mmHg, and LDL-cholesterol less than 100 mg/dl. Among Caucasians, 11.6 percent met all three goals, while only 5.1 percent of African Americans and 7.9 percent of Hispanics did. Examples of other

factors associated with suboptimal control of all three measurements included higher body mass index (BMI, a measure of weight relative to height), lower level of education, and longer duration of diabetes. Individuals who used diabetes medication also were less likely to meet all three goals than individuals who were not on diabetes medication. This seemingly contradictory result may be due, in part, to medication being prescribed to those individuals with longer duration of diabetes whose disease was at a more advanced stage and thus more difficult to control.

As reported in the 2008 edition of *NIDDK's Recent Advances & Emerging Opportunities*, first year results of the trial showed that overweight or obese people with diabetes who had received a year of intensive lifestyle intervention—including regular individual and group counseling, structured meal plans, and customized exercise programs—lost an average 8.6 percent of their initial body weight. By comparison, a similar group that received standard diabetes education and support lost only 0.7 percent of their body weight. This 7 to 10 percent weight loss had a big clinical impact. HbA1c, blood pressure, and LDL-cholesterol improved in both groups, but participants in the lifestyle intervention group saw greater reductions. In the lifestyle group, the percent of people meeting all three ADA goals more than doubled. From a beginning average of 7.3 percent, the mean HbA1c level dropped to 6.6 percent in the lifestyle intervention group, versus 7.2 percent in the group that received the usual care. Lifestyle intervention group participants also reduced their use of diabetes, blood pressure, and lipid-lowering medicines, while improving control of these factors.

*Bertoni AG, Clark JM, Feeney P, Yanovski SZ, Bantle J, Montgomery B, Safford MM, Herman WH, and Haffner S; The Look AHEAD Research Group: Suboptimal control of glycemia, blood pressure, and LDL cholesterol in overweight adults with diabetes: the Look AHEAD Study: J Diabetes Complications 22: 1-9, 2008.*

*The Look AHEAD Research Group: Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: One-year results of the Look AHEAD trial: Diabetes Care 30: 1374-1383, 2007.*

**Hearing Loss Is Common in People with Diabetes:** A recent study has found that hearing loss is about twice as common in adults with diabetes compared to those who do not have the disease. NIH scientists analyzed data from hearing tests administered from 1999 to 2004 to participants in the National Health and Nutrition Examination Survey, which is conducted by the Centers for Disease Control and Prevention (CDC). Over 5,100 survey participants aged 20 to 69 completed a hearing exam that used a machine to measure hearing sensitivity across a range of sound frequencies—low, middle, and high frequency. These participants also completed a questionnaire in which they were asked to report if they had ever been told by a doctor that they had diabetes. After accounting for differences in age, the prevalence of low- or mid-frequency hearing impairment of mild or greater severity in the worse ear was about 21 percent among 399 adults with diabetes, compared to about 9 percent among 4,741 adults without diabetes. For high frequency sounds, mild or greater hearing impairment in the worse ear was found in 54 percent of those with diabetes, compared to 32 percent of those who did not have the disease. Greater hearing impairment in people with diabetes was evident as early as ages 30 to 40. The association between diabetes and hearing loss was observed even after accounting for other factors (in addition to age) that are known to affect hearing, including race, ethnicity, income level, noise exposure, smoking, and the use of certain medications. The researchers also evaluated a subset of participants and found that adults with pre-diabetes had a 30 percent higher rate of hearing loss compared to those with normal blood glucose tested after an overnight fast. Although the study did not directly address how diabetes may cause hearing loss, evidence from other studies suggests that the nerve and blood vessel damage that occurs in diabetes and leads to eye, kidney, and nerve complications may also affect the inner ear. This study of a large, nationally representative population sample indicates that hearing loss may be an under-recognized complication of diabetes that health care providers should be aware of in managing the care of people with diabetes.

*Bainbridge KE, Hoffman HJ, and Cowie CC: Diabetes and hearing impairment in the United States: Audiometric evidence from the National Health and Nutrition Examination Survey, 1999 to 2004. Ann of Int Med 149: 1-10, 2008.*

**Depression and Diabetes—Clues to the Relationship Between Physical and Mental Health:** Scientists have determined that depression and type 2 diabetes

can influence one another, and are making strides in understanding the biology behind this relationship. In previous studies, depression and depressive symptoms were observed to be more common among people with diabetes than in the general population. But does a diagnosis of diabetes increase the risk of developing depression? Does depression increase the risk of developing diabetes? In one study to understand the relationship between these two complex conditions, scientists analyzed the mental and the physical health of men and women enrolled in the National Heart, Lung, and Blood Institute's Multi-Ethnic Study of Atherosclerosis. Each volunteer participated in multiple examinations over an average of 3 years, including evaluations of their blood glucose levels, development and/or progression of diabetes, and quality of life to assess their mental well-being. Since many variables are likely to affect both diabetes and depression, the scientists also documented factors like age, ethnicity, gender, body mass index, socioeconomic factors, lifestyle, and measures of metabolic and inflammatory markers.

The investigators examined the data to determine the frequency of development of diabetes among participants who already had symptoms of depression, and the development of depressive symptoms among participants diagnosed with diabetes. The scientists noted that people with multiple depressive symptoms had a modestly increased risk of developing type 2 diabetes. These results suggest that it may be important to consider future studies to determine whether interventions that treat depression may be of benefit in preventing type 2 diabetes. In addition, the investigators observed that individuals being treated for type 2 diabetes had a significantly greater risk of developing depressive symptoms. These results suggest that physicians treating people with type 2 diabetes should consider routinely screening their patients for depressive symptoms.

*Golden SH, Lazo M, Carnethon M, Bertoni AG, Schreiner PJ, Roux AVD, Lee HB, and Lyketsos C: Examining a bidirectional association between depressive symptoms and diabetes. JAMA 299: 2751-2759, 2008.*

**Intensive Lowering of Blood Glucose May Be Harmful to Patients with Type 2 Diabetes and a High Risk of Cardiovascular Disease:** A large clinical trial has shown that, for individuals with type 2 diabetes who were also at especially high risk of heart disease and stroke, the use of intensive therapy to lower blood glucose (sugar) levels to near normal levels led to increased mortality and did not significantly reduce major cardiovascular events.

Type 2 diabetes is characterized by high blood glucose levels, and having diabetes increases the risk of many serious health complications, including cardiovascular disease. In addition, people with type 2 diabetes may be overweight and have blood pressure and lipid or cholesterol levels that further add to their cardiovascular disease risk. Prior studies suggested that reducing blood glucose levels to those found in adults without diabetes may reduce the risk of cardiovascular events, such as heart attack and stroke, among people with type 2 diabetes. However, a randomized clinical trial was needed to determine the validity of this hypothesis.

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial, led by the National Heart, Lung, and Blood Institute with support from the NIDDK, enrolled over 10,000 adult volunteers who had type 2 diabetes and an especially high risk of cardiovascular disease. The trial was designed to test three treatment approaches to decrease the high rate of major cardiovascular events observed in people with this combination of health problems. The treatment approaches included intensive lowering of blood glucose levels, intensive lowering of blood pressure, and treatment of blood lipids (i.e., fats like cholesterol). Each of the intensive therapies was compared with a more standard therapy. While the study to test the effect of intensively lowering blood glucose levels has ended, the two other studies are currently ongoing.

Blood glucose levels are measured by a test called hemoglobin A1c (HbA1c), which reflects a person's average level of blood glucose in the previous 2 to 3 months. The ACCORD trial randomly assigned participants, who had on entry an average HbA1c of 8.1 percent, to either intensive therapy to lower HbA1c to a target of below 6.0 percent—a level close to normal for adults without diabetes—or to standard therapy to achieve an HbA1c target of 7.0-7.9 percent. ACCORD participants received medications to lower their blood glucose levels; the type, number, and dosages of the drugs varied, depending on the participants' individual needs and the HbA1c goal.

Participants in the intensive therapy group achieved, on average, HbA1c values lower than standard therapy group participants; half achieved an HbA1c of less than 6.4 percent after 1 year. After following volunteers for an average of 3.5 years, however, the researchers observed that intensively lowering blood glucose levels actually increased mortality in this group. Moreover, it did not significantly reduce major cardiovascular events compared to standard therapy. Although patients in both the intensive and standard groups had lower mortality than reported in other studies of similar patients, the NIH, on the advice of a committee monitoring the study, decided to end this part of the study, and all participants were switched to treatment with standard therapy.

Participants in the ACCORD trial had already had diabetes for an average of 10 years and were either at particularly high risk for a cardiovascular event (two or more risk factors beyond diabetes) or had been diagnosed with cardiovascular disease before entering the study. Thus, the results of this study may not apply to patients with type 2 diabetes who are not similar to the ACCORD participants. After the results of this study were released, diabetes experts agreed that the blood glucose level goal currently recommended by the American Diabetes Association (an HbA1c level of 7 percent) continues to be appropriate for the general population with diabetes. However, less aggressive therapy may be appropriate for individuals with limited life expectancy, cardiovascular disease, or frequent low blood glucose reactions. Importantly, people with diabetes should talk with their doctor before making any changes to their treatment regimen.

*The Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH Jr, Probstfield JL, Simons-Morton DG, and Friedewald WT: Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 358: 2545-2559, 2008.*

## REGULATORS OF METABOLISM IN HEALTH AND DISEASE

**A Novel Pathway To Promote New Blood Vessel Growth:** Scientists have discovered a novel molecular pathway in mice that promotes new blood vessel growth, a process called angiogenesis. Angiogenesis is critically important to the development and growth of a healthy embryo. It is also important for health in adults; numerous diseases are caused by either excessive or insufficient angiogenesis. Research previously showed that low levels of oxygen in the body are detected by a group of proteins called "hypoxia inducible factors." These proteins activate the production of vascular endothelial growth factor, or VEGF, which stimulates angiogenesis so that the body receives sufficient supplies of oxygen and nutrients from the blood. Researchers have now demonstrated that another protein, called PGC-1alpha, is also a key regulator of angiogenesis. The scientists first showed that, in cell culture, levels of this protein increase when the cells are exposed to low levels of oxygen or nutrients. Increased levels of PGC-1alpha in turn lead to increased levels of VEGF and other factors known to be involved in promoting angiogenesis. Then, to determine if PGC-1alpha regulates angiogenesis in an animal model, the researchers studied two different groups of genetically-engineered mice: one group produced high levels of PGC-1alpha in their skeletal muscle and the other group lacked the protein altogether. The researchers observed that angiogenesis was accelerated in the mice that had high levels of the protein, whereas angiogenesis was impaired after injury in the mice that lacked it. These results suggest that PGC-1alpha is an important regulator of angiogenesis in a mouse model. Surprisingly, the protein does not stimulate angiogenesis through the well-known signaling pathway involving the hypoxia inducible factors. Rather, it uses an alternate route—a novel signaling pathway that involves a protein called ERR-alpha, which regulates whether certain genes are turned on or off. The two proteins work together to increase levels of VEGF, which in turn promotes angiogenesis.

Decreases in blood supply to the heart, brain, and limbs are a leading cause of morbidity and mortality throughout the world, and scientists are actively trying to develop therapies to target angiogenesis. The discovery of a second pathway involved in new blood vessel growth, which involves PGC-1alpha and ERR-alpha, may result in new opportunities for therapy. PGC-1alpha has already been well studied for its role in diabetes and obesity, and molecules that increase its activity are in development.

*Arany Z, Foo S-Y, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH,*

*Rosenzweig A, and Spiegelman BM: HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. Nature 451: 1008-1012, 2008.*

**Powering Up Mice with PEPCK-C:** A fascinating new discovery in energy metabolism may point the way to new therapeutic approaches for obesity. In humans and other mammals, the use and storage of energy—or energy metabolism—is driven by millions of chemical reactions taking place within cells. Some reactions release energy to power daily activities, for example, while others transform it into fat and other energy storage molecules. Now, scientists have found that, in mice, changing the amount of a single protein in just one tissue leads to a profound change in energy metabolism. The protein, an enzyme called PEPCK-C, helps some body tissues make glucose (sugar) and related energy-rich molecules for immediate use or storage. While it plays a key role in the functions of liver, kidney, and fat tissue, the PEPCK-C enzyme is not normally present in high amounts in skeletal muscle, a major site of energy metabolism. In a recent study, researchers used genetic engineering to create mice that overexpress the PEPCK-C gene in their skeletal muscle. These "PEPCK-C mice" make about 100-fold more active enzyme in their skeletal muscle than normal. When compared to "control" mice that don't have extra PEPCK-C, the PEPCK-C mice were not only much more active in their cages, but they also were able to run up to 30 times farther on a treadmill. Moreover, although they ate about 60 percent more food, the highly active adult PEPCK-C mice weighed only half as much as the control mice and had only one-tenth the body fat. When the researchers examined skeletal muscle cells from the PEPCK-C mice, they saw that the cells had many more mitochondria—the so-called powerhouses of the cell—which control much of cellular energy metabolism. The mice also had significantly more intramuscular fat, which the mitochondria can convert to energy—possibly explaining in part how these mice could fuel their high activity. Finally, despite their higher metabolism, the PEPCK-C mice were actually reproductively active longer and lived longer on average than the control mice. Strikingly, the results of PEPCK-C overexpression in skeletal muscle observed in this study stand in sharp contrast to the effect of higher-than-normal levels of the same enzyme in mouse fat tissue, which were found in earlier studies to cause obesity. The mechanisms underlying these intriguing and contrasting results in mice have yet to be fully explained. However, the study provides new opportunities for exploration to understand energy metabolism and, perhaps eventually, to reduce the onset or effects of overweight and obesity.

*Hakimi P, Yang J, Casadesus G, Massillon D, Tolentino-Silva F, Nye CK, Cabrera ME, Hagen DR, Utter CB, Baghdy Y, Johnson DH, Wilson DL, Kirwan JP, Kalhan SC, and Hanson RW: Overexpression of the cytosolic form of phosphoenolpyruvate carboxykinase (GTP) in skeletal muscle repatterns energy metabolism in the mouse. J Biol Chem 282: 32844-32855, 2007.*

**A New Chemical Tool for Studying a Protein Implicated in Type 2 Diabetes:** Following synthesis in the cell, proteins are often modified by addition of other chemicals that give them important, new biochemical properties. One common type of protein modification is glycosylation—the addition of sugars. A recent NIDDK intramural study sought to provide tools for better understanding an enzyme that catalyzes the reverse process—deglycosylation—for certain sugars on certain proteins. A mutation in the gene that encodes the enzyme, *O*-GlcNAcase, has been implicated in type 2 diabetes in a Mexican American population. *O*-GlcNAcase is found in two forms in the cell, one long and one short. Although the long form of the enzyme is easily studied using previously described biochemical inhibitors, scientists lacked an inhibitor that is effective against the short form. This new study describes an inhibitor that is significantly more potent against the short form of *O*-GlcNAcase, providing a useful tool for studying this important enzyme, and potentially for understanding the role of the enzyme in type 2 diabetes.

*Kim EJ, Amorelli B, Abdo M, Thomas CJ, Love DC, Knapp S, and Hanover JA: Distinctive inhibition of O-GlcNAcase isoforms by an alpha-GlcNAc thiolsulfonate. J Am Chem Soc 129: 14854-14855, 2007.*

## CYSTIC FIBROSIS RESEARCH

**New Animal Model To Study Cystic Fibrosis:** Scientists have generated a new animal model to advance research on cystic fibrosis (CF). Animals are important in research because they can be used to study underlying mechanisms of disease and to test

new therapies before they are tested in people. Animal models are most useful if they closely mimic the human disease. This has not been the case for CF, because mice with the human CF mutation, for example, do not have the same characteristics of human disease, thus limiting their usefulness in CF research. Scientists sought to generate an animal model that more closely resembles human CF. They chose pigs, because pig and human anatomy and physiology are comparatively similar. The scientists genetically engineered pigs to lack the gene that is mutated in CF, called *CFTR*. The newborn piglets lacking *CFTR* had remarkably similar characteristics to newborn humans with CF, including abnormalities involving the pancreas, intestine, gallbladder, and liver. Lung disease in CF is caused by infection and inflammation. Which comes first remains an important question. At birth, researchers found no evidence of infection or inflammation in the pigs, a situation similar to newborn humans with CF. Scientists can use the pig model to gain a greater understanding of how lung disease develops in CF. Because the new pig model closely mimics the human disease, it is an important new resource for scientists studying CF. The pig model provides new opportunities to enhance understanding about CF and to develop new treatment strategies.

*Rogers CS, Stoltz DA, Meyerholz DK, Ostedgaard LS, Rokhlina T, Taft PJ, Rogan MP, Pezzulo AA, Karp PH, Itani OA, Kabel AC, Wohlford-Lenane CL, Davis GJ, Hanfland RA, Smith TL, Samuel M, Wax D, Murphy CN, Rieke A, Whitworth K, Uc A, Starner TD, Brogden KA, Shilyansky J, McCray PB Jr, Zabner J, Prather RS, and Welsh MJ: Disruption of the CFTR gene produces a model of cystic fibrosis in newborn pigs.* Science *321: 1837-1841, 2008.*

## STORY OF DISCOVERY

# *Ability To Predict Type 1 Diabetes Offers Hope for Disease Prevention*

Type 1 diabetes is a devastating disease that most often strikes during childhood, and invariably lasts for the rest of one's life. During every day of the lives of the millions of people with this disease worldwide, consistent attention and vigilance is required to ward off devastating diabetic complications that shorten and reduce the quality of their lives. Therefore, a key goal of NIDDK research is to develop ways to prevent type 1 diabetes from occurring in the first place. Toward realizing that goal, scientists have cleared a critical hurdle by learning how to identify people who are likely to develop the disease.

Being able to predict who will get type 1 diabetes is of obvious importance in identifying people who would benefit from prevention strategies once they are developed. But in fact it is also a key step in the development of interventions to prevent the disease. With the ability to predict type 1 diabetes risk, it becomes feasible to conduct multiple trials in those at risk, so as to increase the possibility of finding the best prevention approach. This is precisely what is being accomplished today through programs like Type 1 Diabetes TrialNet, led by NIDDK, and TRIGR (Trial to Reduce IDDM in the Genetically at Risk), led by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Both programs are supported in part by the Special Statutory Funding Program for Type 1 Diabetes Research.

The scientific achievement of predicting type 1 diabetes was developed through decades of efforts by scientists in several disciplines—immunology, genetics, and epidemiology—working in several countries. Although the clinical appearance of type 1 diabetes is often sudden, with symptoms developing over weeks or days, researchers now know that the disease frequently develops gradually and silently over many years. A key advance was the ability to detect the autoimmune hallmarks of the disease prior to the actual development of type 1 diabetes. Researchers in the 1960s recognized that people with diabetes often make antibodies to insulin, a hormone produced by pancreatic beta cells that are aberrantly destroyed in type 1 diabetes, necessitating treatment with exogenously-supplied insulin. Because these antibodies often arose prior to insulin treatment, the scientists correctly surmised that the people were actually developing antibodies to the insulin being made by their own bodies. Antibodies against one's own proteins are termed "autoantibodies," and are a hallmark of autoimmune diseases like type 1 diabetes.

Indeed, researchers later discovered that people with type 1 diabetes often produce antibodies not only to insulin, but also to several other proteins produced by pancreatic beta cells. Significantly, the appearance of autoantibodies nearly always precedes the onset of overt symptoms of type 1 diabetes, when a person still has an adequate number of insulin-producing beta cells to control blood glucose. Testing for the presence of beta cell autoantibodies therefore became a promising approach to predicting the disease before its clinical appearance.

Several scientists, including NIDDK-supported researchers, worked to turn the discovery of autoantibodies into a useful tool by developing robust, standardized autoantibody tests. Importantly, they recognized that the simple presence or absence of an autoantibody does not provide as much information as accurate measurement of the levels or titer of antibody in the blood. Assays to measure antibodies can now be performed such that each test has a very low false-positive rate. Although onset of the disease

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is usually preceded by creation of antibodies to at least one of these proteins, at any given time a person destined to develop type 1 diabetes only makes autoantibodies to a variable subset of them. The presence of any one of these autoantibodies signals substantially elevated risk, and risk increases as the number of autoantibodies rises.

But type 1 diabetes is such a complex disease that, to be accurately interpreted, an autoantibody test needs to be viewed in the context of more information about the patient. It has long been known that people with a parent, brother, or sister with the disease are more likely to get type 1 diabetes than the population at large. However, most people with such relations will not get the disease, and many people without a known relative who has the disease will. The reasons for this are complex, but an important part of the answer stems from the fact that several genes turn out to predispose a person to type 1 diabetes, while several others actually have a protective effect.

The first major breakthrough in the genetic part of the puzzle came in the 1970s, with the discovery that two particular versions of a gene called *HLA*, which makes a key immune recognition protein, are much more common in people who have type 1 diabetes, suggesting they increase the likelihood of the disease. It was later discovered that certain other versions of this highly variable gene can help protect against the disease. Still other versions of HLA are more neutral in their impact. A person can acquire a high-risk version from one parent, and a protecting version from the other. The overall effect of the HLA variants accounts for a very large proportion of the genetic risk for type 1 diabetes.

With the knowledge of HLA and autoantibody associations with type 1 diabetes, NIDDK-supported scientists designed a prevention trial, the NIDDK-supported Diabetes Prevention Trial Type 1 (DPT-1), which successfully used genetic and autoantibody tests to predict risk for developing type 1 diabetes. To identify "those at risk," the researchers first selected thousands of people who have a close relative with type 1 diabetes, and then screened them for autoantibodies. Those with autoantibodies were then tested for the protective version of HLA. People who had autoantibodies and no protective HLA were tested for their response to glucose, to see whether they were already displaying signs of diabetes. Indeed, some already had the disease, and simply did not know it. The rest fell into two categories: those with a normal response to a glucose challenge were considered to have a "moderate" (26-50 percent) chance of developing type 1 diabetes within 5 years; those with a response to glucose that was weaker (but did not meet the definition of overt diabetes) were considered to have a greater than 50 percent chance of developing the disease within 5 years. Although the specific prevention strategies tested in this trial did not turn out to have a broadly protective effect, the researchers' estimates of risk for type 1 diabetes, based on their screening, proved to be remarkably accurate. Thus, the DPT-1 trial was enormously valuable in demonstrating that it is possible to identify those at high risk for type 1 diabetes—enabling researchers to conduct further studies to test new prevention strategies.

NIDDK-supported scientists are continuing to discover potential new ways to improve prediction of diabetes risk. For example, researchers recently identified another autoantibody that, when combined with tests for the previously-known autoantibodies, improves the predictive power of this approach. (Please see the advance on a new autoantibody for type 1 diabetes described earlier in this chapter.) Researchers are continuing to discover other genes that impact the probability of developing type 1 diabetes. At current count there are over 40 such genes, largely discovered through the efforts of the Type 1 Diabetes Genetics Consortium, made possible by support from NIDDK and the Special Statutory Funding Program for Type 1 Diabetes Research. Individually, none of these new genes has as large

an impact as HLA, but collectively their effect is significant.

With further genetic, autoantibody, or other predictive markers and tools, it may be possible to define risk for type 1 diabetes even more precisely, and to extend such predictive tests to the population as a whole. Such predictive markers may also help scientists identify potential environmental triggers of the disease. Improved tests to assess risk not only would facilitate additional research on prevention strategies, but could also advance research on ways to reverse the disease in its earliest stages and, importantly, enable the resulting interventions to benefit more people.

## STORY OF DISCOVERY

# *Diabetes and Cardiovascular Disease*

Seminal clinical trials have revealed the power of good control of blood glucose (sugar) early during the course of type 1 and type 2 diabetes to reduce later risk for eye, kidney, and nerve complications. Now, clinical trials are examining the more complex relationship between blood glucose control and cardiovascular disease (CVD) in type 2 diabetes. One recent study showed that more intensive control than currently recommended, targeting near normal blood glucose levels, can be dangerous in those with long-duration type 2 diabetes with established CVD or at high risk of developing CVD. Two other recent trials found neither cardiovascular harm nor benefit of moving from "good" to near-normal glucose levels. However, another study found that targeting good glucose control early in the course of disease can reduce cardiovascular risks decades later for many patients with type 2 diabetes. Similar cardiovascular benefits emerging long after a finite period of intensive glucose control were reported previously for individuals with type 1 diabetes. Because CVD is the leading cause of death in people with type 2 diabetes, identification of ways to reduce this risk is particularly important. There is very strong evidence that blood pressure and cholesterol control can markedly reduce CVD, but the effects of glucose control on CVD in type 2 diabetes remained an open question. Taken together, the new results refine the approach to treating diabetes and demonstrate the importance of tailoring therapy to individual patient characteristics.

**Diabetes Increases the Risk of Death from Cardiovascular Disease**

An estimated 23.6 million Americans have diabetes, about 5.7 million of whom have not been diagnosed.[1] Type 1 diabetes, which accounts for 5-10 percent of diagnosed diabetes cases, is an autoimmune disease that often begins in childhood or early adulthood, although it can strike at any age. The majority of people with diabetes have type 2 diabetes—a form

**Drug Therapies for Type 2 Diabetes**

There are many medications available to help people with type 2 diabetes lower their blood glucose. These medications fall into several classes:

- Insulin: moves glucose from blood into cells
- Metformin: reduces output of glucose from the liver and reduces insulin resistance
- Thiazolidinediones: reduce insulin resistance, by a different mechanism than metformin
- Sulfonylureas: promote release of insulin by the pancreas
- Meglitinides: promote release of insulin by the pancreas (shorter and faster acting than sulfonylureas)
- D-phenylalanine derivative: promotes release of insulin
- GLP1-analogs: stimulate production of insulin and slow gastric (stomach) emptying
- DPP-4 inhibitors: slow destruction of GLP1 and stimulate production of insulin
- Amylin analogs: slow glucose absorption from intestine, reduce glucose production by liver, and decrease appetite
- Alpha-glucosidase inhibitors: interfere with digestion and utilization of carbohydrates like starch and table sugar

Other promising therapeutic approaches are currently in development.

of the disease that is typically associated with excess body weight and older age. In part due to the increase in childhood obesity, however, children increasingly are being diagnosed with type 2 diabetes. Both type 1 and type 2 diabetes are also influenced by genetic susceptibility. While both forms of diabetes are characterized by excessively high levels of glucose in the blood, type 1 diabetes and type 2 diabetes have different causes and are treated differently, particularly at disease onset. From the moment of diagnosis, because their insulin-producing cells have been destroyed, people with type 1 diabetes must depend on exogenous insulin, provided by injections or an insulin pump, for survival. Type 2 diabetes, in contrast, is often managed with changes in diet and exercise in its early stages. Insulin-producing cells may still be functioning in type 2 diabetes, but not sufficiently to overcome the insulin resistance that characterizes this form of the disease. A wide variety of prescription medications have been developed to help lower blood glucose in people with type 2 diabetes. (See inset box). Because these drugs act in various ways to lower blood glucose levels, some may be used in combination with others. Many people with type 2 diabetes also need to take insulin to optimally control their blood glucose levels, especially after having the disease for many years.

Despite markedly different causes and treatment options, type 1 diabetes and type 2 diabetes share a common outcome: excess glucose in the blood gradually leads to damaged blood vessels in organs throughout the body. Injury to small blood vessels, known as microvascular disease, increases the risk of blindness, kidney failure, nerve damage, and lower limb amputation. Injury to larger blood vessels, known as macrovascular disease, leads to elevated rates of heart attack, stroke, and other cardiovascular complications in people with diabetes. In general, two out of three adults with diabetes will die of cardiovascular disease or stroke—a risk that is two to four times higher than that for people without diabetes.[1] For people with type 1 diabetes, the risk of death from CVD may be as much as 10-fold greater than the general population of the same age.[2,3] This elevated risk of cardiovascular death shortens the expected life span of people with diabetes by several years.

**Long-Term Benefits of Intensive Glucose Control Established for Microvascular Complications**

Diabetic complications result from many years of gradual glucose-mediated damage to blood vessels. Thus, clinical trials of new therapies for preventing complications are designed to follow participants' health outcomes over long periods of time following initial treatment.

In 1983, the NIDDK's Diabetes Control and Complications Trial (DCCT) was launched with 1,441 volunteers with type 1 diabetes randomly divided into two groups. One group received what was standard insulin therapy at the time—one or two insulin injections per day. The other group was taught to manage their blood glucose intensively with frequent monitoring of glucose levels and multiple insulin injections daily or use of an insulin pump. The study was designed to test the ability of intensive glucose control to reduce eye damage and other microvascular complications. The study relied on a blood test (HbA1c) which gauges the average blood glucose over the previous 2 to 3 months. A normal HbA1c is below 6 percent. Throughout the study the average HbA1c value in the standard therapy group was 9.1 percent, whereas in the intensive therapy group the value was 7.3 percent—a significant difference in glucose control.

This difference, when maintained over an average of 6.5 years, yielded multiple health benefits: participants in the intensive therapy group exhibited lower rates of eye disease (76 percent reduction in risk), kidney disease (50 percent reduction), and nerve damage (60 percent reduction). Thus, the intervention to improve glucose control was clearly an effective means to lower the risk of microvascular complications in type 1 diabetes. However, because the DCCT participants were relatively young and healthy at

the start of the trial, and because CVD typically takes a longer time to develop than other diabetes complications in patients with type 1 diabetes, it was not possible for researchers to assess the effect of intensive glucose control on cardiovascular risks during the 10 years of the trial.

Longer follow-up demonstrated additional benefits. At the conclusion of the DCCT, participants returned to the care of their regular health care providers. However, researchers continued to observe the health of more than 90 percent of the DCCT participants in an ongoing follow-up NIDDK effort called the Epidemiology of Diabetes Interventions and Complications Study (EDIC). By continuing to observe these well-characterized patient groups, the investigators hoped to determine whether the interventions that had worked so well to reduce microvascular disease risk might also yield a long-term benefit of reducing CVD.

In the EDIC study, the HbA1c levels of the study groups gradually equalized over time as glucose control in the original conventional therapy group improved, while that of the intensive therapy group worsened. Intriguingly, the EDIC initially found that differences in risk for microvascular complications between the original study groups persisted for at least 8 to 10 years, *even though the difference in HbA1c levels disappeared*. Then, in 2005, EDIC investigators reported for the first time that intensive glucose control during the DCCT trial period could also reduce long-term CVD risks in type 1 diabetes. Twelve years after the DCCT had ended, members of the original intensive therapy control group had a *42 percent lower risk for heart disease and a 57 percent lower risk for non-fatal heart attacks, strokes, or death from a cardiovascular event* compared with those who had been in the standard treatment group.

A similar trial for type 2 diabetes was conducted in the United Kingdom (U.K.) from 1977 to 1997. In the U.K. Prospective Diabetes Study (UKPDS), which was supported in part by NIDDK, more than 4,000 newly-diagnosed type 2 diabetes patients were stratified by body weight and randomly assigned to one of four treatment groups: conventional therapy, primarily through dietary changes, or intensive therapy to lower blood glucose levels to close to normal using one of the following three diabetes medications: (1) insulin; (2) a sulfonylurea drug; or (3) metformin. (Only participants who met the trial definition of overweight could be randomly assigned to primary metformin treatment in the UKPDS.) Like the DCCT, the UKPDS demonstrated that intensive therapy to control blood glucose and lower HbA1c levels could reduce the risk of microvascular disease in people with diabetes. UKPDS results suggested that intensive therapy might also confer a benefit with respect to CVD, but, at the conclusion of the intervention—patients were followed for an average of 10 years—the differences were not statistically significant. Therefore, an important question remained unanswered as to whether intensive control could protect people with type 2 diabetes from CVD.

**Long-Sought Information Emerges on Glucose Control and Cardiovascular Disease**

Because of its substantial impact on the health and lives of people with diabetes, researchers have long sought effective strategies to prevent or manage diabetic CVD. Several clinical trials had proven that carefully controlling blood pressure and cholesterol levels—both of which contribute to CVD risk—substantially reduces cardiovascular events in people with type 2 diabetes. At the conclusion of the intervention, the UKPDS, the first major clinical trial to examine the effects of intensive glucose control in type 2 diabetes, fell short of proving that improved control of blood glucose levels reduced CVD.

Because the DCCT and the UKPDS trials had proven that good glucose control reduced microvascular complications in both type 1 and type 2 diabetes, subsequent expert guidelines for blood glucose management recommended an HbA1c target of 7 percent, the level of control targeted in UKPDS and

proven to reduce eye, kidney, and nerve complications. Widespread acceptance of those recommendations meant that any subsequent attempt to prove glucose control could lessen CVD must study even more stringent control so that participants would not be put at increased risk of microvascular disease.

During the past decade, several studies were begun to answer this key question, most notably the Action to Control Cardiovascular Risk in Diabetes Study (ACCORD), which is led by the National Heart, Lung, and Blood Institute with NIDDK support. ACCORD was designed to test three treatment approaches to decrease the high rate of CVD among adults with established type 2 diabetes who are at especially high risk for heart attack and stroke. More than 10,000 patients with type 2 diabetes were assigned to one of two regimens for blood glucose control: now-standard therapy designed to attain an HbA1c value of 7.0-7.9 percent, or intensive therapy with the intent of lowering HbA1c levels to below 6.0 percent. After patients had been treated for an average of 3.5 years, the intensive therapy arm was halted 18 months ahead of schedule due to a higher rate of deaths and no significant reduction in cardiovascular events in this treatment group.

Two other studies, an industry sponsored trial (ADVANCE) and the Veterans Administration Diabetes Trial (VADT), also compared the effects of standard and intensive blood glucose control on CVD in participants with longstanding type 2 diabetes similar to the ACCORD participants. Although neither of these studies found increased mortality with intensive therapy, they both failed to find any significant reduction in cardiovascular events.

The results of the three recent trials generated huge interest in the medical community and their full implications are still being explored. Further analyses over the next year may help to clarify some factors, such as patient characteristics and treatment regimens, contributing to the differences, but may not identify the cause of the excess deaths in the ACCORD trial.

While the ACCORD trial demonstrated the danger of intensive glucose management to near normal glucose levels in patients with longstanding type 2 diabetes who were at especially high risk of CVD, it did not address the question of cardiovascular benefit of good glucose control instituted shortly after diagnosis when good control can be achieved with simpler diabetes control regimens. The best evidence of the benefits of early treatment comes from the recently reported long-term follow-up of the UKPDS participants. There were no early adverse effects of intensive glucose control in the newly-diagnosed type 2 diabetes patients studied in the UKPDS. Three-quarters of UKPDS participants were observed for 10 years after the end of the original intervention trial. In 2008, the UKPDS follow-up study reported similar benefits for type 2 diabetes patients as had been seen in EDIC for type 1 diabetes. The intensive therapy groups had persistent reductions in microvascular complications and substantial reductions in risk for heart attack compared to those assigned to standard therapy. Intensive therapy participants also had a lower overall risk of death during the course of the study. In the UKPDS follow-up study, as in EDIC, the HbA1c levels between groups became equal for most of the follow-up period. Thus, a period of intensive diabetes management to control glucose levels appears to confer enduring benefits in terms of reducing diabetic complications—including CVD—even if an individual's glucose control subsequently becomes less stringent. This phenomenon, which has been termed "metabolic memory" or the "legacy effect," provides a powerful motivation for most diabetes patients to maintain their glucose levels as close to normal as possible early in the disease.

**One Treatment Approach Is Not Suitable for All People with Diabetes**

The results of the DCCT/EDIC and UKPDS represent landmark advances in validating intensive glucose

management as a strategy to prevent microvascular and cardiovascular complications in both type 1 and type 2 diabetes. But ACCORD and other large clinical trials of blood glucose control and cardiovascular risk in type 2 diabetes arrived at a seemingly conflicting conclusion. On the surface, the ACCORD outcome seems at odds with the UKPDS finding that intensive glucose control is protective in terms of reducing cardiovascular risks, including death, in people with type 2 diabetes. However, there are important differences between the studies. UKPDS participants had a median age of 53 years and were newly diagnosed with diabetes at the time of enrollment. In contrast, the ACCORD cohort was older, with an average age of 62 years, and had been living with diabetes for a median duration of 10 years. ACCORD participants were also at especially high risk of CVD, and more than a third had already experienced at least one cardiovascular event before the trial began. Moreover, the ACCORD "intensive" therapy protocol attempted to reduce HbA1c values to "near normal" (i.e., non-diabetic) levels, a considerably more aggressive approach to glucose control than the "intensive" therapy regimens of the UKPDS and DCCT. Viewed together, the results of ACCORD and UKPDS suggest that a *personalized* approach to glucose control in type 2 diabetes might be needed—one that takes into account a person's duration of diabetes, the presence or absence of diabetes complications, risk of low blood glucose, other complicating illnesses and life expectancy, as well as other health, behavioral, and social factors.

**Conclusions**

The recent results of long-term clinical trials to reduce diabetes complications are expanding our knowledge of the best ways to manage diabetes. Despite some challenges, progress is being made in improving glucose control and reducing both micro- and macrovascular complications related to both type 1 and type 2 diabetes. Further investigation is needed, since no current treatment regimens fully replicate the tightly regulated control of glucose levels found in people without diabetes.

Type 1 and type 2 diabetes are complex chronic diseases that have multiple clinical presentations, variability in their rates of progression, and variability in susceptibility to development of chronic micro- and macrovascular complications. Strategies for controlling blood glucose to prevent complications may need to be modified for different groups of patients or even for a single patient as their disease progresses. Such strategies must also take into account other therapies to manage CVD risks, such as drugs that normalize blood pressure, reduce blood lipid levels, or alter blood coagulation.

As the number of people with diabetes in the U.S. continues to climb, the NIDDK investment in long-term clinical trials to optimize diabetes management will help reduce the burden of CVD and premature death in this large segment of the population. In addition, basic research to understand the phenomenon of metabolic memory will shed light on the way intensive glucose control early in the course of diabetes can pay off in terms of fewer complications years later. In time, it may be possible to reproduce the effects of metabolic memory even in patients with poorly controlled diabetes and, thereby, help all people with diabetes achieve better health and longer lives.

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*[1] http://www.cdc.gov/diabetes/pubs/factsheet07.htm*

*[2] Krolewski AS, et al: Am J Cardiol 59:750-755, 1987.*

*[3] Dorman JS, et al: Diabetes 33:271-276, 1984.*

# RNAi-based Therapeutic Strategies for Metabolic and Inflammatory Diseases

*Dr. Michael Czech*

*Dr. Michael Czech is a Professor and Chair of Molecular Medicine and a Professor of Biochemistry and Molecular Pharmacology at the University of Massachusetts Medical School in Worcester, Massachusetts. He received his Ph.D. in Biochemistry from Brown University. Dr. Czech has published nearly 300 papers and has served, or is presently serving, on the editorial boards of dozens of journals. He has also served on the NIH endocrinology study section and on the Howard Hughes Medical Institute review panel. Dr. Czech was the recipient of the Grodsky Basic Research Award from the Juvenile Diabetes Research Foundation International in 1997, the 1998 Elliot P. Joslin Medal in diabetes research, and the 2000 Banting Medal of the American Diabetes Association. The following are highlights from the scientific presentation that Dr. Czech gave to the NIDDK's Advisory Council in May 2008.*

Understanding the process by which the body slowly becomes resistant to the hormone insulin, as is the case in type 2 diabetes, is critical to developing effective therapeutics for the disease. Recent research has revealed a link between insulin resistance and the inflammatory response of the immune system. As the body takes in excess calories, fat cells, known as adipocytes, increase in size to store the extra fat. Eventually, the adipocytes become overloaded and begin to release molecules that attract inflammatory cells, specifically macrophages. Macrophages are important in initiating the inflammatory response; they engulf foreign pathogens, such as bacteria or yeast, and secrete molecules that affect the behavior of other immune system cells and that attract additional inflammatory cells to the site of the pathogen. However, inflammation does not only occur when there is an obvious infection. A chronic state of inflammation can occur when macrophages are continually recruited to adipocytes, as in the case of obesity. In this state and with the adipocyte's ability to store fat exceeded, the muscle begins to take up the excess fat. The build up of fat in the muscle disrupts the ability of insulin to stimulate the transport of glucose (sugar) from the blood into muscle, leading to insulin resistance.

Dr. Czech's presentation moved from an initial fundamental discovery to an innovative strategy for its clinical application. He discussed his approach to understanding how cells become resistant to insulin and the role of the inflammatory response in insulin resistance. He shared how his laboratory has utilized a revolutionary technique—ribonucleic acid (RNA) interference, or RNAi—to identify novel molecules critical to these processes. Dr. Czech and his team are exploring the use of this technique as a potential therapy for insulin resistance. Dr. Czech also remarked that this research was made possible by NIDDK's Diabetes Genome Anatomy Project (DGAP), a unique and multi-dimensional initiative for basic research in diabetes. DGAP was designed to facilitate interactions and coordinate a number of investigators at multiple institutions, with projects aimed at understanding the interface between insulin action, insulin resistance, and the genetics of type 2 diabetes.

**Using RNAi To Identify Novel Proteins in Insulin Resistance**

Dr. Czech and his colleagues sought to identify proteins that mediate the interactions between

adipocytes and macrophages and to understand their role in the balance of blood glucose and fat levels. Such information could reveal new drug targets to break the link between obesity and insulin resistance. To uncover these proteins, the scientists used a technique based on the phenomenon of RNA interference. This technique involves designing small molecules, known as small interfering RNAs or siRNAs, that reduce levels of a specific protein by interacting—or interfering—with the genetic material that encodes the protein, to prevent the protein from being made. The scientists thus could design specific siRNAs to reduce the level of a protein and see whether insulin-mediated glucose uptake was affected. With this technique, they screened hundreds of different proteins in mouse adipocytes to determine whether any had a role in insulin action.

Several specific proteins were identified that Dr. Czech and his colleagues never expected to be involved in insulin resistance. One of these is called MAP4K4 (shorthand for mitogen activated protein 4 kinase). Dr. Czech's laboratory subsequently demonstrated that MAP4K4 blocks insulin-stimulated glucose transport through a mechanism that also involves an inflammatory response protein called TNF-alpha. This positions MAP4K4 at the interface between adipocytes, where MAP4K4 can be found, and macrophages, which secrete TNF-alpha. MAP4K4 is also located in other types of cells, and Dr. Czech's laboratory and others have identified additional roles for this protein in muscle and in macrophages, placing MAP4K4 in three key tissues involved in insulin resistance in obesity.

**Developing RNAi as a Potential Therapeutic**

Once a role had been identified for MAP4K4 in inflammation and insulin-dependent glucose uptake, Dr. Czech wanted to explore whether targeting this protein, using the power of RNAi, could have therapeutic potential for diabetes. The investigators decided to target levels of MAP4K4 protein in macrophages, rather than in muscle or adipocytes, because they hypothesized that insulin resistance results from the stimulation of inflammation by MAP4K4 in macrophages. In addition, because macrophages—and inflammation—are involved in many diseases, such as rheumatoid arthritis, colitis, inflammatory bowel disease, cardiovascular disease, and atherosclerosis, developing a strategy for therapy in the macrophage might be applied to many other diseases.

Dr. Czech explained that RNA interference as a potential therapeutic may have several advantages over traditional small molecule drugs, which interact with proteins. In traditional drug development there are a relatively limited number of proteins that can be targeted, as the small molecules normally tested as drug candidates are only effective if they can bind (attach) to the targeted protein. By contrast, RNAi works by interfering with genetic material encoding proteins, not the proteins themselves, and scientists think that there may be fewer structural constraints for this type of interaction. With RNAi, therefore, levels of any protein encoded in the human genome could theoretically be targeted and reduced. Second, traditional small molecule drugs can sometimes bind non-targeted proteins. Because siRNAs are extremely specific in their targets, off-target—and potentially toxic—effects can be minimized. Additionally, siRNAs are made from materials that are native to the body and have not shown toxicity thus far in animal models.

As Dr. Czech noted, an ideal therapeutic would be delivered orally for the patient's ease. An orally delivered drug faces many obstacles on its way to the target tissue: it needs to pass through the acidic environment of the stomach, be absorbed by the gut, and enter the bloodstream. An ideal therapeutic would be specifically delivered to the targeted tissues, thereby avoiding any toxicity due to misdelivery. To address these challenges, Dr. Czech took advantage of special cells called "M cells," which are located within the small intestine, and devised a way to get siRNA to these cells.

The M cells constantly sample the digestive cavity of the intestine looking for particles like bacteria and yeast that may have been ingested. Upon finding these, M cells are able to bind the particles, internalize them, and expel them where nearby macrophages are waiting to devour them. With this system, Dr. Czech utilized the normal biology of the intestine to efficiently direct his RNAi therapeutic to the macrophages.

Dr. Czech's laboratory needed to generate a safe vehicle to deliver the siRNA to an animal being studied. Their efforts led to the development of hollow, porous, tiny (micron-sized) shells made of a substance called beta1,3-D-glucan, which is recognized by proteins on both the M cells and the macrophages, permitting these cells to take in the shells. Beta1,3-D-glucan is a non-toxic material made by yeast cells and has been sold as a human dietary supplement for many years. Layering the siRNA within the hollow center of the shell allows five to six layers of siRNA to be put into each of these particles. Therefore, the scientists could use multiple combinations of siRNA at one time and target several different genes, or use one siRNA at a higher dose. Dr. Czech and his colleagues termed these shell particles "GeRPs" or Glucan-encapsulated siRNA particles.

**Proof of Principle: Using RNAi To Target MAP4K4 in Animals**

This technology required multiple tests to determine whether it could be used as a potential therapeutic in animals. To begin, Dr. Czech and his colleagues needed to ascertain whether the macrophages would even ingest the GeRPs— the first step in this strategy. To do this, the scientists added a fluorescent label to the GeRPs and gave them orally to mice. Using a fluorescence microscope, they were able to see that the macrophages had taken in the GeRPs and that a single macrophage could ingest multiple GeRPs. Another exciting aspect of this technology is that it harnesses the macrophages in the gut to transport the GeRPs. These macrophages are part of the body's lymphatic system, which enables them to travel throughout the body. This prompted Dr. Czech and his colleagues to assess if they could find GeRPs inside macrophages located in various tissues of the mouse body. After 8 days of feeding the mice GeRPs, the scientists observed the fluorescent GeRPs in the lungs, liver, and spleen. From this result, Dr. Czech and his laboratory concluded that they are able to target multiple tissues in the mouse body with this technology.

Dr. Czech's next step was to examine whether GeRPs with siRNA directed to MAP4K4 led to a reduction in the levels of MAP4K4 proteins within the tissues of the mice. In spleen, liver, and lung, the scientists were able to see a reduction in the levels of MAP4K4 as they had hoped. Did this reduction in MAP4K4 protein levels affect the inflammatory response though, as Dr. Czech had predicted? The scientists again fed the mice GeRPs with siRNAs to MAP4K4 and then gave the animals a toxic chemical that mimics a bacterial infection in order to stimulate the inflammatory response. When mice without the siRNAs were given this chemical, their macrophages stimulated an excessive inflammatory response, leading to a very large release of the inflammatory protein TNF-alpha, which was fatal to the animals. However, by feeding the mice siRNA to MAP4K4, Dr. Czech and his colleagues were able to block this storm of TNF-alpha, halting the inflammatory response to the chemical, and protecting the mice. This exciting result demonstrated that the orally administered siRNAs were not only delivered to the correct cell, the macrophage, and carried to multiple tissues, but that these siRNAs also targeted MAP4K4 specifically and altered the mouse inflammatory response.

Does using this technology to target MAP4K4 reduce inflammation in fat tissue and affect insulin-mediated glucose transport into cells? For these preliminary experiments, Dr. Czech and colleagues used obese mice that are highly insulin-resistant and delivered MAP4K4 siRNA-containing GeRPs to the mice by

injection. The scientists looked at various tissues to determine the location of GeRP-filled macrophages and evaluated whether the mice were still resistant to insulin with a test called a "glucose tolerance test." They found, to their surprise, that the fat tissue of these mice was the main tissue that had macrophages with GeRPs in them. This indicated that, in these obese mice, the primary inflamed tissue is the fat tissue— macrophages are largely recruited to this tissue. Dr. Czech and his laboratory also observed a decrease in levels of MAP4K4 protein in the macrophages recovered in this tissue. In addition, these mice were better able to metabolize glucose, indicating that the insulin resistance of these obese mice could be ameliorated. These experiments suggested that delivery of MAP4K4 siRNA to obese mice could have a profound effect on glucose metabolism throughout the body.

**Conclusion**

Dr. Czech's presentation illustrated the power of RNAi technology to identify novel proteins involved in insulin resistance. These proteins are potential targets for drug therapy, as they are found at the interface between fat cells, muscle, and the inflammatory response. One particular protein, MAP4K4, is especially interesting due to its location in all of these tissues. In addition, Dr. Czech showed his laboratory's approach to using siRNAs as a therapeutic modality. By targeting siRNA to MAP4K4 within the macrophages of a mouse with an innovative delivery vehicle, the scientists were able to both block the inflammatory response and alter the insulin resistance in obese mice. Thus, Dr. Czech and his colleagues have developed a technology to deliver RNAi *in vivo* in mice. They plan to build on the studies to determine whether the therapeutic has a similar result in other animals. Dr. Czech's research reveals the exciting potential for a new method of therapy for numerous diseases, including type 2 diabetes.

## PATIENT PROFILE

# Charlotte Cunningham

*With Type 1 Diabetes, "Time Is of the Essence"*

**Charlotte Cunningham**

Late in the summer of 2005, Lilo Cunningham noticed that her then 10-year-old daughter, Charlotte, was beginning to drink copious amounts of water. This seemed unusual to Lilo because Charlotte was not fond of drinking water. "But no matter where we went, she was always looking for a water fountain," says Lilo. Lilo also noticed that Charlotte was using the bathroom more frequently.

Lilo recognized these changes in Charlotte's behavior as potential symptoms of diabetes. As two of Lilo's sisters have sons with the type 1 form of the disease, Lilo decided not to take a chance. Within days of her observations, Lilo made an appointment with Charlotte's pediatrician and, sure enough, learned that Charlotte's blood sugar level was 680—about seven times above normal.

Charlotte was diagnosed with type 1 diabetes—previously known as juvenile diabetes—a devastating illness that often strikes in infancy, childhood, or young adulthood.

The diagnosis was frightening, but Lilo was able to turn to her sisters for advice. In addition to offering many practical suggestions for dealing with diabetes on a day-to-day basis, one of Lilo's sisters, who is very active in the Juvenile Diabetes Research Foundation International (JDRF), informed her that several diabetes research trials were under way. She suggested that the Cunninghams might want to investigate these trials for Charlotte.

Because the Cunninghams were informed of several clinical trials shortly after Charlotte's diagnosis, she was eligible to participate in a clinical trial specifically designed for newly-diagnosed patients. The therapy being tested in this trial may slow down the progression of the disease, which could reap long-term benefits for patients and make it easier for them to control their blood sugar levels.

*"The more we can slow the progression of this disease and keep Charlotte healthy, the better chance she has of leading a longer, healthier life."*

Controlling blood sugar levels is critical. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT) demonstrated that intensive blood sugar control offers remarkable long-term benefits when it comes to preventing or delaying complications frequently associated with type 1 diabetes, including eye, nerve, kidney, and cardiovascular disease.

Charlotte, now 13 years old and 3½ years post-diagnosis, shows no signs of complications from diabetes. "Time is of the essence," says Lilo. "The more we can slow the progression of this disease and keep Charlotte healthy, the better chance she has of leading a longer, healthier life."

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**About the Study**

Type 1 diabetes occurs when a person's immune system mounts a misguided attack and destroys the insulin-producing beta cells found in the pancreas. Insulin is critical for the body to absorb sugar from the blood and to use it for energy. Those with type 1 diabetes need daily administration of externally-supplied insulin, either by injection or with a pump, and must monitor their blood sugar levels vigilantly. Researchers have discovered, however, that many individuals diagnosed with type 1 diabetes still make detectable amounts of insulin, even many years after they are diagnosed. The DCCT also showed that people with type 1 diabetes who still made some of their own insulin had fewer long-term disease complications, as well as reduced incidents of dangerously low blood sugar (hypoglycemia) from administration of too much insulin. These observations suggest that preserving patients' remaining beta cell function, so that they still produce some of their own insulin, could have dramatic, long-term health benefits.

*"We had an incredibly positive experience with Charlotte's study. We were exposed to so many people who know so much about this disease—we learned so much!"*

The trial in which Charlotte is participating is trying to do just that. A previous NIDDK-supported clinical trial indicated that an antibody, called hOKT3gamma1(Ala-ala) or "anti-CD3," halted the destruction of insulin-producing beta cells in a small number of newly-diagnosed patients. Anti-CD3 alters the signal that triggers the disease-causing immune cells to attack the insulin-secreting cells. Charlotte is participating in a trial where researchers are determining if an additional treatment of anti-CD3 will provide further benefit, beyond that of the single treatment. This trial is being conducted by the Immune Tolerance Network, which is led by the National Institute of Allergy and Infectious Diseases, in collaboration with the NIDDK's Type 1 Diabetes TrialNet. Both networks also receive funding from the Special Statutory Funding Program for Type 1 Diabetes Research. Because one of the requirements for participation in this particular trial was that patients enroll within 8 weeks of their diagnosis, the Cunninghams are very grateful that a family member counseled them to act quickly after Charlotte's diagnosis.

"We were fortunate that Charlotte was diagnosed so early and was able to participate in this trial," says Lilo. "As a result, she's perhaps making more insulin than the average person in the early stages of diabetes and is doing very well."

The trial requires Charlotte to be infused daily over a 14-day period with the anti-CD3 antibody. Each daily infusion takes between 15 to 30 minutes, and is administered into Charlotte's upper arm. Charlotte received this 14-day set of infusions two times; the second treatment followed 19 months after the first. Charlotte returned to the trial site every 3 months in between the treatments and for 12 months following the second treatment. These visits were to monitor her response to the treatment and included a physical examination, a blood test, and a test to measure her insulin response. Except for a rash between her fingers, which lasted only 1 day, Charlotte has experienced no side effects from the treatment.

When asked about her overall experience in the trial study, Charlotte responded, "It was very cool." Not the typical response one would expect from an adolescent, but Charlotte has handled her diabetes extremely well from the beginning.

**Lilo and Charlotte's Message: Don't hesitate. Act quickly.**

When it comes to diabetes, Lilo and Charlotte's message to others is clear and simple: At the first sign of symptoms, do not hesitate; act quickly.

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"If you have any suspicions or notice anything wrong with your child, go for a blood test [at your pediatrician's office] and follow up immediately," says Lilo. "If this study succeeds in allowing Charlotte to retain the ability to produce some of her own insulin, even for a little while longer than she might have otherwise, it will help to delay, reduce, and possibly even prevent the secondary complications that often accompany type 1 diabetes." "And make sure you check your blood sugar level regularly," adds Charlotte.

*"Having diabetes hasn't really affected me much when I'm doing sports…my coaches are very understanding and let me do what I need to do to take care of myself."*

Lilo has not observed symptoms in other family members, but that does not mean she was going to take chances. The Cunninghams enrolled their two other children, Charlotte's 16-year-old brother and 19-year-old sister, in a study as well—the TrialNet Natural History Study. This study is screening relatives of people with type 1 diabetes to determine what level of risk these family members have for developing the disease. These studies are being conducted to learn more about the causes and indicators of risk for the development of type 1 diabetes. So far neither one of Charlotte's siblings appears to be at increased risk. "But if either of them should show signs of the disease, I would enroll them in a clinical trial in a heartbeat," Lilo says. "We had an incredibly positive experience with Charlotte's study. We were exposed to so many people who know so much about this disease—we learned so much!" When asked her thoughts on participating in the trial, Charlotte proudly says, "I'm an example of how diabetes research is helping people."

**About Charlotte**

Since February 2008, Charlotte's need for injected insulin has increased dramatically. According to Lilo, it is hard to say exactly what is going on. "Charlotte is in the midst of puberty, which could mean her body is requiring more insulin because of hormonal changes," she says. Nineteen months after her first treatment, Charlotte received her second and final 14-day infusion as part of the trial. The good news is that, even though Charlotte needs more external insulin, tests performed in May 2008 (12 months after Charlotte's last treatment and nearly 3 years after her initial diagnosis) indicate that she is still producing some insulin. Because her need for external insulin is increasing, Charlotte is exploring the possibility of using an insulin pump, a portable device that injects insulin at programmed intervals. She says she is excited about the prospect of using the pump.

If anything, Charlotte's life has become more active, rather than less, since being diagnosed with diabetes. Prior to her diagnosis, Charlotte played tennis and basketball. Now she has added surf boarding, lacrosse, and softball to her repertoire of physical activities. "Having diabetes hasn't really affected me much when I'm doing sports," she says. "I need to make sure my blood sugar count is okay both before and while I'm playing, but my coaches are very understanding and let me do what I need to do to take care of myself."

In the meantime, at the time this story was written, Charlotte was preparing to go to summer camp with 70 of her peers, all of whom have diabetes. She has been to the camp twice before and says she likes it a lot. "We meet with meal planners and check our blood sugar regularly, but mostly it's a regular, fun camp," Charlotte explained. Like any 13-year-old, Charlotte simply wants to lead as active and normal a life as possible.

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# Verner Thomas

## *DEPLOY Lifestyle Interventions Delay, Prevent Type 2 Diabetes*

**Verner Thomas**

At age 61, retired and overweight, Verner Thomas joined the YMCA to try to improve her health. On one of her visits, she was handed a brochure asking if she'd like to participate in something called the "Diabetes Education & Prevention with a Lifestyle Intervention Offered at the YMCA," or DEPLOY. DEPLOY is testing a diabetes prevention model in which YMCA employees are trained to help people who have risk factors for type 2 diabetes lose weight and increase their physical activity.

With support from NIDDK and in cooperation with the YMCA, the DEPLOY program is building on previous NIDDK-supported research that demonstrated that, in people at high risk, an intensive lifestyle intervention can be an extremely effective means of preventing or delaying the onset of type 2 diabetes.

*"My daughter is really proud of me," says Verner. "She says 'Momma, you look better and feel better. You're just a much more vibrant person. I want you to keep this up!'"*

And Verner is a reaffirmation of those findings.

Prior to involving herself in the program, Verner's blood sugar and weight strongly indicated that she was at high risk of developing type 2 diabetes within the next 1 to 2 years. Additionally, her cholesterol levels were abnormally high. Today, 2 years after being introduced to the DEPLOY program, Verner exercises regularly, eats a more disciplined diet, and in general is more conscious of the lifestyle that she leads. As a result, she has lost weight, lowered her blood sugar levels, improved her cholesterol levels, and, best of all, has remained diabetes free.

"My daughter is really proud of me," says Verner. "She says 'Momma, you look better and feel better. You're just a much more vibrant person. I want you to keep this up!'"

After speaking with Verner, one gets the feeling that her daughter was preaching to the choir. Ms. Thomas is well aware of the positive changes she's made in her life as a result of DEPLOY.

**Type 2 Diabetes—Reducing the Burden**

Diabetes is a chronic, common, and costly disease that is robbing many Americans of good health and quality of life. Type 2 diabetes—once known as adult-onset diabetes, or non-insulin-dependent diabetes

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mellitus—is the most common form of the disease. It primarily affects adults, but it can develop in childhood and adolescence. Older age, overweight, and inactivity are strong risk factors for type 2 diabetes; heredity plays an important role as well. People with diabetes have blood sugar levels that are above normal. Over the years, high blood sugar damages nerves and blood vessels, leading to serious health complications such as heart disease, stroke, blindness, kidney disease and kidney failure, the need for lower limb amputations, and gum infections.

Ominously, Verner is hardly alone when it comes to being at high risk for type 2 diabetes. People who, like Verner did, have blood sugar levels higher than normal, but not high enough to be classified as diabetes, are considered to have "pre-diabetes." In addition to 23.6 million Americans who already have type 2 diabetes, the Centers for Disease Control and Prevention estimates that at least another 57 million have pre-diabetes, and thus are at high risk of progressing to type 2 diabetes.

Importantly, there is a window of opportunity to reverse course on the way to developing type 2 diabetes. Spearheaded by the NIDDK, the landmark Diabetes Prevention Program, or DPP, was a clinical trial that showed that in overweight people with pre-diabetes, type 2 diabetes can be prevented or delayed through use of the diabetes medication metformin, or through a lifestyle intervention leading to moderate weight loss through diet and exercise. The immense success of the intensive lifestyle intervention, which showed a 58 percent reduction in risk of developing diabetes, has led to new research studies and programs testing ways to effectively translate these results into interventions that can be widely and effectively implemented to prevent diabetes in those at risk.

**Translating DPP with the DEPLOY Program**

Verner's mother had diabetes, as did her grandmother. She has a family history on her father's side of high blood pressure, as well as a history of obesity on both sides of her family. Verner also understands that, as an African American, she has a 1.8-fold increased risk of developing type 2 diabetes compared to non-Hispanic whites.

"This program (DEPLOY) makes you aware of what you should be doing and what you're not doing to protect yourself against type 2 diabetes," says Verner. "It motivated me to take better care of myself—how I eat and exercise….I wasn't as faithful in my exercising until I got into the program."

Operating out of local YMCAs, and led by primary investigator Dr. Ronald Ackermann of the Indiana University School of Medicine, DEPLOY takes groups of 8 to 12 people who, like Verner, have pre-diabetes and other risk factors, and puts them through a series of classroom-style meetings that focus on knowledge building and skill development to help them set goals, self-monitor, and problem-solve around their pre-diabetes. Major goals of the program include a 5 to 7 percent reduction in baseline weight, and 150 minutes per week of moderate level physical activity similar to brisk walking. The program is based on the lifestyle intervention that proved so effective at delaying or preventing type 2 diabetes among participants in the DPP.

Verner first started with DEPLOY in January 2006. Since then, in addition to remaining diabetes free, her weight has gone from 219 pounds down to 177, and her blood pressure has gone from 133/90 to 110/72 (normal is 120/80 or lower). Her body mass index (BMI) has also dropped from 33 to 29. BMI is a measure of weight relative to height. A BMI of 30 or more in an adult is considered obese. Verner thus is no longer obese—a significant achievement. She remains determined to get her BMI still lower. But in all categories, including her sugar and cholesterol levels, Verner's numbers are heading in the right direction. Her fasting blood sugar has fallen from 111 to 90 milligrams per deciliter, meaning she no longer has pre-diabetes—and for that she is grateful.

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"I feel healthier and stronger today than I did 2½ years ago," says Verner. "I've gotten a lot of compliments since I lost the weight, and psychologically I feel better. I'm more open, social, and outgoing," she adds with a satisfying chuckle.

She admits that she also still faces some challenges. "My problem is sweets," says Verner. "When I'm under stress I fall back on them." But she says that for the most part she's been able to keep her sweet tooth under control, as well as the rest of her diet.

To meet their goal of moderate weight loss, participants in DEPLOY are counseled to increase physical activity and to reduce their intake of fat and total calories. The diet and exercise interventions are flexible and sensitive to individual, cultural, and community differences where they are implemented. As part of her diet, Verner eats fish occasionally, and in addition, she says, "I only eat one egg a month. I get my protein from peanut butter, beans, and meat substitutes."

**Looking to the Future**

Researchers are making major discoveries in how to predict who will develop type 2 diabetes and its complications; how to personalize individual treatments; and how to use this information to preempt disease onset and development of complications (see box). Public health efforts founded on these discoveries can hopefully help to reduce the burden of type 2 diabetes and its complications in the future. Research programs such as DEPLOY—and the hard work and dedication of the participants—are an important part of making this future real for people at risk for type 2 diabetes.

DEPLOY has a strong fan in Verner: "I'm dedicated to maintaining my regime of eating healthily and exercising regularly," she says, and she is sure that DEPLOY helped point her in that direction.

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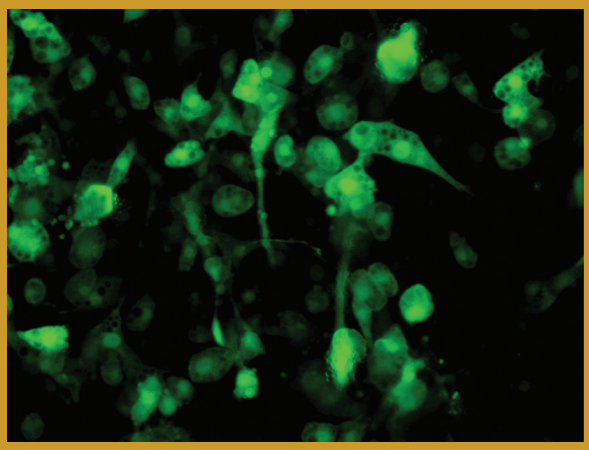
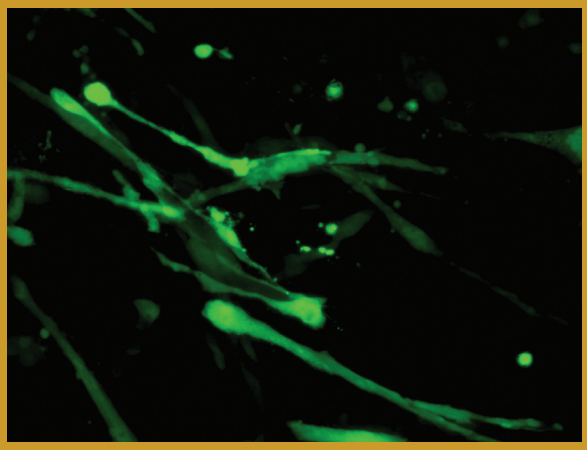
**The Beneficiary of Decades of Research**
Verner Thomas and the millions of other Americans who are either at high risk or already have type 2 diabetes are the beneficiaries of decades of research.

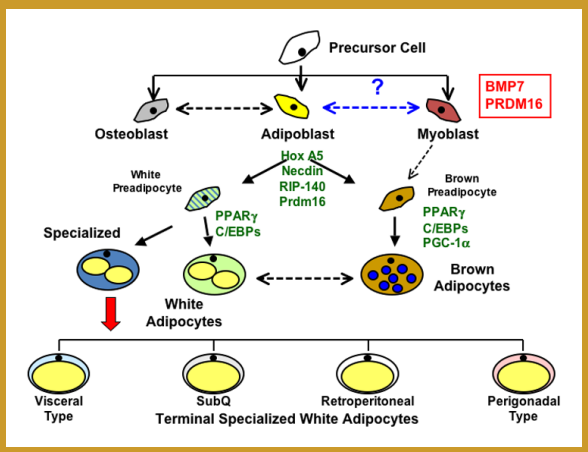
Thirty years ago there were no proven strategies to prevent type 2 diabetes or its complications, and the only treatments, now obsolete, caused dangerously low blood sugar reactions and weight gain in patients.

Today, as a result of research, diabetes is better understood, new and more effective treatments are available, and type 2 diabetes and its complications can be delayed and in some cases, even prevented. For example:

- Researchers now know that obesity is a strong risk factor for type 2 diabetes, and have a new understanding of the molecular links between obesity and insulin resistance, a condition that prevents the body from effectively using insulin.
- Risk factors other than obesity have been identified and can be targeted.
- Newly-identified diabetes genes will enhance researchers' ability to identify and intervene in those at risk.
- Based on clinical research demonstrating the health benefits of early detection and therapy, Medicare now covers testing for diabetes.
- New drug development has been aided by an NIH-supported clinical trial that validated an indicator, called hemoglobin A1c, that reflects average blood sugar control over a 2 to 3 month period.
- New oral medications that target the specific metabolic abnormalities of type 2 diabetes are available.
- The Diabetes Prevention Program (DPP) demonstrated that type 2 diabetes can be prevented or delayed in those at risk. The benefits of the DPP were seen in all ages, all racial and ethnic groups, in women with history of gestational diabetes, and in people with diabetes risk genes.
- The landmark DPP findings are being actively disseminated to the public by the National Diabetes Education Program.
- Kidney disease resulting from diabetes can now be detected earlier by standardized blood tests to estimate kidney function and monitor urine protein excretion.
- With timely laser surgery and appropriate follow-up care, people with advanced eye disease related to diabetes can reduce their risk of blindness by 90 percent.








**NIDDK-supported research highlighted in this chapter sheds new light on the origin of different types of fat in the body. For example, researchers have discovered a new role for a protein, called PRDM16, as a master switch between the development of brown fat cells and muscle cells. Brown fat cells burn fat molecules to generate heat. The other type of fat, white fat, tends to store excess calories, and is associated with obesity-related diseases. To gain insight into how brown fat cells are formed, scientists recently assessed the effects of depleting the protein PRDM16 from mouse cells that ordinarily develop into brown fat cells. With normal levels of PRDM16, these cells developed into brown fat cells (top panel). Unexpectedly, when depleted of PRDM16, these cells did not develop into fat cells, as predicted, but rather became muscle cells (middle panel). The bottom panel is a diagram summarizing the complexity of steps and factors involved in fat cell development. In the diagram, PRDM16 and another factor called BMP-7, which is also discussed in this chapter, regulate this process at an early step in development, when stem cells have the potential to become precursor cells for bone (osteoblast), fat (adipoblast), or muscle (myoblast). The diagram also shows the steps and factors that determine whether fat precursor cells develop into white or brown fat. This chapter contains additional new information regarding the formation of fat cells, and the potential implications of this research for treating obesity.**

*Images of cells are courtesy of Dr. Bruce M. Spiegelman and reprinted by permission from Macmillan Publishers Ltd: Nature, 454: 961-967, copyright 2008. Diagram of fat cell development courtesy of Dr. C. Ronald Kahn, Joslin Diabetes Center and Harvard Medical School.*

# Obesity

*Obesity has risen to epidemic levels in the U.S. 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] Furthermore, while obesity and overweight have risen in the population in general, the greatest increases observed over approximately the past two decades have been in the prevalence of extreme obesity; those who are severely obese are most at risk for serious health problems.[3] Levels of childhood overweight and obesity have also escalated in the past several decades. Obesity affects approximately 16 percent of children and teens ages 2 through 19.[1,4,5] These children are at risk for developing serious diseases both during their youth and later in adulthood. Overweight and obesity also disproportionately affect racial and ethnic minority populations, and those who are socio-economically disadvantaged.

The increased prevalence of obesity in the U.S. is thought to result from the interaction of genetic susceptibility with behavior and factors in the environment that promote increased caloric intake and sedentary lifestyles. Thus, the NIDDK supports a multidimensional research portfolio on obesity, ranging from basic studies to large clinical trials. For example, researchers are elucidating the hormones and other signaling molecules that influence appetite, satiety, and energy expenditure, and that link obesity to type 2 diabetes and other adverse health conditions. With imaging technology, scientists have explored changes in brain activity elicited by the sight of food, and how this brain activity is affected by weight loss. Research on body fat has led to surprising new findings about the origins and formation of different types of fat tissue—not only "white fat," which stores fat molecules and is associated with obesity, but also "brown fat" tissue, which burns fat molecules to generate heat. Research is also revealing molecular links between metabolism, appetite, and the circadian rhythm. Investigators are continuing to develop and test behavioral and environmental interventions to prevent or treat obesity in children. Other research addresses potential medical intervention strategies, including observational studies to evaluate the risks and benefits of bariatric surgery as a treatment for severe obesity. The NIDDK additionally supports studies of eating disorders that are associated with obesity in some people.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter. To help bring the results of research to the public and health care providers, the NIDDK also sponsors education and information programs. Given the importance of the obesity epidemic as a public health problem, and its relevance to the mission of the NIDDK, the Institute continues to play a leading role in the NIH Obesity Research Task Force. Co-chaired by the Directors of the NIDDK and the National Heart, Lung, and Blood Institute, the Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices. With extensive input from external scientists and the public, the Task Force developed the *Strategic*

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

[2] *Ogden CL, et al: JAMA 295: 1549-1555, 2006; National Center for Health Statistics. Obesity Among Adults in the United States—No Significant Change Since 2003-2004. Data Brief Number 1. Hyattsville, MD: Public Health Service. 2007.*

[3] *Flegal KM, et al: JAMA 288: 1723-1727, 2002; Flegal KM and Troiano RP: Int J Obes Relat Metab Disord 24: 807-818, 2000; Freedman DS, et al: JAMA 288: 1758-1761, 2002.*

[4] *Ogden CL, et al: JAMA 299: 2401-2405, 2008.*

[5] *For children and adolescents, this document uses the terms overweight and obesity interchangeably to refer to a BMI at or greater than the 95th percentile on growth charts (which are based on previous national surveys).*

*Plan for NIH Obesity Research*, published in August 2004 (http://obesityresearch.nih.gov/About/strategic-plan.htm). The NIH is currently supporting a spectrum of research studies consistent with the recommendations of the *Strategic Plan*.

## MOLECULAR CONTRIBUTORS TO OBESITY

**In the Eye of the Beholder—The Sight of Food Elicits Different Brain Responses after Weight Loss, Due to Changes in the Hormone Leptin:** Scientists have gained new insight into why food can appear particularly irresistible to someone struggling to maintain a reduced body weight, and their research has implications for obesity treatment. Building on the knowledge from previous studies that weight and fat loss reduces leptin levels, the scientists used brain imaging to assess whether weight reduction also modulates brain responses to the sight of food, and whether any such changes can be reversed by the administration of replacement doses of leptin. In the first phase of the study, using a special liquid formula diet, the scientists helped six volunteers who were obese lose 10 percent of their body weight. They then had the volunteers maintain their reduced weight on a carefully-monitored diet. During the weight maintenance phase of the study, the volunteers received leptin injections for several weeks and placebo injections for several weeks so that the effects of the hormone as compared to placebo could be assessed for each person. The scientists used functional magnetic resonance imaging, or fMRI, which provides an image of the brain that identifies regions of high activity. The researchers looked at the activity in different parts of the volunteers' brains as they viewed foods, including fruits, vegetables, grains, and sweets; and, as a control, non-food objects like a cell phone. The sight of the food affected activity in several parts of the volunteers' brains. Comparing images taken before and after weight loss, the scientists found that weight loss altered the mental response to food, resulting in an increase in brain activity in some areas and a decrease in others. Prior research had shown that the affected areas of the brain are associated with a variety of emotional and sensory responses to food, decision making, and other behaviors related to food. The scientists also discovered that leptin administration reversed many of the weight loss-induced changes in brain activity. Thus, food is perceived differently depending on the eye—or in this case, the leptin levels—of the beholder. This research advances knowledge of the many biologic processes that conspire to make the body regain lost fat: earlier studies had shown that weight loss leads to increased hunger and also causes the body to expend less energy and muscles to work more efficiently, so that fewer calories are burned. The identification of leptin as having a key role in all of these processes augments the evidence that leptin replacement therapy may help people maintain weight loss.

*Rosenbaum M, Sy M, Pavlovich K, Leibel RL, and Hirsch J: Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. J Clin Invest 118: 2583-2591, 2008.*

**Rev-ing Up Metabolism and Fat Cell Development:** Scientists have recently uncovered intriguing new roles in metabolism for a key protein controlling the circadian clock. In animals and humans, the internally driven circadian clock regulates many behaviors and bodily processes—including sleep/wake cycles, changes in blood pressure, and body temperature fluctuations—to harmonize these activities with daily, rhythmic changes in the environment, most notably day/night cycles. Not surprisingly, metabolic functions, such as the synthesis of glucose (sugar) and fats and the release of glucose into the blood, are also controlled by the circadian clock—but how that is accomplished at the molecular level has been poorly understood. Now, researchers have evidence that a protein called Rev-erb-alpha links the circadian clock and metabolism. Rev-erb-alpha is a key regulator of the circadian clock, controlling in a rhythmic fashion how much protein is made by a "master" clock gene. In their experiments, the researchers found that Rev-erb-alpha is highly dependent for its regulatory activity on another molecule called heme. The heme molecule is integral to many metabolic pathways, and its concentrations in the cell oscillate in a circadian rhythm. Through a series of biochemical tests and experiments in cells, they observed that, if heme was present, Rev-erb-alpha functioned normally. However, if they removed heme or used a mutant Rev-erb-alpha protein that could not bind heme, the protein's activity was diminished. At the same time, the researchers discovered that Rev-erb-alpha regulates not only a

master circadian clock gene, but also two key genes involved in glucose and fat metabolism—and that this activity also depends upon Rev-erb-alpha's ability to bind to heme. Thus, the interaction of heme with Rev-erb-alpha is a strong candidate for a coordinator of the circadian clock and metabolism. In a related study, researchers from the same laboratory demonstrated that Rev-erb-alpha is also important to the maturation of fat cells from their precursor cells. Through studies using fat cell precursors grown in the laboratory, the researchers found that the Rev-erb-alpha protein is present in high amounts at the onset of maturation, but its levels quickly drop as maturation proceeds. The initial increase and sudden decrease in levels of Rev-erb-alpha appears to be necessary to kick-start fat cell maturation and then allow it to move forward. From these studies, Rev-erb-alpha thus appears to play a key role not only in the circadian clock, but also in metabolism and in cellular development. Further study of these newly-discovered roles for Rev-erb-alpha will help investigators better understand the interrelationships between the circadian clock, metabolism, and cellular differentiation and how they might influence each other in both health and disease.

*Yin L, Wu N, Curtin JC, Qatanani M, Szwergold NR, Reid RA, Waitt GM, Parks DJ, Pearce KH, Wisely GB, and Lazar MA: Rev-erb-alpha, a heme sensor that coordinates metabolic and circadian pathways. Science 318: 1786-1789, 2007.*

*Wang J and Lazar MA: Bifunctional role of Rev-erb-alpha in adipocyte differentiation. Mol and Cell Biol 28: 2213-2220, 2008.*

**Rats Susceptible To Developing Obesity from a High-Calorie Diet Exhibit Reduced Density of Brain Cell Connections:** Scientists have shown that rats susceptible to obesity from a high-calorie diet have fewer brain cell connections than control rats in a key part of the brain that regulates body weight, known as the hypothalamus. This reduced density of brain cell connections was seen in both young and adult animals, indicating that the defect occurs early in life and persists into adulthood. Specifically, the scientists were interested in the brain cell connections that form in response to the hormone leptin. Leptin plays a key role in the development of brain cell connections in the hypothalamus and, in addition, serves as a sensor to the brain regarding appetite regulation whereby increased leptin levels depress appetite and promote satiety. Interestingly, most people who are obese have high leptin levels but are resistant to leptin signaling, including its influence on appetite. Similarly, in this rat model of diet-induced obesity, the obese rats also are resistant to leptin signaling. The scientists hypothesized that the leptin-resistant state of the rats may be due to abnormal development of the hypothalamic brain cell connections, specifically from the arcuate nucleus of the hypothalamus (ARH). Using a fluorescent tracer, the scientists were able to visualize the connections from the ARH to another part of the hypothalamus also known to be important in body weight regulation. The scientists compared these connections in brains of diet-induced obese rats and in other rats termed "diet-resistant" because they do not gain excess weight when fed the same high-calorie diet. The brain cell connections between these two parts of the hypothalamus were two- to four-fold less dense in diet-induced obese rats as compared to the diet-resistant animals. The initial experiment was conducted in rats 12-16 days old, but the same pattern was observed in adult rats. Furthermore, maternal diet did not influence the density of the connections. This result indicated that the genetic predisposition of the offspring held greater influence over the density of the connections than the environmental effects of the mother's diet. To investigate the role of leptin in the formation of these connections, the scientists added leptin to samples of brain tissue from the rats. They found that leptin caused the brain cells to form fewer connections in the brain tissue from rats prone to diet-induced obesity, in comparison to diet-resistant rats. The combined results of these and other experiments suggest that the leptin-resistant state of diet-induced obese rats may begin with decreased brain cell connections formed during neonatal development of the ARH, a key area that relays leptin signaling to other parts of the hypothalamus. Therefore, with fewer connections to transmit the leptin signal, the brain is less able to respond to leptin levels, even in very high amounts. The research presented here provides new avenues to investigate the complex brain pathways involved in obesity, eating behavior, and the regulation of body weight using emerging technologies in brain imaging and related fields.

*Bouret SG, Gorski JN, Patterson CM, Chen S, Levin BE, and Simerly RB: Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. Cell Metab 7: 179-185, 2008.*

**The Power of Hunger—the Hormone Ghrelin Harnesses Brain Cells' Energy Machines To Increase Appetite:** Scientists have discovered that the hormone ghrelin induces hunger through a process involving mitochondria, components of cells that generate energy for biologic processes. In particular, the researchers found that ghrelin acts via a mitochondrial protein called UCP2. Ghrelin is produced in the gut; its levels rise just before meals to induce food intake, and then fall just after a meal. When ghrelin travels from the gut to the brain, it latches onto specific brain cells and sets in motion an elaborate set of molecular signaling pathways that not only increase hunger, but, as shown in this new study, also ensure that the target brain cells will have ample energy to keep sending their message to "start eating," even though the message needs to be sent at times when the body may be comparatively low on energy. Prior research indicated that UCP2 is present in the same brain cells that serve as docking sites for ghrelin, and both UCP2 and ghrelin levels rise during fasting. Based on this guilt-by-association, the researchers explored whether UCP2 may somehow assist ghrelin. To do this, they assessed ghrelin's actions in normal mice and in mice that lacked UCP2 due to a genetic mutation. They found that ghrelin increases the number of mitochondria in brain cells, which in turn contributes to an increase in overall mitochondrial activity. Focusing specifically on a group of brain cells known to promote increased food intake, the scientists next observed that ghrelin causes these cells to fire off more signals, increase production of molecules associated with hunger, and inhibit another type of brain cell known to dampen appetite. All of these effects of ghrelin were dependent upon UCP2, as they were greatly attenuated in the mutant, UCP2-deficient mice. Other experiments showed that UCP2 affects how much food the mice consume.

Having elucidated the importance of mitochondria and UCP2 to ghrelin's effects on appetite, the scientists next sought to determine what UCP2 may be doing to assist ghrelin. Increased mitochondrial activity can lead to increased production of a destructive metabolic byproduct called reactive oxygen species (ROS, often referred to as "free radicals"), which UCP2 has previously been found to help eliminate. The scientists compared the levels of ROS in the brains of normal and UCP2-deficient mice. In normal mice, although ghrelin increased mitochondrial use of fatty acids as fuel, ROS levels did not increase. In mice lacking UCP2, however, ghrelin administration led to higher levels of these toxic byproducts. The scientists reasoned that UCP2 may enable appetite-inducing brain cells to keep working by eliminating toxic byproducts which would otherwise inhibit them. They also found that ghrelin boosts UCP2 production in normal mice, enabling further scavenging of ROS. Through these and other experiments, the scientists have shed new light on how ghrelin induces food intake. Ghrelin causes certain brain cells to increase their appetite-promoting activities, and increases mitochondrial burning of fatty acids to provide the cells with energy to drive these activities. UCP2 helps rid the cell of the resulting toxic byproducts, to ensure that their adverse effects do not interfere with the cell signaling that increases appetite and, ultimately, food consumption. These novel insights may lead to new approaches to help reduce overweight and obesity.

*Andrews ZB, Liu Z-W, Walllingford N, Erion DM, Borok E, Friedman JM, Tschöp MH, Shanabrough M, Cline G, Shulman GI, Coppola A, Gao X-B, Horvath TL, and Diano S: UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. Nature 454: 846-851, 2008.*

**A Role for the Immune System in Protecting Against Obesity and Insulin Resistance:** One of the processes associated with obesity and the insulin resistance that leads to type 2 diabetes is inflammation of highly metabolic tissues such as liver and muscle, and other metabolic tissues such as fat. In this process, immune system cells called macrophages are activated to migrate to these tissues and release signals that induce inflammation and promote insulin resistance. However, not all macrophages have this effect. Those that do not are said to be "alternatively activated," and are more common in lean animals than in those that are obese. Now, researchers have identified key molecular triggers of the alternative activation pathway, and identified a subset of liver macrophages called Kupffer cells which, when alternatively activated, have a key role in preventing insulin resistance in over-fed mice. Further investigation will be required to understand the genetic and environmental signals that trigger the pathway to alternatively activate macrophages and the precise mechanism by which these cells modulate insulin sensitivity. If these results prove to be relevant to human tissues as they are in mice, therapeutic

stimulation of the alternative activation program might be an effective means to treat obesity and prevent insulin resistance.

*Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW, and Chawla A: Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab 7: 496-507, 2008.*

## DETERMINANTS OF "BROWN" FAT FORMATION

**Brown or Brawn—Brown Fat and Muscle Cells Share Common Origins, with Fate Determined by PRDM16:** In a surprising finding, scientists have discovered that skeletal muscle cells and brown fat cells can arise from the same precursors. They also identified the biologic switch, a protein called PRDM16, that directs the precursors to develop into brown fat. Previously, it was thought that the two major types of fat tissue, brown and white, shared the same developmental origins. However, in many ways they are quite different. White fat cells serve as the body's energy reserves by storing fat, and they additionally secrete a variety of signaling molecules. Excess white fat tissue is associated with obesity and related diseases. By contrast, brown fat cells burn fat molecules to dissipate heat, and might thus protect against obesity. Brown fat cells also share similarities with muscle cells; for example, both burn fat in mitochondria, the components of the cell that generate energy to power biologic processes or to provide body heat.

To understand how brown fat cells are formed, scientists recently assessed the effects of depleting PRDM16 from mouse cells that were thought to be precursors of brown fat. Unexpectedly, brown fat precursors depleted of PRDM16 did not morph into white fat cells, as predicted, but rather became muscle cells. The scientists then hypothesized that brown fat and muscle cells might have the same precursors. To determine whether this was the case, they designed a way to visualize cells that arose from these precursors. Their approach was to genetically engineer mice so that their muscle cell precursors contained a readily visible marking that would be inherited during development by cells that derived from these precursors. Once the mice had grown, the scientists found that the brown fat and skeletal muscle cells—but not the white fat cells—contained this marking. Finally, the scientists found that excess production of PRDM16 in muscle cell precursors could divert their development from muscle cells into brown fat. Thus, the researchers concluded that both brown fat and muscle cells share a precursor and that PRDM16 plays an important role in determining whether the precursor gives rise to brown fat or muscle.

Scientists also recently gained new understanding of how PRDM16 can both turn on brown fat-specific genes and turn off genes important for white fat cells. It does so by associating with different sets of additional regulatory proteins. Interestingly, brown fat cells can arise within white fat tissue under certain conditions, such as extended exposure to cold or stimulation of some nervous system responses, and these types of brown fat cells appear to have a different origin, unrelated to muscle. These studies provide novel insights into the development of brown fat, and may inform the development of a new intervention strategy for obesity: generating more brown fat cells to burn excess calories.

*Kajimura S, Seale P, Tomaru T, Erdjument-Bromage H, Cooper MP, Ruas JL, Chin S, Tempst P, Lazar MA, and Spiegelman BM: Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes Dev 22: 1397-1409, 2008.*

*Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, and Spiegelman BM: PRDM16 controls a brown fat/skeletal muscle switch. Nature 454: 961-967, 2008.*

**Building Brown Fat with BMP-7:** A new discovery in fat cell research may point the way to new therapeutic options for obesity. In mammals, not all fat, or adipose tissue, is the same. White adipose tissue stores extra calories as fat for later use and is the tissue associated with obesity, while brown adipose tissue, or brown fat, actually burns fat to generate heat, keeping an animal warm and slim. Until recently, it was thought that in humans, only newborns had brown fat, but new findings suggest that adults actually retain some as well. In a recent study, researchers sought out factors that determine the generation of brown fat from

precursor cells. Working with laboratory-grown fat precursor cells from mice, they found that treatment with a molecule called bone morphogenetic protein 7, or BMP-7, is sufficient to drive precursor brown fat cells to develop into active brown fat cells. BMP-7 treatment suppressed cellular factors that normally inhibit brown fat cell development and induced production of key molecules that drive brown fat cell maturation—including UCP1, a signature protein found in brown fat cells that is essential for generating heat. Precursor brown fat cells treated with BMP-7 also increased by five-fold the number of their mitochondria, the cellular powerhouses that enable them to burn fat. In contrast, precursor white fat cells from mice were not affected by BMP-7 treatment. Demonstrating that BMP-7 is important to brown fat tissue development in animals, the researchers found that mice genetically engineered to have no BMP-7 had very little brown fat and produced little or no UCP1 protein as compared with normal, BMP-7-producing siblings. Increasing the amount of BMP-7 in mice had the opposite effect. The researchers found that administering extra BMP-7 to mice via a genetically engineered virus not only increased their brown fat mass, but also significantly increased whole body energy expenditure and basal body temperature—leading to a significant reduction in weight gain when compared with control mice. Although these experiments were all performed with mouse cells and in mice, now that brown fat has been found in humans, the findings suggest that BMP-7 may prove to be a therapeutic target to help counteract obesity in humans in the future.

*Tseng Y-H, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN, and Kahn CR: New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454: 1000-1004, 2008.*

## LIFESTYLE INTERVENTION TO REDUCE OBESITY IN CHILDREN

### Strategy To Reduce Screen Time for Young Children—Beneficial Effects on Body Weight:

With a combination of advanced technology, stickers, and other incentives, researchers have developed a new strategy to help parents limit the hours their young children spend transfixed in front of a television or computer game—and thus reduce their risk for gaining excess weight. Increasing evidence has linked television viewing with childhood obesity. The researchers focused on reducing screen time in children ages 4 to 7 in part because intervening early may help prevent later health problems. At the start of the study, the children were at or above the 75th percentile for body mass index (BMI, a measure of weight relative to height) for their age and gender, and they spent 14 hours or more each week watching television or playing computer games. Families were randomly assigned to either the intervention or control group. For the intervention group, the researchers attached a "TV Allowance" device to all televisions and computers in the home, and provided each family member with an individual code to permit screen time. The devices were programmed to allow only a certain amount of screen time for the participating child, and to reduce the child's weekly screen time by half over the first several months of the study. Parents also gave the children stickers and praise for their progress, as well as other incentives. In the control group, children did not have these types of limits on screen time; instead, their families received newsletters with tips on parenting, activities, and recipes.

The study showed that television and computer use can be reduced substantially in young children, and that these reductions can beneficially affect BMI. After 6 months and 12 months of the study, children in the intervention group had lower BMIs than those in the control group. Among the children from families of lower socioeconomic status, the intervention was particularly beneficial: the researchers observed a substantial difference in BMI between the intervention and control groups, and the improvement in BMI lasted for the full 2 years of the study. This result is encouraging given the increased risk for obesity in socioeconomically disadvantaged children. The researchers also found that reduced screen time correlated with the children's eating fewer calories, although not with changes in physical activity. The intervention's overall effect on BMI was not large. However, by inducing even modest reductions in BMI and television watching behavior at a young age, this type of relatively inexpensive intervention, in combination with other efforts, may ultimately help reduce risk for health problems associated with childhood obesity.

*Epstein LH, Roemmich JN, Robinson JL, Paluch RA, Winiewicz DD, Fuerch JH, and Robinson TN: A randomized trial of the effects of reducing television viewing and computer use on body mass index in young children. Arch Pediatr Adolesc Med 162: 239-245, 2008.*

## POTENTIAL MEDICAL INTERVENTION STRATEGIES TO COMBAT OBESITY AND ITS ASSOCIATED CONDITIONS

**Drugs Boost Exercise Endurance in Mice:** Researchers have identified two drugs that, in mice, seem to confer many of the healthful benefits of long-term exercise, giving them more fat-burning muscle and better endurance. These findings might eventually lead to better treatments for certain muscle disorders, frailty, obesity, and other conditions in which exercise is known to be helpful but not always practical.

Scientists have long searched for drugs that mimic or enhance the effects of exercise and its known therapeutic benefits. Several years ago, scientists identified a potential drug target—a signaling protein known as PPARdelta—which regulates several fat-burning genes in muscle cells. The researchers created genetically-engineered mice that produced high levels of PPARdelta in their muscles. The animals' running endurance nearly doubled, with their muscles developing more fat-burning, fatigue-resistant muscle fibers than normal mice. In a new study, the researchers used drugs, rather than genetic engineering, to enhance the effects of PPARdelta and another exercise-related molecule, AMPK. The scientists first gave oral doses of a PPARdelta-activating drug to mice for several weeks. Initial results were disappointing. The drug by itself had no impact on the animals' running endurance in a treadmill test. However, when the scientists added exercise training to the mix—having the mice run on a treadmill for nearly an hour daily for a month—the treated mice were able to run up to 75 percent farther than mice that received exercise training alone. The treated mice developed nearly 40 percent more fatigue-resistant muscle fibers than untreated animals. In addition, gene activity in their muscle cells was strikingly similar to the genetically-engineered mice in previous studies.

The researchers also tested the effects of the protein AMPK, which is known to be activated by exercise and is involved in regulating many other genes. The mice received daily doses of the drug AICAR, which activates AMPK. Surprisingly, 4 weeks of AICAR treatment alone activated exercise-related genes and enhanced running endurance by 44 percent in sedentary mice. In fact, the drug allowed sedentary animals to run longer and farther than animals that had received weeks of exercise training. Either drug alone activated a unique subset of exercise-related genes. However, the greatest rise in gene activity occurred when the PPARdelta-activating drug was combined with exercise training, which activates AMPK. This drug-exercise pattern of gene activity—dubbed an "endurance gene signature"—led to the greatest improvement in endurance. Because the muscles of humans and mice use similar genetic pathways, these findings could eventually lead to improved therapies. However, the drugs' effects on human muscles and endurance must still be tested.

*Narkar VA, Downes M, Yu RT, Embler E, Wang Y-X, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, Kang H, Shaw RJ, and Evans RM: AMPK and PPARdelta agonists are exercise mimetics. Cell 134: 405-415, 2008.*

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**Encouraging Metabolic Results in Test of a Candidate Therapeutic for Cardiovascular Disease Risk:** Scientists have recently shown that a compound that mimics some of the effects of a naturally-occurring hormone has metabolic benefits in people with high cholesterol, without causing adverse effects on the heart in a short-term study. The compound is designed to work like thyroid-stimulating hormone (TSH), a key metabolic regulator. People who make too little of their own TSH have numerous health problems, including obesity, high cholesterol, osteoporosis, and impaired kidney function. TSH may thus appear to be an attractive therapy to counteract some of these conditions, even in people who do not have low TSH levels. However, administering too much TSH can cause dangerously elevated heart rates and arrhythmias, as well as other health problems. Therefore, researchers have worked to develop

compounds with some of the salutary effects of TSH, without its potentially serious side-effects. Such TSH-like molecules have been successfully tested in overweight animals, where they proved to induce, without toxicity, weight loss and improvements in blood lipids. In the current study, scientists tested one of the TSH-like compounds in a small number of people with elevated cholesterol. They found that it significantly lowered LDL ("bad")-cholesterol, without causing the obvious heart problems typically observed in people with elevated TSH. The treatment did not result in weight loss or other obvious bodily changes during the 2 week test. Statins are popular and effective medications that also lower LDL-cholesterol, but they are poorly tolerated by some people. The TSH-like compound works by a different mechanism than statins, and therefore may prove to be an option for people who cannot take those medications. Although the results obtained in this study are very preliminary—long-term tests will be required to determine whether this potential therapeutic provides enduring benefits without simultaneously causing other health problems—these findings are exciting because they provide hope for a new therapy to combat heart disease.

*Berkenstam A, Kristensen J, Mellström K, Carlsson B, Malm J, Rehnmark S, Garg N, Andersson CM, Rudling M, Sjöberg F, Angelin B, and Baxter JD: The thyroid hormone mimetic compound KB2115 lowers plasma LDL cholesterol and stimulates bile acid synthesis without cardiac effects in humans. Proc Natl Acad Sci USA 105: 663-667, 2008.*

# *Dr. Rudolph Leibel and Dr. Natasha Leibel: Combating Obesity and Diabetes Is a Family Affair*

**Dr. Rudolph Leibel and Dr. Natasha Leibel (Photo credit: Charles Leduc)**

When it comes to furthering the understanding and treatment of diabetes and obesity, it is difficult to imagine a more passionate and devoted team than that of Dr. Rudolph Leibel and his daughter, Dr. Natasha Leibel, at the Naomi Berrie Diabetes Center at Columbia University Medical Center.

Dr. Rudolph Leibel (referred to as Rudy in this profile) is the Co-Director of the Naomi Berrie Diabetes Center and Professor of Pediatrics and Medicine at Columbia University. He has dedicated his research career to studying the regulation of body weight in rodents and humans and the genetic basis of type 2 diabetes and obesity. He has had research support from the NIDDK for over 25 years and has served as a member of the NIDDK Advisory Council and the NIDDK Clinical Obesity Research Panel. Dr. Natasha Leibel (referred to as Natasha in this profile) is a pediatric endocrinologist specializing in the treatment of diabetes in children. Together, father and daughter exemplify the concept of translational research at its best. Rudy's work in the laboratory is leading to a greater understanding of the causes of obesity and type 2 diabetes and is also providing insight into potential treatments. Natasha is at the forefront of putting this new research knowledge into practice as she cares for children with diabetes.

**Dr. Rudolph Leibel: At the Forefront of Research on Obesity and Diabetes**

While this father and daughter duo work in the same field—and only a few floors apart in the same building—they each arrived at their career through their own distinct path. Rudy, a world-renowned scientist who has made many contributions to the fields of diabetes and obesity research, started out his medical school training thinking that he would have a very different career path. "I wanted to be a psychiatrist," he states.

However, at the end of medical school, he decided to enter pediatrics—a decision that would eventually lead to his interest in laboratory research.

Following his general pediatric residency, Natasha's birth, and 2 years in the U.S. Army, Rudy started an NIH fellowship in endocrinology (the study of diseases related to the glands and hormones of the body) at Massachusetts General Hospital in Boston. Endocrinology was very exciting to him. As he recalls, "There was plenty of opportunity to study the origins of clinical diseases and to make new discoveries—very little was known about the hormones involved in many of the endocrine diseases. Assays to measure these hormones were just becoming available."

Rudy recalls one particularly memorable experience in the clinic over 30 years ago, when he received a visit from an obese child and his mother—a visit that would dramatically change his career path. At that time, obese children were frequently referred to pediatric endocrinologists because most people assumed that childhood obesity was associated with thyroid or adrenal problems, which is rarely the case.

Rudy could not find anything wrong with the child and, therefore, counseled the mother on healthier eating and physical activity. The mother was unimpressed with what

she determined was Rudy's lack of knowledge about her son's condition and she made a point of telling him so with some creative language.

Rudy says, "I was shocked for a number of reasons, as you might imagine, but, as I thought about it, I really had to agree with her. There was indeed a collective lack of understanding among scientists and clinicians about the causes of obesity." Rudy decided, "If I were going to remain in this field and help obese children, I needed to do something to move the field forward and advance knowledge about the regulation of body weight and the underlying causes of obesity."

A short time after this experience in the clinic, Rudy made an impromptu visit to the laboratory of Dr. Jules Hirsch, an expert in obesity and the properties of fat tissue in the body, at Rockefeller University in New York City. This visit further inspired Rudy to pursue the regulation of body weight in a research setting. The entire family supported Rudy's interest in research and moved to New York—even if it meant moving from a big house in Boston to a small apartment in New York.

Rudy states, "I stayed in research much longer than I might have because of a real passion for the work and a desire to follow it to completion…I originally went to Rockefeller on a 2 year leave of absence; however, this leave has now lasted 35 years."

Rudy's dedication and passion have led to the identification of several genes involved in the regulation of body weight and diabetes. For example, his lab collaborated in the discovery of the leptin gene. This discovery is just one of many key contributions that he has made to increasing understanding of the underlying causes of obesity and diabetes.

**Dr. Natasha Leibel: At the Forefront of Treating Children with Diabetes**

"I made the conscious decision to go to medical school while I was in college," Natasha replies, when asked when she first was interested in medicine.

Her father, however, thinks that her interest in medicine and her passion for helping others came about at an earlier age. "When we lived in Boston, I was practicing in a hospital and seeing a lot of patients. Because I was so busy, patients would often call my home asking for medical advice, and Natasha would listen to me talking to them," Rudy remembers. "As a little girl of 6 or 7 years old, Natasha would answer the phone and provide advice to patients about the treatment of acute conditions such as diarrhea and high blood sugar," he says. Although Rudy felt, "she gave rather solid advice," he stresses that his wife would always intervene and explain, "That was not the Dr. Leibel you were looking for." Rudy states, "I'm not saying that led her to a career in medicine but I don't think she was dissuaded by any of it."

Growing up, Natasha was very much aware of her father's career and the importance he placed on science as an essential part of education. She also observed his compassion and kindness toward others and, from this, knew that she also wanted to be in a profession where she could make a real difference in the lives of other people. "I decided that medicine was a good fit for me because of the many opportunities within the discipline and also because it would provide me with a chance to give back to society," said Natasha.

During medical school, Natasha felt especially drawn to pediatrics because, as she states, "I really enjoyed working with families. Children, in particular, are so vulnerable, especially when they are sick." Like her father, she was fascinated by the field of endocrinology; however, she is facing a new set of challenges. While Rudy is on the forefront of research, Natasha is on the forefront of clinical care where she specializes in caring for children with diabetes, including children with type 2 diabetes. Type 2 diabetes was previously called "adult-onset" diabetes because it primarily affected older adults. However, the disease is increasingly being diagnosed in children—a trend associated with the rising rates of pediatric obesity.

Natasha states, "The development of type 2 diabetes in children is so new that there are many questions regarding the optimal treatment of diabetes in this young population." To answer these questions, Natasha is involved in the NIDDK's Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study. The TODAY study seeks to identify the best treatment approach for type 2 diabetes in children and teens.

Natasha also cares for patients with type 1 diabetes, an autoimmune disease that most often occurs in infancy, childhood, and adolescence. Therefore, whether treating

a child with type 1 or type 2 diabetes, Natasha has the difficult task of caring for children living with a chronic and burdensome condition. Natasha says, "I get to be a part of these families' lives, which is a wonderful gift as a doctor." She also knows how difficult living with diabetes is for these children. Natasha is passionate about helping her patients and making a difference in their lives.

**Bringing the Clinic and Laboratory Together for Patients**

Some of Natasha's patients have the opportunity to meet and work with the researchers, including Rudy, who also are working in the laboratory to advance understanding of diabetes causes and treatment. Natasha says, "It is amazing to bring the children who we take care of to the laboratory to talk with the scientists. It is very encouraging for the children to see that there are people working so hard to try to understand their disease."

In addition, the Naomi Berrie Diabetes Center hosts a summer diabetes camp where Natasha notes, "The high point of the week is bringing the children upstairs to the laboratory." While Rudy and Natasha do not advertise their father/daughter relationship during these visits, the children often figure it out. Rudy and Natasha feel that some of the children are especially heartened to learn that a child of someone who has been working in the field of diabetes research for so long has gone on to pursue clinical training in this area. In fact, some of the participants in the diabetes camp are so inspired by the scientists and clinicians that Rudy and Natasha feel that they may be stimulating a future generation of pediatric endocrinologists. The Diabetes Center also sponsors summer students who are in high school, college, or medical school, to work in the research laboratories. Some of these students are also patients in the Diabetes Center. This program gives them an opportunity to contribute to a better scientific understanding of their disease.

**Following Your Passion**

Since Rudy and Natasha both pursued medical training in the same subspecialty—and Rudy was even one of Natasha's teachers in medical school—it may seem as if Rudy's career path had a major influence on Natasha's choice of profession. On the contrary, she explains, the way her father has lived his life has had a much bigger impact on Natasha than his medical and scientific pursuits.

"Growing up," Natasha explains, "my sister Alexis, who is also a physician, and I were always encouraged to find what we were passionate about and what we enjoy doing. Certainly, that was the example we saw him live by," she says of Rudy, "which was to follow his passion and to work really hard." Natasha continued, "He has been my role model because of the way he has lived his life and the challenges he has taken on. His enthusiasm and dedication are a constant source of inspiration."

Being in the same area of medicine does provide its advantages though, for both Natasha and Rudy. Natasha states, "It is an honor to work with him, and a great bond we have being in the same profession. Although I may have 'helped' him as a young girl with his patients, I certainly go to him all the time with questions about my patients. He is a phenomenal clinician." Likewise, Rudy is extremely proud of Natasha and her accomplishments in the profession they share.

Working only a few floors apart, Natasha says that, "People always ask us if we eat lunch together." She cautions that this is not the case because she and her father have fundamentally different views about lunch. Rudy skips lunch, while Natasha makes lunch a priority. In fact, when Natasha started at the Naomi Berrie Diabetes Center in 2005, Rudy joked that he would take her to lunch in 2007. Natasha laughs and says, "He did, but I ended up paying because he forgot to bring money." It looks like the next lunch date is scheduled for sometime this year.

In the meantime, both father and daughter are working tirelessly in the laboratory and in the clinic to combat obesity and diabetes. Even if they don't meet every day for lunch, Rudy and Natasha do see each other and talk about their work. Natasha confided, "We do talk frequently about science and he is a great source of knowledge. Mostly now he likes to talk about his grandchildren, though." And, she adds about her inspiring physician-scientist father, "He is a great babysitter!"

## STORY OF DISCOVERY

# *Leptin as a Treatment for Lipodystrophy: A Translational Success Story*

This story begins with an obese mouse and ends with a medical treatment for people who may lack fat tissue altogether. The common link that ties together these two very different entities is a hormone called leptin. Identifying this link was a result of the collaboration among many investigators over several years, including NIDDK-supported scientists at universities, scientists in the NIDDK Intramural Research Program, industry researchers, and many others. This translational success story is a demonstration of how exciting discoveries in the laboratory are used to improve the health of people.

**The Obese Mouse and the Discovery of Leptin**

In 1950, scientists identified a new mouse model that was extremely obese. They called the unknown gene causing the obesity "*ob*." By the 1980s, the identity of the *ob* gene was still unknown, but it was becoming more and more apparent that research on genetic contributors to obesity was critically important to pursue. Therefore, the NIDDK sought to support research to identify obesity-related genes in rodents, including the *ob* gene. The Institute sponsored a workshop on this topic and developed an initiative to solicit research applications. In 1989, the NIDDK awarded a grant to Dr. Jeffrey Friedman through this initiative. Dr. Friedman's subsequent pioneering research led to the 1994 discovery of the mouse *ob* gene. The hormone produced by this gene was named "leptin," a term that derives from a Greek word meaning thin. Because the *ob* mutant mouse was obese, the scientists realized that the normal *ob* gene—and the hormone it encodes—must contribute to leanness.

The landmark discovery of leptin unleashed a wave of new research advances in fat biology and metabolism. Researchers found that leptin is secreted by fat cells and released in proportion to the amount of fat. These observations drastically altered the former view of normal fat tissue as simply a passive "fat storehouse." Research fueled by this 1994 discovery also led to the identification of a number of other substances that, like leptin, are secreted by fat cells and influence appetite and metabolism.

Studies demonstrated that obese animals deficient in leptin, including mice carrying the mutant form of the *ob* gene, lost weight when given the hormone. Therefore, researchers postulated that leptin treatment might also be useful for human obesity. There are, in fact, very rare instances of complete deficiency of leptin in humans that result in morbid obesity from infancy. Leptin treatment in these individuals caused substantial weight loss, providing hope for improved quality of life and longevity.

Unfortunately, in clinical studies done at that time, leptin administration was not effective in treating the vast majority of cases of human obesity, which are not due to leptin deficiency. In most cases, obesity results from a complex interaction among genetic variation (potentially involving many genes not yet identified) and the environment. Obese individuals, in fact, usually have very high levels of leptin, probably a consequence of the many fat cells secreting it. The inability of the high levels of leptin to decrease body weight suggests that the more common forms of obesity are associated with a resistance to leptin's actions. Although these results were disappointing, scientists did not give up in their quest to use this new knowledge to benefit people.

**Testing Leptin as a Treatment for Lipodystrophy**

Scientists in the NIDDK's Intramural Research Program had broad experience with respect to

## STORY OF DISCOVERY

studying people with various forms of insulin resistance. Using this experience and knowledge, they identified a patient population—people with lipodystrophy—who could potentially benefit from leptin treatment.

Lipodystrophy is actually a group of disorders with disparate origins but with a common set of characteristics. Individuals with lipodystrophy lack fatty tissue in the face, neck, or extremities. They sometimes have central obesity and sometimes lack fat tissue altogether. While lipodystrophy is characterized by the loss of fatty tissue in certain areas of the body, tissues such as liver and muscle exhibit significant abnormal accumulation of fat, which impairs metabolic activity. These patients also exhibit resistance to the effects of insulin and are thus at high risk of developing diabetes. They may also have a range of lipid abnormalities. Treatment of lipodystrophy has included the administration of insulin, oral hypoglycemic (blood glucose lowering) agents, and lipid-lowering drugs. In spite of treatment, patients with lipodystrophy continue to have severely high levels of triglycerides, leading to recurrent attacks of acute inflammation of the pancreas; severe problems controlling blood glucose levels, posing risks of diabetic eye and kidney disease; and fat accumulation in the liver, which can result in cirrhosis and liver failure.

Because many people with lipodystrophy have low leptin levels, and because research had demonstrated beneficial effects of leptin on insulin sensitivity and fat metabolism in a number of tissues, researchers in the NIDDK Intramural Research Program and their collaborators investigated whether leptin treatment could ameliorate conditions associated with lipodystrophy. In two small clinical studies of individuals with lipodystrophy treated for short periods of time (3-8 months), leptin therapy had dramatic benefits. In one study of female patients with different forms of lipodystrophy, most of whom also had type 2 diabetes, leptin therapy improved blood glucose levels, lowered triglyceride levels, and decreased liver fat content. In another study, leptin therapy markedly improved insulin sensitivity, lowered lipid levels, and decreased liver fat content in individuals with severe lipodystrophy who also suffered from poorly controlled type 2 diabetes. Patients in these studies were able to reduce or discontinue their diabetes medications.

Seeing such dramatic results, the researchers next examined the effect of long-term leptin therapy (12 months) in patients with severe forms of lipodystrophy and poorly-controlled diabetes. Long-term leptin therapy had similarly remarkable results. Patients had improved blood glucose and blood lipid levels, and decreased fat in their livers. The patients also reported a dramatic reduction in their appetite, which led to moderate reductions in their weight. In addition, patients were able to discontinue or reduce their diabetes medications. These exciting results suggested that leptin was an effective treatment for severe lipodystrophy.

The scientists also examined the effect of leptin on other metabolic abnormalities associated with lipodystrophy. For example, female patients often have irregular or absent menstrual cycles. Leptin treatment was found to be corrective of that condition—eight of eight female patients achieved normal menstrual function following leptin therapy. In a study of 10 patients, leptin effectively improved liver function and reduced liver fat content in people with lipodystrophy and nonalcoholic steatohepatitis, a progressive metabolic liver disease. In a study of 25 patients with lipodystrophy, researchers found that a surprisingly high number had some form of kidney disease. Leptin treatment was found to improve their kidney function. Thus, leptin corrected a broad range of metabolic defects associated with lipodystrophy.

Lipodystrophy can either be inherited or acquired, and can be complete (near total lack of fat) or partial (fat loss in certain parts of the body). Clinical trials

conducted by scientists in the NIDDK Intramural Research Program and their collaborators examined leptin treatment for various forms of lipodystrophy and found that leptin effectively treated all forms tested. These results suggest that leptin is generally effective for treating lipodystrophy, independent of the underlying cause.

**Testing Leptin for Treating Lipodystrophy: A Team Effort**

The clinical trials testing leptin therapy for lipodystrophy conducted by the NIDDK Intramural Research Program required numerous collaborators, and spawned new collaborations. Leading this effort was Dr. Phillip Gorden, a former NIDDK Director who returned to the laboratory to continue his research. Because leptin was manufactured by industry, the Intramural Research Program and the NIDDK Office of Technology Transfer and Development worked with industry to obtain the leptin needed for the studies. In addition, because lipodystrophy affects the liver and kidneys, scientists in the Intramural Research Program with expertise studying those organs were valuable contributors to the studies. Furthermore, collaborators external to the NIDDK have studied the genetic underpinnings of different forms of inherited lipodystrophy; several genes have now been identified. Finally, many of the patients were evaluated and treated at the NIDDK's Metabolic Clinical Research Unit, a new facility in the NIH Clinical Center that enables scientists to make precise metabolic measurements. It was only through the contributions of all of these collaborators that this translational success story came to fruition.

**Looking to the Future**

Looking to the future, scientists are continuing research on leptin and exploring approaches for its use in treating other diseases and disorders. As described in this story, knowledge gained from studying a common condition, obesity, led to the discovery of leptin and a treatment for a very rare disease, lipodystrophy. Scientists are now coming full circle by building on the successful clinical studies with leptin in lipodystrophy and applying it to research on common diseases. For example, the NIDDK Intramural Research Program is conducting studies to examine leptin's effects on treating people with other forms of severe insulin resistance and other common metabolic conditions. If leptin proves effective in these cases, these studies would be an example of how research on rare diseases may additionally benefit people with more common diseases and syndromes. The discovery of leptin has led—and continues to lead—to a cascade of exciting and unexpected findings with broad implications for improving health.

# Food Intake and Body Weight: Regulation by Apo A-IV in the Brain

*Dr. Patrick Tso*

*Dr. Patrick Tso is Professor of Pathology, Associate Director of the Cincinnati Obesity Research Center, and Director of the Center for Lipid and Atherosclerosis Research at the University of Cincinnati College of Medicine. Additionally, he is the Director of the Cincinnati Mouse Metabolic Phenotyping Center, funded by NIDDK. Dr Tso is a highly respected researcher in the area of lipid (fat) metabolism, a field in which he has worked for over 20 years. At the September 2008 meeting of the NIDDK Advisory Council, Dr. Tso shared insights from his exciting research on how food intake and body weight are regulated by apolipoprotein A-IV (apo A-IV). The following are highlights of his presentation.*

**Feeling Full after a High-Fat Meal: The Discovery That Apo A-IV Regulates Food Intake**

How does a high-fat meal make one feel full? Dr. Tso described his laboratory's research to understand what causes satiety and the insights that have emerged from these studies about the role of a small biologic factor called apo A-IV, which is made in humans and animals. Intrigued by the dramatic increase in intestinal apo A-IV production known to occur after ingestion of dietary fat, Dr. Tso and one of his post-doctoral fellows, Dr. Kazuma Fujimoto, investigated a potential role for apo A-IV in satiety. The researchers conducted their experiments in rodents, and began with a focus on a body fluid called lymph, which varies in composition depending upon what food has been ingested. After a fatty meal, lymph from the abdomen contains an abundance of apo A-IV along with fat absorbed from the food. To assess whether this fluid might curtail food intake, they compared different samples of lymph, some with fat and some without. After administering the lymph samples intravenously into fasting rats, they assessed how much the rats subsequently ate. In describing this study, Dr. Tso shared an anecdote about the experimental design. He recounted that Dr. Fujimoto was concerned that the rats might be too "worried" to eat if a person was nearby. So, Dr. Fujimoto decided to speak to each rat for 15 minutes every day (in his native Japanese) to help the animals feel comfortable around him. This procedure evidently worked, as the rats did eat—but those who had been given the fat-containing lymph ate significantly less. Thus, something in the lymph was signaling that a relatively small meal would be sufficient. The researchers then investigated which component of the lymph might be causing this satiety effect: the fat, or the apo A-IV. After a series of additional experiments, they discovered that it was apo A-IV.

**Site of Action: The Brain**

Dr. Tso next sought to discover where in the body apo A-IV exerts its effect to reduce food intake. Although apo A-IV was originally found to be produced in the intestine, substantial regulation of appetite occurs in the brain. Thus, Dr. Tso, with another post-doctoral fellow, Dr. Koji Fukagawa, explored whether apo A-IV could reduce food intake when infused directly into the brain. The answer was yes, as determined by further studies in rats.

Building on this research, Dr. Tso and another of his post-doctoral fellows, Dr. Min Liu, found that apo A-IV is not only produced in the intestine, but it is also synthesized in a part of the brain, the hypothalamus, known to play a crucial role in the control of food intake and body weight. In further experiments in rats, they demonstrated that excess apo A-IV in the brain,

from infusions, reduces body weight in parallel to its effect on satiety.

**From Feeding to Fullness: Elucidating Biologic Pathways in the Brain**

Having illuminated the role of brain apo A-IV in regulating food intake, Dr. Tso and his colleagues next asked: What regulates apo A-IV? They first tested whether brain apo A-IV, like apo A-IV in the intestine, is affected by feeding and fasting. From studies in rats, the researchers found that apo A-IV levels in the brain substantially decreased after fasting, a result that is consistent with their findings regarding the role of apo A-IV in satiety; an animal that had not eaten for a day should not feel full. The researchers next explored the effects of different types of food on apo A-IV levels in the brain. When the rats, after fasting, were given their standard "chow," apo A-IV levels in the brain did not change significantly. If the animals instead ate high-fat food, their brain apo A-IV levels greatly increased.

Dr. Tso and his laboratory also discovered that apo A-IV levels in the brain fluctuate with the circadian rhythm—the day/night cycles. Levels of this satiety-inducing factor were lowest at night, when rats typically eat, and peaked during the day, when rats normally do not eat. Thus, the daily rise and fall of apo A-IV levels mirrored the animals' feeding patterns. The researchers then explored whether the changes in apo A-IV levels were caused by the cycles of light and dark per se, or by the concomitant cycles of feeding and fasting. When they shifted the rats' meal times to the daylight hours (by providing food only during the day), the researchers found that apo A-IV levels changed, too. Dr. Tso concluded that it was the cycles of feeding and fasting that affected apo A-IV levels, rather than daylight and darkness. That is, under normal conditions, apo A-IV levels are low at night (when food is typically available), and the rats are thus able to eat. Their ingestion of food causes apo A-IV levels to rise by the morning hours, which in turn makes the rats too full to eat during the day. After not eating for a while, the apo A-IV levels fall again so that by night time, the rats become hungry and eat.

To further explore the pathway by which apo A-IV causes satiety, Dr. Tso and his research team investigated whether apo A-IV interacts with the hormone leptin. Mice deficient in leptin are strikingly obese, and this hormone also plays a critical role in body weight regulation in humans. The researchers measured apo A-IV levels, and the effects of fasting and feeding, in normal mice and mice that lacked leptin (as a result of a genetic mutation). In the leptin-deficient mice, levels of apo A-IV in the brain were lower than in the normal mice. Additionally, when leptin-deficient mice were given a high-fat meal, the levels of brain apo A-IV did not increase as in normal mice. The scientists then injected leptin into the deficient mice, and found that this led to a restoration of normal levels of apo A-IV. From these and other experiments, the researchers concluded that leptin regulates apo A-IV, and that leptin and apo A-IV interact to reduce food intake and body weight.

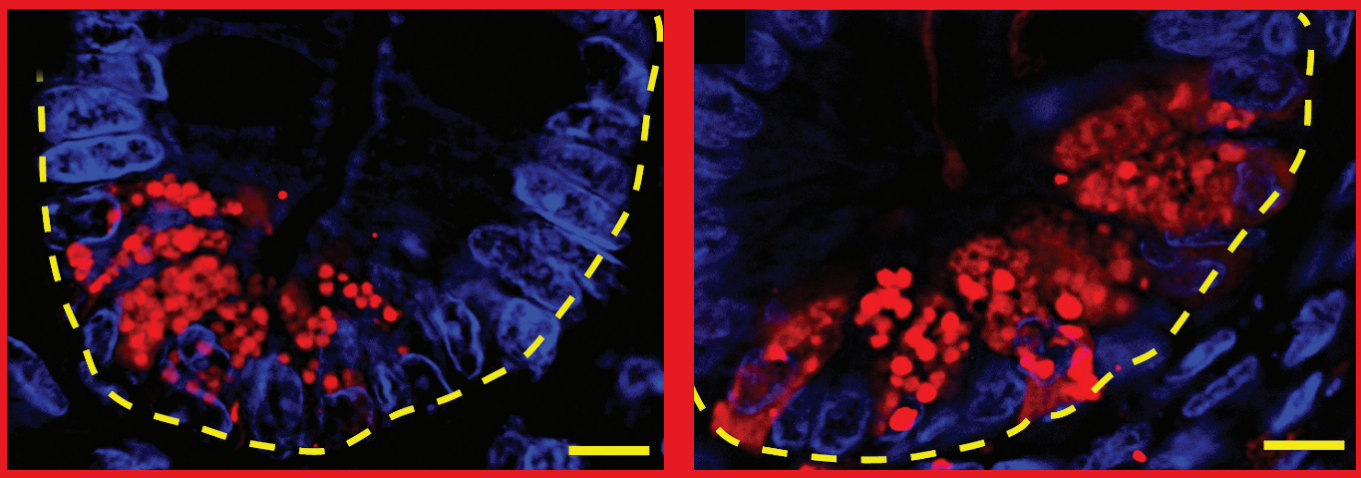
Dr. Tso's research team then turned their attention to other factors in the brain known to regulate food intake, collectively referred to as the melanocortin system, to determine whether these factors interact with apo A-IV. Again using rodents as a model system, the researchers found that apo A-IV and a major component of the melanocortin system, called POMC, are present in the same brain cells, and both apo A-IV and POMC levels are low during fasting. Administering apo A-IV led to an elevation in POMC levels as well. This research, together with additional studies, demonstrated that apo A-IV also interacts with the melanocortin system to inhibit food intake.

**Conclusions—Apo A-IV**

Dr. Tso's research on apo A-IV has yielded novel insights into the regulation of food intake and satiety. In concluding his presentation, Dr. Tso noted that apo A-IV has other functions as well, related to fat metabolism and other biologic processes. By

shedding light on the regulation of satiety, this research will also advance understanding of what could go awry in obesity, with implications for potential intervention approaches.

*Dr. Tso acknowledged the contributions of the scientists who worked with him on these studies when they were post-doctoral fellows in his laboratory: Drs. Kazuma Fujimoto, Koji Fukagawa, and Min Liu. Additionally, Dr. Tso thanked his long-time collaborator on this research, Dr. Stephen Woods, who is also a Professor at the University of Cincinnati.*



**Autophagy is an important process that the cell uses to degrade damaged cellular components and to help eliminate some pathogenic bacteria. These images show the effect of the Crohn's disease-associated mutation *ATG16L1* in Paneth cells from human participants who do (right panel) or do not (left panel) carry the mutated gene. The Paneth cell, a type of intestinal cell, is important in defending against the microbial flora. The protein Atg16 is responsible for proper placement of the autophagy machinery within the cell. When Atg16 is mutated, an increased proportion of Paneth cells contain disorganized or diminished granules (red dots) which serve as a storage depot for antimicrobial peptides and a protein called lysozyme. This new finding sheds light on previously unrecognized abnormalities in Paneth cell storage granules in the intestinal crypt in people with Crohn's disease who carry the mutated *ATG16L1* gene. Additional information on autophagy and its role in Crohn's disease can be found in this chapter. Also described in this chapter, NIDDK-supported research suggests that autophagy is triggered by an abnormal aggregation of mutant proteins in alpha-1-antitrypsin deficiency disease. Autophagy has also been implicated in anemia due to abnormal red blood cell maturation, as described in the chapter on Kidney, Urologic, and Hematologic Diseases.**

*Images provided by Drs. Thaddeus S. Stappenbeck and Herbert W. Virgin IV and reprinted by permission from Macmillan Publishers Ltd: Nature, 456: 259-263, copyright 2008.*

# Digestive Diseases and Nutrition

*Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. These conditions include disorders of the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. Disorders of the digestive tract exact a significant toll on many Americans each year. For example, approximately 135 million people each year suffer from non-food-borne gastroenteritis, a typically infectious inflammation of the GI tract associated with such symptoms as diarrhea, nausea, and vomiting.[1] Additionally, liver and biliary diseases affect a large portion of the population and represent a huge burden, in terms of quality of life as well as health care costs, such as the estimated 6 billion dollars spent annually in the U.S. on gallbladder disease care.[1] NIDDK-supported scientists are vigorously pursuing research to understand how widespread these diseases are across the U.S., to identify the causes of these diseases and how they progress, and to test new interventions for treatment and prevention of these costly diseases, including drugs, surgery, and behavior modification.*

Digestive diseases that affect the GI tract include inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis. These diseases are marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. IBD often strikes early in life, with a peak age of onset in adolescence or young adulthood. To address this condition, surgery may be required, including removal of the affected region of the intestine. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to the development of IBD. Helping to catalyze the design of novel therapeutic strategies will be the continued discovery of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences. Research on controlling intestinal inflammation has potential benefits not only for patients with inflammatory bowel diseases, but also for those at risk of developing colorectal cancer. Screening programs for colorectal cancer are aimed at reducing mortality through early detection, particularly in those individuals at higher risk.

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 cell, is associated with a heightened risk of esophageal cancer, the most rapidly rising cancer in the U.S. Gastroparesis is another functional bowel disorder that 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.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the small intestine is damaged when the immune system reacts to the protein gluten—a component of wheat, barley, and rye. This reaction interferes with the ability to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, growth failure. The only

[1] *Sandler RS, et al: Gastroenterology 122: 1500-1511, 2002.*

current treatment for celiac disease is maintenance of a 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 gastrointestinal tract are increasingly appreciated as 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 host cells, and with nutrients ingested by their 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 liver is an organ within the digestive system that performs many centralized functions in the body, including metabolism and distribution of nutrients such as fats. When the liver is functionally compromised by disease, this can have serious adverse impacts 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 more commonly affect adults—such as a form of nonalcoholic fatty liver disease known as nonalcoholic steatohepatitis (NASH). Some are caused by viral infection, such as hepatitis B and C, or by genetic mutations, such as alpha-1-antitrypsin deficiency, while others arise from diverse factors such as autoimmune reactions, drug toxicity, and other, unknown triggers. Many of these forms of liver disease, 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. The number of livers available from deceased donors is limited. Therefore, research is of critical importance to identify and treat liver disease, preserve liver function in people with liver disease, and explore treatment options beyond cadaveric liver transplants, including the use of tissues from living donors.

The number of overweight and obese Americans has risen dramatically in the past two decades and is now at epidemic levels. Obesity is associated with numerous serious diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular factors that control appetite, metabolism, and energy storage, they are identifying potential targets for the development of new pharmacologic agents 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 increased physical activity and improved diet. (Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)

## GENETICS OF INFLAMMATORY BOWEL DISEASES

**The Number of Genes Associated with Crohn's Disease Soars to 30 and Beyond:** Using genome-wide association methodology and combined research data, three international research groups conducted a joint study that identified 21 new genes or gene regions associated with Crohn's disease (CD), bringing the total number to more than 30. Crohn's is a complex disease involving genes, the immune system, and microbes that interact to cause the intestinal inflammation. Although the precise number of CD-associated genes is not known, scientists estimate that 100 or more genes having varying levels of influence on disease susceptibility may be implicated in CD. Importantly, even genes with modest contributions to disease risk may have significant consequences. The three research groups had previously conducted individual genome-wide association studies that identified 11 genes associated with CD. In this joint study, data from the previous investigations were combined to create a large study cohort capable of detecting the more subtle CD variants. The new study's analysis of the combined data identified the 11 original CD genes and discovered 21 additional CD genes, bringing the total number of CD-associated genes to 32. The scientists confirmed the results of the new study by conducting a replication analysis with an independent group of

individuals with CD, and a mixture of family-based and population-based healthy (control) participants. The findings of this multi-national study demonstrate the advantage of very large study cohorts in detecting genes that may each have only modest effects in complex diseases, but that may interact with environmental factors and perhaps with each other to influence disease susceptibility. Although the functions of some of these CD variants are unknown, several are related to biochemical pathways that promote inflammation. All of the genes and genomic regions identified by this study provide important information for understanding the molecular architecture of CD and identifying potential targets for future therapies.

*Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ; The NIDDK IBD Genetics Consortium, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot J-P, de Vos M, Vermeire S, Louis E; The Belgian-French IBD Consortium; The Wellcome Trust Case Control Consortium, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, and Daly MJ: Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 40: 955-962, 2008.*

### Specialized Intestinal Cells and Crohn's Disease:

Scientists have recently discovered that a gene associated with Crohn's disease plays an important role in specialized intestinal cells, known as Paneth cells. Crohn's disease, a type of inflammatory bowel disease, is a chronic disorder that causes inflammation of the digestive tract resulting in abdominal pain and diarrhea. Previous research revealed that some patients with Crohn's disease harbor a mutation in a gene called *ATG16L1*. This gene is involved in an important biologic process called autophagy, which cells use to degrade damaged cellular components and to help eliminate some pathogenic bacteria. But it was not known how the ATG16L1 protein contributes to Crohn's disease onset and/or progression. Because the ATG16L1 protein is responsible for proper placement of the autophagy machinery within mammalian cells, researchers created mice having reduced levels of ATG16L1 to begin to determine the role of this protein in autophagy in the intestine. Reduced levels of ATG16L1 had no effect on the overall appearance of the intestine, but there were obvious changes in Paneth cells that line the intestinal wall. Paneth cells have granules filled with antimicrobial agents which are secreted in response to invasive bacteria. In contrast to mice with normal levels of ATG16L1, mice with reduced levels of this protein contained disorganized granules and marked changes in their granule-secreting pathways, as well as other defects. Examination of mice deficient in a different autophagy gene (*ATG5*) revealed similar Paneth cell abnormalities, verifying that the autophagy process is involved in the granule secretion pathway of Paneth cells. Further investigation revealed that intestinal Paneth cells from patients with Crohn's disease contained disorganized or diminished granules, which was similar to that seen in mice with reduced levels of ATG16L1. Thus, this study demonstrates previously unrecognized abnormalities with respect to Paneth cells and provides additional understanding of a compromised host defense in Crohn's disease.

*Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S, Stone CD, Brunt EM, Xavier RJ, Sleckman BP, Li E, Mizushima N, Stappenbeck TS, and Virgin HW IV: A key role for autophagy and the autophagy gene Atg16L1 in mouse and human intestinal Paneth cells. Nature 456: 259-263, 2008.*

## SCREENING FOR COLON CANCER

### Disparities Found in the Prevalence of Colon Polyps for African Americans and Caucasians:

New research suggests that more extensive screening for pre-cancerous polyps may help address disparities in rates of colon cancer. African Americans are at greater risk of developing colorectoral cancer and dying from this disease than are Caucasians. The reasons for these disparities are not clear. However, colon cancer is frequently preventable if pre-cancerous polyps are detected and removed before they can form tumors. Therefore, scientists designed a study to gain insight into whether there are differences between African Americans and Caucasians in the number of polyps and polyp location as detected by colonoscopy. They reasoned that any differences found could inform

recommendations for earlier or enhanced screening. Screening colonoscopy data were analyzed from subsets of African American and Caucasian men and women who had no prior symptoms of colorectal disease. The analysis revealed that the African Americans had greater numbers of pre-cancerous polyps (polyps 9 millimeters or larger) than the Caucasians. The number of pre-cancerous polyps detected in African American women younger than 50 years was found to be particularly high. Although all people over age 60 were more likely to have polyps located in the upper (proximal) colon than younger patients, African Americans over age 60 had a greater number of proximal polyps than Caucasians of the same age. This study provides important information showing that African Americans who do not yet show symptoms of colorectal disease are more likely than Caucasians to have pre-cancerous colon polyps. Evidence linking upper colon polyps to age suggests that people over age 60—both African Americans and Caucasians—would benefit more from colonoscopy, a procedure which examines the entire colon. This is significant because another technique for detecting polyps—the sigmoidoscopy—does not screen the proximal colon. Notably, this study suggests that increased early colonoscopy screening for African Americans has the potential to significantly reduce their disproportionate burden of colon cancer.

*Lieberman DA, Holub JL, Moravec MD, Eisen GM, Peters D, and Morris CD: Prevalence of colon polyps detected by colonoscopy screening in asymptomatic black and white patients. JAMA 300: 1417-1422, 2008.*

## BARRETT'S ESOPHAGUS RISK FACTORS

**Fruits and Veggies Help Protect Against Barrett's Esophagus:** A recent study shows that eating more fruits and vegetables, which are rich in antioxidants as well as other substances that may affect health, is associated with a reduced risk of developing Barrett's esophagus (BE). BE is a condition in which the cells lining the esophagus transform into a type of cell that is found in the intestine. Patients with BE are at high risk of developing a form of cancer called esophageal adenocarcinoma, a cancer that has risen substantially in the U.S. in recent decades. Previous studies revealed an association between eating more fruits and vegetables and having a decreased risk of developing esophageal cancer, as well as an association between dietary antioxidants (which are among the many substances found in fruits and vegetables) and reduced risk for esophageal adenocarcinoma. The current study was designed to determine if a higher intake of fruits and vegetables, and either dietary or supplemental antioxidants, was associated with lower risk of BE. The study began by interviewing three groups of volunteers: (1) patients recently diagnosed with BE and other study participants; (2) healthy individuals; and (3) individuals with a history of gastroesophageal reflux disease (GERD), a condition that is often a precursor to BE. All study participants completed a "food frequency" questionnaire to assess their dietary habits during the previous year. Participants' diets subsequently were analyzed according to the number of servings of fruits and vegetables they consumed and whether or not they took antioxidants in the form of dietary supplements. Comparison of the diets of BE patients with those of the "healthy" group revealed that eating more fruits and vegetables correlated with a reduced risk of BE, with a greater benefit achieved from more daily servings. In contrast, dietary supplements containing antioxidants did not lower the risk of BE, even for those who ate few servings of fruits and vegetables. Comparison of the diets in the "GERD" participants with the healthy group showed that fruit and vegetable consumption was also associated with reduced risk of GERD. The results of this study indicate that eating more fruits and vegetables is associated with a reduced risk of BE, which may in turn reduce the risk of developing esophageal cancer.

*Kubo A, Levin TR, Block G, Rumore GJ, Quesenberry CP, Buffler P, and Corley DA: Dietary antioxidants, fruits, and vegetables and the risk of Barrett's esophagus. Amer J of Gasteroenterol 103: 1614-1623, 2008.*

## IRRITABLE BOWEL SYNDROME CONTRIBUTORS

**New Insights into the Pain Associated with Irritable Bowel Syndrome:** Researchers have found that patients with irritable bowel syndrome (IBS) perceive visceral pain associated with IBS differently than healthy volunteers, and exhibit altered brain activity responses to both pain and anticipation of pain. In addition, pain responses are heightened in IBS patients who have experienced physical abuse.

IBS is a relatively common, painful, and distressing disorder characterized by cramping, abdominal pain, bloating, constipation, and diarrhea. IBS affects more women than men. It is a debilitating disorder that can prevent individuals from working, attending social events, or traveling even short distances. The coping mechanisms that are employed by individuals to cope with pain are determined by the degree of pain that is anticipated. Inhibition mechanisms are employed to minimize discomfort when the expected pain is tolerable, whereas amplification mechanisms are used to enhance arousal and vigilance when the expected pain is dangerous. Researchers thus designed clinical studies to evaluate the pain responses of IBS patients, explore whether IBS patients exhibit increased sensitivity to anticipated visceral pain, and determine if prior abuse plays a role in the intensity of pain experienced by some IBS patients.

In one study, functional magnetic resonance imaging (fMRI) was used to evaluate the brain activity of women with IBS and healthy women volunteers in response to anticipation and delivery of mild and moderate pain induced by rectal distention. When compared to healthy volunteers, IBS patients experienced a heightened perception of pain and altered brain activity both when anticipating the pain, which was cued, and during the actual painful rectal distention procedure. Brain activity patterns of the healthy volunteers corresponded to known inhibition responses that would help to minimize pain perception. When the IBS patients were anticipating the pain, the brain imaging showed less of this inhibitory response, which then correlated with increased brain responses during the actual pain. These results confirm a hypersensitivity to visceral pain in IBS patients and suggest a relationship among pain perception, altered brain activity in response to pain stimuli, and the anticipation of pain.

In a second study, researchers assessed whether a history of sexual or other physical abuse affected the degree of pain IBS patients reported and, using fMRI, whether prior abuse correlated with differences in brain responses to painful stimuli. The responses to induced pain of women with IBS who had been abused were compared to those of women with IBS who did not have abuse histories as well as to women who did not have IBS. Women with both IBS and a history of abuse reported significantly greater pain in response to rectal distention, and they exhibited brain activity patterns that demonstrated reductions in pain inhibition responses and other alterations in brain activity associated with pain. Analyses of the increased perceived pain and the decreased pain inhibition of patients with histories of abuse revealed that abuse has a synergistic effect that intensifies IBS-related pain. This finding is consistent with the poorer treatment outcomes observed in IBS patients who have been abused.

Visceral pain has a major impact on the lives of individuals with IBS. In these studies, scientists explored pain perception of IBS patients, gaining new insights into patient responses to anticipated and perceived pelvic pain and the effects of prior abuse on IBS-related pain. The findings of these studies provide researchers with greater understanding of IBS-related pain, which may lead to improved treatment strategies and outcomes.

*Ringel Y, Drossman DA, Leserman JL, Suyenobu BY, Wilber K, Lin W, Whitehead WE, Naliboff BD, Berman S, and Mayer EA: Effect of abuse history on pain reports and brain responses to aversive visceral stimulation: An fMRI study. Gastroenterology 134: 396-404, 2008.*

*Berman SM, Naliboff BD, Suyenobu B, Labus JS, Stains J, Ohning G, Kilpatrick L, Bueller JA, Ruby K, Jarcho J, and Mayer EA: Reduced brainstem inhibition during anticipated pelvic visceral pain correlates with enhanced brain response to the visceral stimulus in women with irritable bowel syndrome. J Neurosci 28: 349-359, 2008.*

## CELIAC DISEASE IN CHILDREN

**New Antibody Test To Detect Early Childhood Celiac Disease:** Scientists have developed a new strategy to facilitate early detection of celiac disease and to help patients and doctors monitor adherence to the gluten-free diet required by people with this disease. The strategy uses a new test to detect antibodies against small modified gluten proteins, called deamidated gliadin peptides (DGP); these antibodies are made aberrantly by the immune systems of patients with celiac disease. Celiac disease affects approximately 1 in 133 individuals in the U.S. When individuals with celiac disease ingest gluten, a component of wheat, rye, and barley, their

immune systems mount a reaction against it, which damages the small intestine. To avoid or repair this damage, individuals with celiac disease must adhere to a gluten-free diet. There is great individual variation in the intensity and spectrum of symptoms associated with celiac disease. Many of the symptoms, including weight loss, short stature, and failure of infants to thrive, are related to malabsorption of nutrients by the damaged small intestine. Therefore, it is especially important to diagnose this disease as early as possible in infants and children to prevent the growth problems associated with celiac disease.

In this study, scientists used a new test to compare levels of antibodies to DGP to levels of another type of antibody made in celiac patients, the transglutaminase autoantibody (TGAA). An autoantibody is an antibody made by the immune system against one of the body's own proteins; celiac is thus considered an autoimmune disease. The antibody levels were tracked over time using blood samples collected from 50 children (6 months to 17 years of age), beginning prior to their disease diagnosis. The children were participating in the NIDDK-sponsored Celiac Disease Autoimmunity Research (CEDAR) study. These children were at high risk for developing celiac disease due to their genetic predisposition to celiac disease and/or another autoimmune disease, type 1 diabetes. The CEDAR study is, in fact, a branch of a larger study, the Diabetes Autoimmunity Study in the Young (DAISY). All of the children studied were TGAA positive, and 39 of the children were also positive for DGP antibodies at the time TGAA was first detected. Notably, in 9 of these children, DGP antibodies were found even before TGAA was detected, demonstrating that the DGP antibody test was indicative of celiac disease in these children before they tested TGAA positive. After the initial evaluations, 30 children elected to follow a gluten-free diet while 20 continued on a normal diet. For the 30 children on the gluten-free diet, both TGAA and DGP antibody decreased over time. However, DGP antibody levels declined more quickly than TGAA levels after starting a gluten-free diet. These results suggest that measurement of DPG antibody may be more useful than measurement of TGAA in monitoring adherence to a gluten-free diet. By following the development of antibodies to DGP and TGAA in children at risk for celiac disease, scientists have shown that the new DGP antibody test will permit earlier detection of celiac disease in certain individuals and will provide a more sensitive tool for evaluating the success of a gluten-free diet intervention.

*Liu E, Li M, Emery L, Taki I, Barriga K, Tiberti C, Eisenbarth GS, Rewers MJ, and Hoffenberg EJ: Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr 45: 293-300, 2007.*

## INHERITED DISEASES OF NUTRIENT METABOLISM

**Menkes Disease—A New Strategy for Therapeutic Design:** Scientists have developed a novel approach to screening for potential treatments for a fatal childhood disease, Menkes disease. Children with this disease suffer seizures, floppy muscle tone, neurodegeneration in the brain, and failure to thrive beginning a few weeks after birth, along with other symptoms. Menkes disease is caused by mutations in a gene that encodes a copper-transporting protein; copper is critical for many biological processes. Although treatment with copper injections can improve outcomes if begun shortly after birth, development may still not be normal, and the disease is often not detected early enough. The scientists first developed a model of this devastating disease in zebrafish, a small fish highly amenable to experimentation. Aptly named "calamity," this zebrafish model has similar symptoms to human Menkes disease. To explore potential new therapeutic approaches, the scientists then considered the nature of the gene and the disease-causing mutations. As is the case with many genes, the protein-coding information occurs in non-contiguous segments along a strand of DNA. Normally, the intervening segments are excised and the protein-coding information joined together in a process called splicing, which occurs in special copies of the gene called mRNA copies. A mutation in the zebrafish gene caused aberrant splicing, resulting in a lack of normal copper-transporting protein. To try to counteract this defect, the scientists next designed a series of molecules that would interact with specific stretches of mRNA around the mutant splice site. After injecting these molecules—called "antisense oligonucleotides"—into zebrafish embryos, they identified a few that reduced the aberrant splicing and restored normal neurologic development. Because

different types of mutations cause disease in different ways, this approach would not ameliorate the effects of all Menkes disease mutations. However, this strategy may eventually lead to new treatments for many patients with this disease and other genetic diseases. It also demonstrates the value of a zebrafish model of human genetic disease for rapid screening of potential therapeutics.

*Madsen EC, Morcos PA, Mendelsohn BA, and Gitlin JD: In vivo correction of a Menkes disease model using antisense oligonucleotides. Proc Natl Acad Sci USA 105: 3909-3914, 2008.*

## LIVER FAT METABOLISM AND CONTRIBUTORS TO NONALCOHOLIC FATTY LIVER DISEASE

**New Role for Molecular Factor in Regulating Liver Fat Metabolism:** Research sponsored in part by the NIDDK has uncovered a new function for a previously identified protein that acts within liver cells to regulate lipid (fat) metabolism. The liver serves as a major metabolic center within the body, receiving nutrients for processing soon after their digestion in the intestine. When carbohydrates are consumed in excess of the body's needs, the liver can incorporate them into lipids such as triglycerides for storage. A long-standing surplus of lipids produced by the liver can contribute to conditions such as the metabolic syndrome that are marked by abnormal lipid levels. Lipid synthesis in the liver is a process that is regulated by several transcription factors that control the production of enzymes involved in carbohydrate and lipid metabolism. The transcription factor XBP1 was originally known to have a key role in a completely different cellular process—turning on genes involved in protein secretion by pancreatic cells and plasma B immune cells. In these studies, however, scientists found that XBP1 plays an additional, functionally distinct role in regulating lipid metabolism in the liver. Scientists used a mouse model, in which XBP1 synthesis was controlled, to isolate the specific actions of XBP1 in liver lipid metabolism. The requirement of this factor for normal lipid synthesis in the liver was demonstrated by the low levels of lipids, such as triglycerides, observed in the liver and bloodstream when XBP1 synthesis was blocked. In contrast, when mice with functional XBP1 were fed a high-carbohydrate diet, they produced high levels of XBP1, which directly activated genes in the liver involved in lipid synthesis. With these studies, researchers have recognized the dual functions of the XBP1 transcription factor as a regulator of both lipid synthesis in the liver and protein secretion in other cells. The opportunity now exists to develop inhibitors of XBP1 action within the liver as a potential treatment for patients with abnormally high lipid levels.

*Lee A-H, Scapa EF, Cohen DE, and Glimcher LH: Regulation of hepatic lipogenesis by the transcription factor XBP1. Science 320: 1492-1496, 2008.*

**Gene Variant Linked to Disparity in Nonalcoholic Fatty Liver Disease Risk:** Genome-wide scanning has revealed gene alterations associated with variation in risk for nonalcoholic fatty liver disease in different ethnic groups. Nonalcoholic fatty liver disease refers to the accumulation of excess fat in the liver in the absence of heavy alcohol consumption, which can lead to liver inflammation, cirrhosis, and the need for transplant. This form of liver disease has become more common in the U.S. population as the numbers of obese and overweight individuals have increased, and is currently the leading cause of liver disease in this country. However, within the general population, some ethnic groups—such as Hispanics—appear to be disproportionately affected by this form of fatty liver disease, while others—such as African Americans—are more resistant to its development. Researchers sought to identify an inherited factor that might explain these risk disparities. To find this genetic factor, they utilized the same technique of genome-wide scanning that has proven so successful recently in identifying genes associated with other diseases through scanning the genomes of participants in a large population-based study called the Dallas Heart Study. Participants were of Hispanic, African American, and European American ancestry. After comparing the genomic data with a noninvasive, imaging-based measure of liver fat and a blood test for liver inflammation, they uncovered a variant in the gene *PNPLA3* that was strongly associated with increased levels of liver fat and inflammation. (The variant resulted in a change in the protein encoded by the gene.) This gene variant was found to be more common among Hispanics, and the variant was also associated with higher levels of liver fat and inflammation in this group. Conversely, a

different variant of the same gene was associated with lower liver fat in African Americans. While the precise effects of the *PNPLA3* gene variants on the function of the encoded proteins remain to be established, researchers are pursuing additional studies to define their proposed roles in liver fat metabolism. Based on their distinctive associations with nonalcoholic fatty liver disease risk amongst ethnic groups in the U.S., these gene variants could provide a basis for needed diagnostic tests to predict who is at greater risk for developing this form of fatty liver disease and whether their disease will progress.

*Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, and Hobbs HH: Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 40: 1461-1465, 2008.*

## HEPATITIS C TREATMENT AND EARLY DETECTION OF PROGRESSION TO LIVER CANCER

### Immune Molecule Gives Clue to Differential Treatment Responses in Chronic Hepatitis C:

The standard treatment for chronic hepatitis C—a combination of the antiviral drugs peginterferon and ribavirin—is known to be less effective in African Americans compared to Caucasians. The reason for this differential effect has been elusive, but scientists recently uncovered an important clue in the form of a molecule that sits on the surface of immune cells and affects their ability to fight infection by the hepatitis C virus (HCV). This research was part of a larger study funded by the NIDDK known as the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C, or "Virahep-C," aimed at identifying reasons for differential responses to these antiviral drugs in some African Americans compared to Caucasian Americans and developing ways to improve treatment.

In this series of experiments, investigators measured levels of an immune cell molecule known as PD-1 in study participants, both before and after receiving antiviral treatment for chronic hepatitis C. PD-1 is short for "programmed death-1," reflecting in part the fact that its presence on cells indicates that they are functionally exhausted. Found on the surface of T cells of the immune system, PD-1 inhibits the ability of these cells to defend against HCV. Participants with persistent chronic HCV were found to have higher pre-treatment levels of PD-1 on their T cells than individuals without HCV. In addition, African American participants for whom this therapy was not effective had higher pre-treatment levels of PD-1 on their T cells than those with successful treatment results. No such differences were found in Caucasian participants who did or did not respond to therapy. Participants from both racial groups who responded to therapy showed a decrease in PD-1 levels over the course of therapy.

Based on these findings, the researchers concluded that high levels of the PD-1 molecule on the surface of immune cells prior to treatment plays an important role in determining whether African American patients, in particular, respond to antiviral therapy for chronic hepatitis C. This study provides some of the first evidence to explain why antiviral therapy against chronic hepatitis C has not been as effective in some racial groups. Through further understanding of how PD-1 and other immune system elements contribute to treatment responses in different racial groups, scientists hope to build the knowledge base necessary to improve future therapy for all patients with chronic hepatitis C.

*Golden-Mason L, Klarquist J, Wahed AS, and Rosen HR: Cutting edge: Programmed Death-1 expression is increased on immunocytes in chronic hepatitis C virus and predicts failure of response to antiviral therapy: Race-dependent differences. J Immunol 180: 3637-3641, 2008.*

**Unique Gene Expression Patterns Found in Early Liver Cancers:** In the U.S. population, hepatitis C virus infection is the leading cause of hepatocellular carcinoma, a form of liver cancer that is increasing in prevalence in this and other countries. Understanding the molecular events within liver cells that drive the development of hepatocellular carcinoma would help in identifying its early origin at a stage where it may respond to more effective, targeted therapies. An international research group with NIDDK support recently conducted a study using liver tissue biopsies from individuals with an early stage of hepatocellular carcinoma due to hepatitis C to identify unique molecular changes. Using technologies such as the gene microarray, they analyzed the genomes (collection of genes) and transcriptomes (collection of messages

made, or "expressed," from the genes, a sign of their activation) in these liver tumors during the early stages of development. The researchers detected changes in the expression of genes that are involved in specific pathways signaling for cellular processes relevant to cancer, such as proliferation or tumor suppression. Different tumors were found to have unique patterns of altered gene expression, which would allow them to be classified by subtype. For example, the gene encoding vascular endothelial growth factor A (VEGFA), which is essential for promoting the growth of new blood vessels to tumors, was found to be overexpressed in some tumors, while other tumors displayed changes in expression of molecules involved in different signaling pathways. Additionally, the researchers found extra copies of large chromosomal segments in some tumors, some of which correlated with increased expression of the VEGFA gene. The investigators were able to use the molecular changes they observed to predict the risk of cancer recurrence after surgical removal of the tumor. These findings provide a wealth of potential biomarkers for predicting treatment response based on characteristics of a particular patient's liver tumor, as well as new targets for developing more personalized liver cancer therapies.

*Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, and Llovet JM: Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Research 68: 6779-6788, 2008.*

## LIVER TRANSPLANT OUTCOMES

**Evaluating Outcomes in Recipients and Donors of Living Donor Liver Transplants:** Investigators from the NIDDK-supported Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) have conducted two studies that quantify the impacts of living donor liver transplantation (LDLT) on patients receiving a transplant, as well as on the individuals who donate portions of their livers for this procedure, using data from a network of transplant centers. The A2ALL study is being conducted at nine transplant centers across the U.S. to provide information on the risks and benefits of LDLT for both recipients and donors.

Compared to liver transplantation using deceased donor organs, which are in limited supply relative to demand, LDLT reduces waiting time. However, it is also a more complex procedure in which only a partial liver can be transplanted, and poses some risks to the healthy donors, who derive no direct benefit from their donation. Thus, it is important to simultaneously assess: (1) recipient outcomes to determine whether there are patient survival benefits from proceeding with a LDLT instead of waiting for a liver from a deceased donor; and (2) donor outcomes to determine the risk of complications from undergoing this voluntary procedure.

In the study of recipient outcomes, records from the centers were analyzed to determine if LDLT increased survival of adult patients compared to those who received a deceased donor liver transplant or no transplant. Study investigators found that, over an average of 4 years of follow-up time, LDLT was associated with lower mortality than the alternative of waiting for a liver from a deceased donor. Patients receiving LDLT were 44 percent less likely to die during the follow-up period than individuals who did not receive LDLT. The study also demonstrated that recipient survival with LDLT increased as the transplantation team became more familiar with the procedure. Once the centers were more experienced in LDLT, defined as performing more than 20 procedures, the mortality risk was 65 percent lower for patients who received LDLT compared to those who did not.

Focusing on the outcomes among the donors of the livers, a second study evaluated reports of complications they experienced across the participating centers. In the period during and after transplantation, 38 percent of donors experienced one or more complications from the procedure. The most common complications, including bacterial infections and biliary leaks (leaking of bile from a defect in the wall of the bile duct), were of low severity, but some were potentially life-threatening and required hospitalization. In contrast to the study of recipient outcomes, greater surgical experience performing LDLT had no effect on minimizing donor complications. Factors that were associated with development of donor complications were high levels of the liver enzyme alkaline phosphatase prior to transplantation, and the need for blood transfusion during the procedure.

Additional studies are planned to assess impacts on donor pain, quality of life, and other predictors of donor complications.

This study provides evidence that, while LDLT can increase survival for patients with end-stage liver disease—especially with improving experience of transplantation centers—it continues to carry some health risks for donors that are not ameliorated by greater surgical experience. These new data will be useful to patients who may be contemplating LDLT relative to other treatment options; to potential healthy volunteer donors considering the risks of the procedure, both for themselves and the recipients, as well as the benefits; and to their physicians.

*Berg CL, Gillespie BW, Merion RM, Brown RS Jr, Abecassis MM, Trotter JF, Fisher RA, Freise CE, Ghobrial RM, Shaked A, Fair JH, Everhart JE, and the A2ALL Study Group: Improvement in survival associated with adult-to-adult living donor liver transplantation. Gastroenterology 133: 1806-1813, 2007.*

*Ghobrial RM, Freise CE, Trotter JF, Tong L, Ojo AO, Fair JH, Fisher RA, Emond JC, Koffron AJ, Pruett TL, Olthoff KM, and the A2ALL Study Group: Donor morbidity after living donation for liver transplantation. Gastroenterology 135: 468-476, 2008.*

# *National Commission on Digestive Diseases*

Diseases of the digestive system span a wide range of conditions—from functional gastrointestinal (GI) and motility disorders, inflammatory bowel diseases, and celiac disease, to liver and gallbladder diseases, to pancreatic diseases and GI cancers. Collectively, these diseases represent an enormous public health burden. A strong commitment to advancing research is required to combat digestive diseases.

Since its establishment by the former NIH Director, Dr. Elias Zerhouni, in 2005, the National Commission on Digestive Diseases made substantial progress toward its goal to improve the health of the Nation through advancing digestive diseases research. The Commission was responsive to the mutual interest in this research area shared by the Congress, the NIH, and the research community. Within the NIH, the NIDDK provided leadership and support for the Commission.

As part of its charge, the Commission assessed the state-of-the-science in digestive diseases and the related NIH research portfolio, in order to identify research challenges and opportunities for inclusion in its long-range research plan for digestive diseases. The Commission's efforts benefited from the diverse expertise of its members, who represented the academic and medical research and practice communities, the patient advocacy community, and the NIH and other Federal health agencies. The research plan was also informed by a separate, parallel, NIDDK report on the current burden of digestive diseases in the U.S.

In 2006, the Commission initiated a research planning process by convening two public meetings, defining the topic areas within digestive diseases research that comprise the research plan, assigning Commission members to chair topical Working Groups, conducting an open call for additional experts to serve as Working Group members, and laying the foundation for the Working Groups' deliberations through the identification of research goals and other recommendations. Dr. Zerhouni addressed the Commission members at their November 2006 meeting to express his support and appreciation for their efforts.

During a subsequent public meeting held by the Commission in June 2007, the Working Group chairs presented their Groups' research recommendations for specific digestive diseases, along with steps to achieve the proposed goals. At the following meeting in November 2007, the Commission considered the entire draft research plan and invited public comments. A formal public comment period held in early 2008 invited additional stakeholder input on the draft research plan, which was posted on the internet. Following incorporation of public input, the Commission convened in May 2008 to discuss the draft research plan, which was subsequently finalized and approved by all members of the Commission. The final research plan, entitled "Opportunities and Challenges in Digestive Diseases Research: Recommendations of the National Commission on Digestive Diseases," will be released in early 2009 in both electronic and print formats. The research plan was transmitted to the NIH Director and Congress, as specified in the Commission's charter, and will also be widely disseminated to the research and health care communities interested in digestive diseases research, including professional and patient advocacy organizations; leaders of NIH Institutes, Centers, and Offices, as well as other Federal agencies; private research organizations; and individual scientists and advocates who provided input through the Working Groups. The Commission's 10-year research plan is intended to guide the NIH—along with the investigative and lay communities—in pursuing important research avenues for combating digestive diseases.

The Commission's research plan will be available in electronic form through its website: *http://NCDD.niddk.nih.gov*. Hard copies of the publication will also be available through the National Digestive Diseases Information Clearinghouse, and ordering information will be provided on the website.

## STORY OF DISCOVERY

# *Hepatitis B Research Progress: A Series of Fortunate Events*

Over the span of a few decades in the U.S., hepatitis B has been transformed from a disease newly infecting 200,000-300,000 individuals annually to one infecting approximately 46,000 individuals in 2006, the most recent year surveyed.[1] This impressive public health achievement can be attributed largely to immunization programs using a safe and effective hepatitis B vaccine, and screening of the blood supply for the virus. Therapy for chronic hepatitis B has similarly improved from a point in time when no effective treatment was available, to the current armamentarium of seven FDA-approved treatment options. These gains in hepatitis B control and care are based on years of careful research, marked by a confluence of serendipity and concerted effort by U.S. and international scientists into understanding the cause and course of hepatitis B, effectively treating it, and preventing its spread. NIH-sponsored research has contributed greatly to advancing knowledge of hepatitis B over the years. This Story of Discovery highlights some of the landmark accomplishments to date in hepatitis B research and their far-reaching impact through translation into improved medical care and public health in this country and around the world.

**Silent Disease with a Global Reach**

Hepatitis B is an inflammation of the liver caused by infection with the hepatitis B virus (HBV), which results from exposure to an infected person or their blood or blood products. Infection with HBV can result in acute or chronic forms of hepatitis. Symptoms can include fatigue, nausea, fever, loss of appetite, stomach pain, diarrhea, dark urine, light stools, and jaundice (yellowing of the eyes and skin). However, hepatitis B often is a "silent disease," quietly inhabiting the body for several decades before provoking symptoms or progressing to cirrhosis (scarring of the liver that prevents normal function) and/or hepatocellular carcinoma (liver cancer). This delayed appearance of symptoms can hinder efforts to detect the disease at an early stage and to prevent further transmission. Common ways in which HBV is passed on include: from mother to baby at birth; sex without use of a condom; use of tainted needles or tools for injection drug use, tattoos, or body piercing; accidental needle-stick; or sharing a toothbrush or razor with an infected person. Receiving a blood transfusion in the U.S. used to be another common mode of transmission, during the 1980s and earlier, prior to effective screening of donor blood for the virus.

Chronic hepatitis B currently affects an estimated 1.25 million people in the U.S., resulting in approximately 5,000 deaths each year.[1] Recent estimates from the World Health Organization indicate that more than 350 million people worldwide have chronic hepatitis B, out of 2 billion infected with the virus.[2] In particular, individuals from parts of the world where hepatitis B is endemic, such as parts of Asia and sub-Saharan Africa, are at increased risk of developing chronic hepatitis B, which is the leading cause of cirrhosis and hepatocellular carcinoma worldwide. People infected with the human immunodeficiency virus (HIV) are also at high risk of being co-infected with HBV, due to common, bloodborne transmission routes.

**Discovery of a Bloodborne Threat to Liver Health**

Hepatitis epidemics, which likely included hepatitis B as one cause, have spanned the course of human history, dating back to antiquity and observations on an epidemic of jaundice by Hippocrates. Yet it wasn't until 1883 that a German scientist first described what was later thought to be this particular form of viral hepatitis in a group of people who had developed jaundice after receiving a smallpox vaccine

## STORY OF DISCOVERY

prepared from human blood. Similarly, an outbreak of hepatitis-related jaundice affecting approximately 50,000 U.S. Army personnel during World War II was later attributed to HBV infection transmitted through a contaminated yellow-fever vaccine, based on research performed in the late 1980s with support from the Veterans Affairs Medical Center, the NIDDK and the National Cancer Institute (NCI) within the NIH, and the National Research Council.

The infectious agent responsible for these hepatitis outbreaks, the hepatitis B virus, was identified by Dr. Baruch Blumberg while working at the NIH in the 1960s—a discovery that later earned him the Nobel Prize in Physiology or Medicine in 1976. Strangely enough, Dr. Blumberg and his laboratory did not set out to find the virus causing hepatitis B. As part of their research to identify forms of blood proteins that differ across populations or ethnic groups, they were testing blood from hemophilia patients who had received multiple blood transfusions. When proteins in a donor's blood are slightly different from those in a recipient's own blood, the body may mount an immune reaction, including the production of antibodies that stick to the foreign proteins. Thus, the scientists were looking for antibodies in the hemophilia patients as potential markers of differences between their blood proteins and the donors'. In this case, however, the scientists would soon learn that some of the antibodies reflected the presence of a bloodborne infectious agent.

In 1963, Dr. Blumberg and Dr. Harvey Alter identified an antibody in the blood of a patient in New York with hemophilia that reacted against a protein in blood collected from an Australian aborigine. This protein was named the "Australia antigen" or "Au." This finding piqued their curiosity as to why a patient in New York would produce an antibody against a protein found in the blood of an individual so geographically, ethnically, and culturally distinct as an aborigine living in Australia. They went on to test samples from individuals around the globe and found the antigen in some of these as well, more commonly in people who had received multiple blood transfusions or were from Asian or tropical regions.

A clue that the Australia antigen might be linked to liver disease came in early 1966 when Dr. Blumberg's group noted that one patient's blood first tested negative and then positive for the antigen—a shift associated with clinical signs of chronic hepatitis in the form of elevated liver enzymes. Also around this time, one of the technicians in Dr. Blumberg's laboratory developed a case of acute hepatitis, which was accompanied by a positive test for the antigen. Additional clinical studies during the late 1960s in the U.S. and Japan also found hepatitis associated with the Australia antigen. Soon after, blood banks in the U.S. and abroad started screening donors to ensure that this apparent bloodborne cause of hepatitis was not passed on to transfusion recipients.

The Australia antigen was confirmed to be part of a virus causing hepatitis B (now known as HBV) in 1970 by a research group in London that visualized viral particles in blood from patients with hepatitis who had tested positive for the antigen. Once the protein heretofore known as the Australia antigen was revealed to be HBV, scientists realized how the hemophilia patient in New York could harbor antibodies to a protein found in the blood of an Australian aborigine; presumably, both individuals were infected at some point with HBV. In the following years, research would continue to yield illuminating details about HBV. For example, further investigations of the Australia antigen identified it as a protein on the surface of HBV. NIH-supported studies also revealed the distinctive circular shape and other characteristics of the HBV genome. Valuable knowledge of the mechanisms HBV uses for infection and replication, and its overall life cycle, was gained from research in unique animal models

that could simulate human HBV infection, such as ducks, woodchucks, and ground squirrels, as well as from cells grown in the laboratory.

Basic research sponsored by the NIH into understanding HBV components, infection strategy, and resulting disease processes would later prove to be essential as a basis for additional prevention strategies, as well as effective diagnostic and treatment approaches.

Investigations of the disease resulting from HBV infection also showed that chronic hepatitis B could lead also to a form of liver cancer known as hepatocellular carcinoma. For example, in 1981, a study sponsored in part by the NCI of over 20,000 Chinese government workers showed that chronic hepatitis B was strongly associated with development of and death from hepatocellular carcinoma after 5 years. Later, in 2005, the National Institute of Environmental Health Sciences' National Toxicology Program would list the hepatitis B virus as a known human carcinogen in its annual Report on Carcinogens.

**Medical Success Story: Effective Prevention of Hepatitis B**

Soon after discovery of the Australia antigen, researchers developed tests to detect HBV in blood that could be applied to diagnosis and screening of populations for the infection. Screening of the donor blood supply for the virus had an important impact on reducing disease transmission in patients requiring transfusions. But for prevention in the general population, a vaccine was needed.

Basic research on the natural history of HBV infection led to the preparation in the 1970s and 1980s of the first hepatitis B vaccines based on heat-inactivated and blood plasma-derived viruses. Clinical research on the plasma-derived vaccine conducted with NIH support showed that it was effective at protecting against HBV infection.

Researchers later developed an improved, "recombinant" version of the vaccine by inserting the hepatitis B surface protein gene into yeast or mammalian cells, which facilitated its purification and preparation for the vaccine. These vaccines also protect against infection by the hepatitis D virus, which requires HBV in order to replicate.

Since the establishment in the U.S. in the 1980s of vaccination programs and donor blood screening for hepatitis B, new cases of acute hepatitis B have declined by more than 80 percent.[3] The immunization strategy initially recommended by the Centers for Disease Control and Prevention (CDC) in 1991 entailed universal vaccination of children. In 1992, Federal programs began routine hepatitis B vaccination of infants, and vaccination of adolescents was added in 1995. The vaccine is also currently recommended for individuals in high-risk groups, such as family members of patients with chronic hepatitis B and individuals who inhabit or emigrate from parts of the world with high rates of infection. Worldwide, beginning in the 1990s, the World Health Organization has called for all countries to add the hepatitis B vaccine to their national immunization programs, which presents a challenge in many parts of the developing world. Multi-national public-private partnerships, such as the Global Alliance for Vaccines and Immunization, are working to improve vaccination rates in these areas.

**Many Treatment Options for Hepatitis B**

Early trials of therapy for hepatitis B focused on the immune-cell chemical interferon. Studies conducted during the 1970s through the 1990s, in the U.S. with NIH support and also abroad, demonstrated the efficacy of treating hepatitis B with interferon, which decreases the stability of HBV genetic material, as well as viral assembly. However, interferon carries potential side effects, including fever, fatigue, headache and muscle aches, and depression. More recently, advances in understanding the viral life cycle and pathogenesis of hepatitis B have paved

the way for identifying new therapeutic agents known as nucleoside/nucleotide analogues, some of which were originally developed to treat HIV infection. These drugs protect against hepatitis B by directly inhibiting replication of HBV through targeting its polymerase enzyme. Currently, seven antiviral drugs are approved by the FDA to treat hepatitis B: interferon-alpha, peginterferon, lamivudine, adefovir, entecavir, telbivudine, and tenofovir. However, no definitive guidance yet exists on the most effective use of these drugs, either alone or in combination. Other issues that remain to be resolved concerning use of these drugs against hepatitis B include how to ensure a lasting response once treatment is stopped and how to avoid the development of viral resistance with long-term treatment, in which the virus mutates over time to escape suppression by the antiviral drug. The drugs also differ in terms of efficacy, safety, likelihood of viral resistance development, method and frequency of administration, and cost. The many HBV types (genotypes) in existence also affect response to therapy and disease progression. New antiviral therapies are currently being tested against hepatitis B in clinical trials.

In addition to pharmaceutical agents, liver transplantation is an effective treatment for individuals with hepatitis B who develop cirrhosis and end-stage liver disease. However, organs for transplant remain in short supply.

To resolve the many issues concerning optimal use of available therapies against hepatitis B, the NIDDK has sponsored several consensus-building conferences that bring together experts in the field to make recommendations based on available evidence. For example, in September 2000 and in April 2006, the NIH sponsored workshops to review current health care practices and develop recommendations for optimal management of hepatitis B. Proceedings from these meetings were published in scientific journals. In October 2008, the NIDDK convened an NIH Consensus Development Conference on Management of Hepatitis B together with the NIH Office of Medical Applications of Research, The Johns Hopkins University School of Medicine, and other entities within the NIH and the Department of Health and Human Services.

The purpose of this 3-day conference was to examine important issues in hepatitis B therapy, including which groups of patients benefit from treatment and at what point during treatment, as well as which groups do not show a benefit. The external experts serving on the conference panel addressed major questions regarding hepatitis B management related to current burden, natural history, benefits and risks of current treatment options, who should or should not be treated, appropriate measures to monitor treatment, and the greatest needs and opportunities for future research on hepatitis B. Additional information on this conference is provided in the accompanying feature on “NIH Consensus Development Conference on Management of Hepatitis B.”

**More To Discover Through Research**

Despite the impressive scientific gains made over the past decades toward preventing and treating hepatitis B, much remains to be learned about this disease, including details of the disease processes associated with HBV infection, as well as ways to optimize approaches to treatment and control. To further advance knowledge of hepatitis B, the NIDDK is funding the Hepatitis B Clinical Research Network. Established in fall 2008, the Network consists of 12 clinical centers, a data coordinating center, a virology center, and an immunology center. The Network is conducting translational research on chronic hepatitis B, focusing on understanding disease processes and applying this knowledge to more effective strategies to treat and control the disease. Its focus has been informed by the research recommendations of recent NIH-sponsored meetings and planning activities on this topic.

## STORY OF DISCOVERY

Through scientific endeavors such as the Hepatitis B Clinical Research Network and investigator-initiated research, conferences, and research planning efforts including the trans-NIH *Action Plan for Liver Disease Research* and the new National Commission on Digestive Diseases' research plan, the NIH is now building upon the extraordinary legacy of past research advances to make additional contributions toward alleviating the burden of hepatitis B, in the U.S. and throughout the world.

[1] *http://www.cdc.gov/hepatitis/PDFs/disease_burden.pdf*

[2] *http://www.who.int/mediacentre/factsheets/fs204/en*

[3] *http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/disease_burden.pdf*

Additional information on hepatitis B and research progress on this disease is available through the following NIH resources:

- NIH Fact Sheet on Hepatitis B, available at: *http://www.nih.gov/about/researchresultsforthepublic/HepatitisB.pdf*
- Materials for the public provided by the National Digestive Diseases Information Clearinghouse (*http://digestive.niddk.nih.gov/index.htm*), including the brochure "What I Need to Know About Hepatitis B," available in electronic or printed form.

# *NIH Consensus Development Conference on Management of Hepatitis B*

In October 2008, the NIH convened an independent panel of experts for a Consensus Development Conference on the Management of Hepatitis B to weigh available evidence on managing hepatitis B and to offer recommendations concerning future research. This conference examined important issues in hepatitis B therapy, including which groups of patients benefit from currently available treatment and at what point during treatment, as well as which groups do not show a benefit. The NIDDK and NIH Office of Medical Applications of Research, and the Johns Hopkins University School of Medicine sponsored the conference, with additional support from the National Cancer Institute, National Institute of Allergy and Infectious Diseases, Centers for Disease Control and Prevention, and Food and Drug Administration.

The consensus panel included experts in fields such as hepatology and liver transplantation, gastroenterology, public health and epidemiology, infectious diseases, pathology, oncology, family practice, internal medicine, and biostatistics, as well as a public representative. The panel's deliberations were informed by presentations during the conference by experts in the field of hepatitis B management, a review of the published medical literature performed by the Agency for Healthcare Research and Quality, and input from conference participants.

Based on available evidence, the panel recommended several avenues for future research relating to hepatitis B management and control, including:

- prospective clinical studies to define the natural history;
- large randomized clinical trials, including those using placebo controls, to test therapies alone and in combination in terms of health outcomes such as liver failure, cancer, and death;
- identification of elevated hepatitis B virus DNA in blood and elevated liver enzymes as the most important indicators for progression to cirrhosis and liver cancer;
- prevention of disease transmission through routine hepatitis B screening for new immigrants who arrive from countries where hepatitis B prevalence is greater than two percent;
- identification of patients with hepatitis B who should or should not be treated based on whether active disease is present; and
- pursuit of translational research on hepatitis B through the new Hepatitis B Clinical Research Network, the research directions of which will be informed by the conference's recommendations.

As an independent report, not a statement of NIH policy, the panel's statement will be used to inform planning of future research on these important challenges and opportunities in hepatitis B management. The panel's full statement and additional information about this consensus conference are available at: *http://consensus.nih.gov/2008/2008HepatitisBCDC120main.htm*

# Liver Disease in Alpha-1-Antitrypsin Deficiency: Organ-specific Complications Arise from a Misfolded Protein

*Dr. David Perlmutter*

*Dr. David Perlmutter is a Vira I. Heinz Professor and Chairman of Pediatrics at the University of Pittsburgh. He is also the Physician-in-Chief and Scientific Director of Children's Hospital of Pittsburgh. Dr. Perlmutter has carried out basic research on alpha-1-antitrypsin deficiency for more than 20 years. His work has led to many new concepts about the underlying causes of liver disease in this genetic condition and has suggested several new concepts for approaches to prevent chronic liver injury, liver cancer, and lung disease that sometimes result from alpha-1-antitrypsin deficiency. Dr. Perlmutter spoke at the January 2008 meeting of the NIDDK Advisory Council to share some insights from his ongoing studies of alpha-1-antitrypsin deficiency. Following are highlights of that presentation.*

Alpha-1-antitrypsin deficiency (a condition also referred to as Alpha-1) is a genetic disorder caused by defective production of the protein alpha-1-antitrypsin (alpha-1AT). It affects about 1 in every 1,800 live births.[1] In normal individuals, alpha-1AT protein is produced in the liver and secreted into the bloodstream. Its main site of action is in the lungs, where it protects the delicate tissue from damage. People with Alpha-1 carry a mutation in the gene encoding alpha-1AT, which results in a protein that retains some of its biological function but is poorly secreted, and thus does not reach the lungs and may accumulate—sometimes forming large aggregates—within the liver.

Mutant alpha-1AT can cause two different medical problems: pulmonary complications such as emphysema may arise because the protein does not perform its function in the lungs; and liver complications such as inflammation and cancer may arise because the mutant protein can build up in the liver. While lung complications are hallmarks of Alpha-1, most patients do not develop serious liver disease; in fact, only 8 to 10 percent of the people with Alpha-1 will do so. This wide variation in the severity of liver symptoms among people with Alpha-1 strongly suggests that additional genetic and/or environmental variables contribute to the development of clinical liver disease. The identity of these factors is unknown. One hypothesis Dr. Perlmutter posed was whether "protected" individuals—those who carry the alpha-1AT mutation but do not develop liver disease—are somehow able to metabolize the mutant alpha-1AT, while patients who are susceptible to liver disease are not. The first questions Dr. Perlmutter addressed concerned the mechanisms by which this mutant protein was degraded in the liver, and whether these pathways were less effective in people whose livers have aggregates of mutant alpha-1AT.

**Alpha-1AT Processing in the Liver**

Using a series of experiments in cultured cells, Dr. Perlmutter and his colleagues found that a metabolic pathway known as the "autophagic pathway" was involved in the degradation of mutant alpha-1AT in the liver. Autophagy is the degradation of a cell's own components by its internal digestive pathways—literally, autophagy is a process by which a cell eats part of itself. It is a tightly-regulated process that plays a part in normal cell growth and metabolism and helps to maintain a balance between the synthesis, degradation, and recycling of cellular components. It is also a major mechanism by which a cell under stress—starvation, for example—reallocates scarce nutrients to essential processes. The autophagic

pathway seemed to be particularly important in the disposal of the very large aggregates of protein found when very high levels of the mutant protein were produced, as the aggregates of alpha-1AT were able to activate the autophagic response.

**Other Pathways for Disposing of Mutant, Misshapen, or "Misfolded" Proteins**

Dr. Perlmutter's research team next turned to two other well-characterized cellular pathways activated in response to the accumulation of misfolded proteins in general to see if they were involved in the metabolism of mutant alpha-1AT. The first was the "unfolded protein response" pathway, which is activated in response to the presence of misfolded or defective proteins. When the researchers looked at markers for activation of the unfolded protein response pathway, however, they were unable to detect increased activity in the presence of mutant alpha-1AT. A second pathway, the "ER overload response" pathway, is activated when the endoplasmic reticulum—a specialized area within the cells where proteins are prepared for secretion—becomes "backed up" with proteins that cannot get out of the cell. In contrast to the unfolded protein response, the ER overload response pathway did show increased activity in the presence of the mutant alpha-1AT.

**Identification of a Novel Pathway Involved in Alpha-1AT Metabolism**

The researchers next asked whether there were any other, previously unknown pathways that might also be involved in a cell's disposal of mutant, misfolded proteins. They reasoned that, when faced with a potentially toxic accumulation of mutant alpha-1AT, a cell may turn on or off certain genes to regulate various metabolic pathways, some of which would help it dispose of the mutant protein. Thus, the researchers engineered mice to produce mutant alpha-1AT in their livers in an inducible manner, and then analyzed the patterns of gene expression (the extent to which genes are turned on or off) in the mouse livers in the absence and presence of the mutant protein. When the mutant alpha-1AT was produced, the expression of 75 liver genes was increased, and the expression of 131 was decreased. Analysis of these response patterns found that these changes in expression involved genes that play a role in various cellular processes.

One gene whose expression was markedly increased in these mice in the presence of mutant alpha-1AT is the "regulator of G-protein signaling 16," also known as RGS16. G-proteins are important mediators of intracellular signals, so changes in the expression of a gene that modulates G-protein activity could have potentially far-reaching effects on a cell. The increase in RGS16 gene expression was associated strongly with the presence of aggregates of the mutant alpha-1AT in the mouse livers. Similar changes in RGS16 expression were seen in samples of human livers from individuals with Alpha-1.

RGS16 seems to be activated in response to the aggregation of mutant alpha-1AT that characterizes Alpha-1 in individuals with liver disease. Therefore, it may be an excellent marker for the distinct form of metabolic stress seen in these patients. RGS16 may also represent a key player in a novel pathway through which autophagy is regulated, making it a potential target for the development of future therapeutic strategies. Future research will further characterize the role played by RGS16 in modulating cellular metabolism in the presence of mutant alpha-1AT.

**A New Model System To Study Alpha-1**

Dr. Perlmutter next described an innovative series of experiments using a model organism to study Alpha-1, the roundworm *Caenorhabditis elegans*. *C. elegans* is a small (about 1 mm long), transparent worm that is used extensively by biomedical researchers. This organism offers a number of benefits as a disease model, both biological and practical. Its genome has been fully sequenced and its genes and their functions are similar to those of mammals. It is relatively easy to work with, reproducing every 3 days and generating many offspring, and it is transparent—facilitating observation

of its inner workings. There are also substantial existing genetic and molecular tools that researchers can employ when using this organism.

Dr. Perlmutter's collaborators, Drs. Gary Silverman and Stephen Pak, constructed fusions of various alpha-1AT genes with a gene encoding "green fluorescent protein," a marker often used by biologists to allow easy visualization of a protein. When they inserted the normal alpha-1AT gene, fused with green fluorescent protein, into the intestinal cells of worms, they saw green fluorescence in the interior of the intestinal tract, indicating that the protein was being properly secreted out of the cells. (In *C. elegans*, the intestine performs many of the functions of the liver.) In worms that produced fusions with the mutant alpha-1AT gene, the green fluorescence was retained within the cell in globules, indicating a failure to secrete, and intracellular aggregation of the protein. Additionally, worms expressing the mutant gene exhibited arrested development at the larval stage, and did not live as long as normal worms or worms expressing the normal alpha-1AT.

But what is responsible for the physiological manifestation of the mutant alpha-1AT? To answer this question, the researchers used a slightly different mutant of alpha-1AT that is non-functional and accumulates within the cells, but does not form aggregates. When this alternate mutant was inserted into worms, there was no growth arrest at the larval stage in these worms. This finding indicates that some of the biological effects seen in worms with the original mutant alpha-1AT require not only the retention of the protein within the liver cells, but also the formation of protein aggregates within cells.

Dr. Perlmutter outlined the next steps in the research he is doing with Drs. Silverman and Pak: the adaptation of the worm model for high-throughput screening for genetic modifiers of disease severity and for potential drug candidates. He described technology that could automatically sort through and characterize large numbers of these tiny worms. Such an approach would allow the rapid screening of hundreds of potential genetic and/or pharmacologic approaches to address the problems seen in Alpha-1.

**Conclusions**

In a subset of patients with Alpha-1, accumulation of aggregates of the mutant protein in the liver causes damage and increases the risk of cancer. The risk for liver disease is heavily influenced by genetic and/or environmental factors that may impact various degradation pathways and other protective cellular responses. Dr. Perlmutter and his colleagues discovered that the autophagic pathway appears to play a particularly important role in disposing of the mutant protein. Finally, Dr. Perlmutter's development of a novel worm model amenable to high-throughput screening may expedite the identification of genetic modifiers and new therapeutic agents.

*Dr. Perlmutter acknowledged the contributions of his collaborators in research, Drs. Silverman and Pak. Gary Silverman, M.D., Ph.D. is The Twenty Five Club Endowed Professor of Pediatrics, Professor of Cell Biology and Physiology at University of Pittsburgh School of Medicine and Chief of Newborn Medicine at University of Pittsburgh Medical Center. Stephen Pak, Ph.D. is Assistant Professor of Pediatrics at University of Pittsburgh School of Medicine. Drs. Silverman and Pak have worked with Dr. Perlmutter to characterize the* C. elegans *model organism in order to elucidate the role of cellular signaling molecules in regulating cell metabolism.*

[1] *Perlmutter DH, et al: Molecular pathogenesis of alpha-1-antitrypsin deficiency-associated liver disease: a meeting review. Hepatology 45: 1313-1323, 2007.*

## PATIENT PROFILE

# Mariah Watts

*Bariatric Surgery—Weighing the Pros and Cons*

**Mariah Watts**

By the time she was 16 years old, Mariah Watts was five-feet eight-inches tall, suffered from sleep apnea, had pre-diabetes—and weighed over 350 pounds. In addition to being unable to breathe well while sleeping (sleep apnea) and facing a high risk for type 2 diabetes, she also had a difficult time with many ordinary activities, such as sitting in seats at school. "I was always so self conscious," says the now 17-year-old who struggled with being overweight for many years, as well as with some of the serious health conditions that accompany obesity. "I wouldn't wish this on anybody," she says.

In January, 2008, Mariah underwent bariatric surgery, an operation that promotes weight loss. Seven months later, Mariah was down to 234 pounds, no longer had trouble breathing or sleeping, and no longer had pre-diabetes. Both she and her family are delighted with these results. "The surgery has changed Mariah's attitude and given her a whole new lease on life," says her mother, Mazie.

Although bariatric surgery can have dramatic health benefits, researchers caution that it also carries substantial risks.

Today, Mariah is voluntarily participating in the Teen Longitudinal Assessment of Bariatric Surgery, Teen-LABS, an observational study supported by the NIDDK to help determine if bariatric surgery is an appropriate treatment option for extremely overweight teens. Mariah enrolled in the study just before her surgery, enabling researchers to assess her health and quality of life in great detail both before and after the surgery, for comparison. Teen-LABS is being conducted at several medical centers in the U.S. and is led by Dr. Thomas Inge, a pediatric surgeon at the Cincinnati Children's Hospital Medical Center.

*Although bariatric surgery can have dramatic health benefits, researchers caution that it also carries substantial risks.*

Although bariatric surgery is not a common procedure in adolescents, its use has been increasing in clinical practice as a treatment for very severe obesity in this age group. Thus, the NIDDK is supporting the Teen-LABS study to collect health outcome data on adolescents who were already planning to have bariatric surgery, so as to evaluate its risks and benefits. (NIDDK does not pay for the surgery.) With these data, future adolescent patients, their parents, and health care teams will be able to make more informed, evidence-based decisions.

## PATIENT PROFILE

**Bariatric Surgery and Adolescents**

Bariatric surgery promotes substantial weight loss by restricting food intake and, in some cases, decreasing the amount of calories and nutrients the body absorbs. This surgery can also reduce the risk for—and in some cases even reverse—type 2 diabetes, a devastating disease.

However, the surgery also comes with substantial risks. Early complications may include bleeding, infection, leaks from the site where the intestines are sewn together (in certain operations), and blood clots in the legs that can progress to the lungs and heart. Later complications may include malnutrition, especially in patients who do not take their prescribed vitamins and minerals, hernias, and other health problems.

*Teen-LABS, an observational study supported by the NIDDK, enrolls teens who are already planning to have bariatric surgery. The study collects health outcome data so that future adolescent patients, their parents, and health care teams will be able to make more informed, evidence-based decisions.*

Therefore, recommended criteria for accepting an adolescent as a candidate for bariatric surgery are more stringent than for adults. Teens considered for this surgery have extreme obesity (as defined by body mass index, a measure of weight relative to height). Many of the adolescents who have enrolled in Teen-LABS so far have weighed 300 or 400 pounds, or more. Additionally, the teens who are candidates for this surgery have serious weight-related health problems, such as sleep apnea, type 2 diabetes, or other conditions. Furthermore, they must have tried other approaches to lose weight, without success.

In addition, experts recommend that potential adolescent patients and their parents should be evaluated to see whether they are emotionally prepared for the operation and the lifestyle changes they will need to make. A teen must be motivated and have strong family support, because the fact is bariatric surgery is not an easy way out to control weight. Even after surgery, patients will have to continue follow-up with health care professionals throughout their lives—and they will need to maintain a lifetime of healthy habits, including eating less food and exercising regularly.

Mariah says she is committed to making those life-long changes.

**A Family Decision**

A number of factors contribute to obesity. These include environmental and behavioral factors as well as genetic susceptibility. In Mariah's case, as in most obese individuals, the exact contribution of each is not known. "My father, his sisters and their children are all big people," says Mariah's mother. In addition, she says Mariah struggled with eating as a child. "I ate constantly," adds Mariah. "I'd get full and 30 minutes later would want to eat again."

By age 11, Mariah weighed between 230 and 250 pounds and started dieting. She went to nutritionists and tried different types of diets. But nothing seemed to work. As time passed, Mazie observed her daughter becoming increasingly depressed and lethargic. "I started taking her to doctors and dieticians," says Mazie. "It was painful to watch her go through all of this."

Finally, as a last resort, Mazie encouraged her daughter to consider bariatric surgery. Mariah, desperate to lose weight, was agreeable to the idea—and did her homework. Mariah researched the procedure so thoroughly over the internet "that I could have done the surgery myself," she adds with a smile.

There are several types of bariatric surgery. Mariah underwent the Roux-en-Y gastric bypass version.

## PATIENT PROFILE

This surgery limits Mariah's food intake because it reduced her stomach to the size of a small pouch. It also decreases the absorption of calories and nutrients by preventing food from contacting most of the stomach and the upper part of her small intestine. The surgery changes the digestive tract so that food is routed directly from the small stomach pouch to a lower part of the small intestine.

According to Mariah, the worst thing about having had the surgery is the fact that she sometimes will eat more than her post-surgery digestive tract can handle, leaving her feeling weak, dizzy, and sweaty. "It's horrible. It feels like you're dying," she says, adding that the feeling lasts for about 25 or 30 minutes. She also adds that she's been learning how to restrict her eating habits further. "When I go to a restaurant now, I know what to eat and what not to eat," says Mariah. And in general, she says she's eating much healthier.

As a result, she has much more energy than she had before. "Before the surgery I used to come home from school, lay around, sleep or watch TV," says Mariah. "Now I find something physical to do every day. My little sister likes when I jump on the trampoline with her. This summer I swam in the family pool all the time and really enjoyed it. And when my friends come over, we go for walks; stuff I didn't do before."

"Mariah is an entirely different person," says her mother. "She's dating for the first time. Life is just more normal. I just see so much joy in her."

**Participating in the Teen-LABS Study**

Shortly after Mariah and her parents decided to go forward with the surgery, Mariah's parents encouraged her to take part in the Teen-LABS study. "The study was something new to us," says Mazie. "We had never experienced anything like this, but we thought it was important for us to get involved so that other parents and their teenage children could make more informed decisions about whether or not to have this type of surgery." Mariah agrees.

*"...We thought it was important for us to get involved [in the Teen-LABS study] so that other parents and their teenage children could make more informed decisions about whether or not to have this type of surgery."*

The Teen-LABS study began in 2007 and is based on the related LABS study, which is assessing risks and benefits of bariatric surgery in adults and is also supported by NIDDK. Over the span of 5 years, Teen-LABS will collect data on teens like Mariah, who had bariatric surgery as adolescents, and will compare this information with data from adult participants in the LABS study who had bariatric surgery as adults, after having been obese since their teen years. Teen-LABS and LABS researchers are collecting information on the pre-operative and 2-year post-operative status of adolescent and adult participants, including measuring body composition, body fat, cardiovascular disease risks, sleep apnea episodes, diabetes indicators, depressive symptoms, quality of life, eating habits, and nutritional status. Additionally, the investigators are storing serum and plasma (components of blood), urine, and genetic samples for future studies.

What would Mariah tell other teens and their families who are considering bariatric surgery? "They need to do their homework first," she says. This homework will become more informative in the future with new data, thanks to her participation in Teen-LABS, along with the other study volunteers. "And," Mariah adds, "they need to commit to eating better and exercising more."

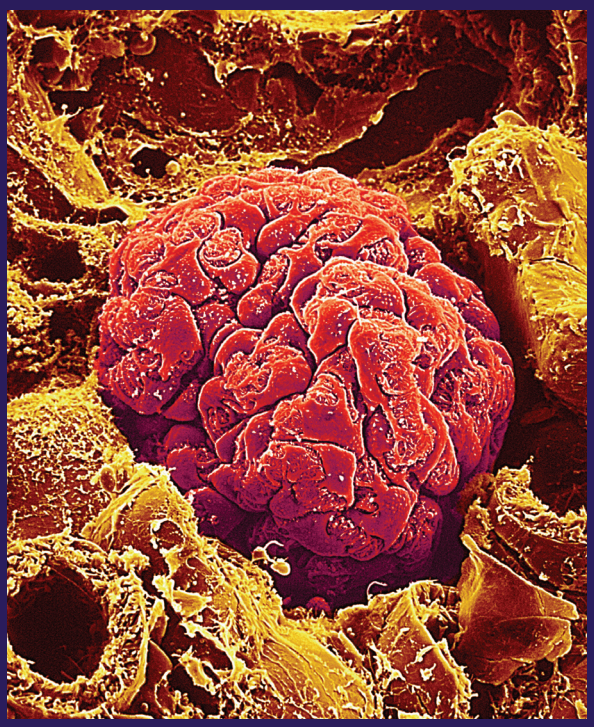
## PATIENT PROFILE

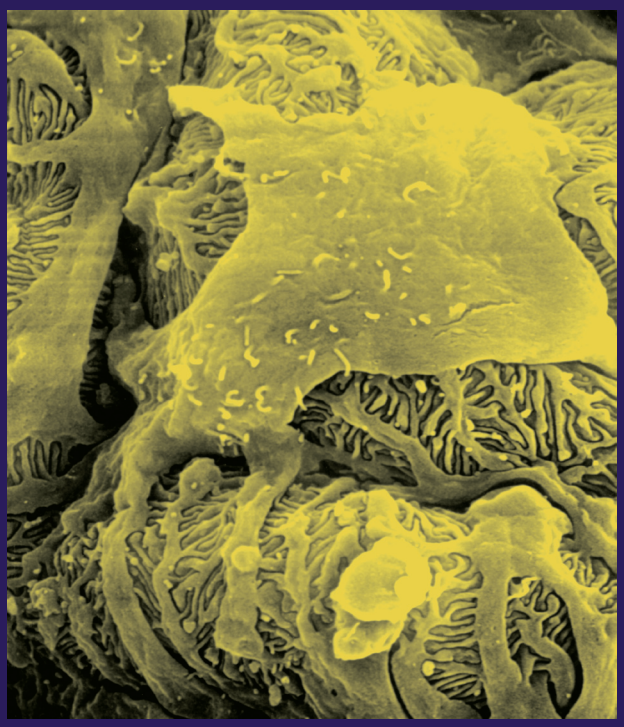
**For additional information about bariatric surgery:**

Bariatric Surgery for Severe Obesity (NIDDK publication)—
*http://win.niddk.nih.gov/publications/gastric.htm*

For more information on the Teen-LABS study—
*http://www.cincinnatichildrens.org/research/project/teen-labs*

As part of its multifaceted research portfolio on the causes, prevention, and treatment of obesity, the NIDDK additionally encourages research to understand how bariatric surgery has its effects. Certain bariatric surgical procedures are associated with remission of diabetes soon after surgery, even before substantial weight loss has occurred. Through increased understanding of potential mechanisms by which alterations in the gut reduce risk for or ameliorate type 2 diabetes in obese individuals, researchers may be able to improve surgical and nonsurgical therapies for obesity-related health conditions.





**The glomerulus, located in center of the image on the left, is a small ball of capillaries that, together with surrounding cells, comprises the basic filtering unit of the kidney. The glomerulus is composed of several cell types, including podocytes, which can be seen in the image on the right. Processes extend from neighboring podocytes and interdigitate with one another, like the interlocking fingers of two hands. Many forms of kidney disease involve damage to podocytes within the glomerulus. As described in this chapter, researchers have recently identified variations near the *MYH9* gene on chromosome 22 that correlate with increased susceptibility to non-diabetic kidney disease in African Americans. The MYH9 protein is found in kidney podocyte cells. This finding may have important implications for the treatment of the very large number of these individuals who bear a disproportionate burden of kidney disease.**

*Left image credit: Susumu Nishinaga/Photo Researchers, Inc.; Right image credit: Dr. Tobias B. Huber, University Hospital Freiberg.*

# Kidney, Urologic, and Hematologic Diseases

*Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the U.S. 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 process about 200 quarts of blood a day to filter out about two quarts of waste products and extra water from the blood, excreting them as urine. In people with chronic kidney disease, the function of these life-sustaining organs is impaired. Kidney disease may 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. At the close of 2006, more than 500,000 patients were receiving treatment for ESRD.[1] An estimated 26 million Americans suffer from chronic kidney disease.[2] The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. If unchecked, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications in several years, as more people begin to develop kidney and other complications of diabetes.

Racial minorities, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease. African Americans are four times more likely and American Indians are twice as likely to develop kidney failure as non-Hispanic whites.[1] Hispanics have a significantly increased risk for kidney failure as well.

The NIDDK supports a significant body of research aimed at increased understanding of 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 to progression of kidney disease to ESRD; and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. Areas of focus include diseases that collectively account for more than half of all cases of treated ESRD. Of special interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related glomerular diseases, including IgA nephropathy and hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program 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. It represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect men and women 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 effort includes basic, clinical, and epidemiologic research on the genitourinary tract. The NIDDK has supported studies in benign and noncancerous urologic disorders and diseases, including benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, pelvic floor disorders, congenital anomalies of the genitourinary tract, and sexual dysfunction.

Benign prostatic hyperplasia, or BPH, is a common, symptomatic condition that increases with age in men. Prostatitis—chronic inflammation of the prostate gland—is a painful condition that accounts for a significant percentage of all physician visits by young

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[1] *U.S. Renal Data System, USRDS 2008 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, 2008.*

[2] *Coresh J, et al: Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047, 2007.*

and middle-aged men for complaints involving the genital and urinary systems. To advance research in these areas, the NIDDK recently released a Prostate Research Strategic Plan which will serve as a guide for future scientific inquiry. The NIDDK is committed to enhancing research to understand, treat, and prevent these common and troubling disorders.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs). NIDDK research includes both basic and clinical projects aimed at understanding UTIs and finding ways to prevent their recurrence. Interstitial cystitis/painful bladder syndrome (IC/PBS) is a debilitating, chronic, and painful bladder disease. The number of individuals suffering with IC/PBS is not known with certainty, but it has been estimated that 1.3 million adults in the U.S. may have the disorder, with more women affected (90 percent) than men.[3] NIDDK-supported basic and clinical research is focused on elucidating the cause(s) 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 sponsors the Interstitial Cystitis Clinical Trials Group/Research Network to conduct clinical studies in IC/PBS. A new initiative, "Multi-disciplinary Approach to Chronic Pelvic Pain," is addressing many of the unanswered questions that impede research progress in both IC/PBS and chronic prostatitis, which share similar symptoms.

A conservative estimate is that approximately 12-13 million Americans, most of them women, suffer from urinary incontinence.[4,5] Many who have the disorder suffer in silence due to embarrassment and lack of knowledge about options available. The clinical field of urinary incontinence has changed dramatically in the last decade with the advent of new surgical procedures that have rapidly been introduced into the field. The NIDDK's Urinary Incontinence Treatment Network has recently completed a trial comparing two minimally invasive surgeries for the treatment of stress urinary incontinence and results are expected by the end of 2009.

Urolithiasis and urinary tract stone disease are frequent causes of visits to health care providers. The NIDDK has a robust interest in this field, ranging from prevention to basic stone formation/dissolution and treatment with improvement of the current minimally invasive treatment modalities of laser or ultrasound lithotripsy or extracorporeal shock wave lithotripsy.

One of the most common causes of kidney failure in children is vesicoureteral reflux. In fact, abnormalities of the genitourinary tract are among the most common birth defects. The NIDDK is conducting a clinical trial to determine if the current practice of long-term antibiotics is necessary for the treatment of these children.

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, and thrombocytopenia. The Institute is also keenly interested in the basic biology and genetic regulation of stem cells, especially 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.

## ELUCIDATING THE GENETICS OF KIDNEY DISEASE

**New Insights into a Common Form of Kidney Disease:** Two recent reports describe the development of new research tools and advances in our knowledge of the mechanisms underlying IgA nephropathy (IgAN). IgAN is a relatively common form of kidney disease arising from the accumulation of IgA—an antibody the body uses to fight infections—in the kidneys. The cause

[3] *Clemens JQ, et al: Interstitial Cystitis and Painful Bladder Syndrome in* Urological Diseases in America *(pp. 125-154). NIDDK, NIH Publication Number 07-5512, 2007.*

[4] *Nygaard I, et al: Urinary Incontinence in Women in* Urological Diseases in America *(pp. 157-191). NIDDK, NIH Publication Number 07-5512, 2007.*

[5] *Stothers L, et al: Urinary Incontinence in Men in* Urological Diseases in America *(pp. 193-221). NIDDK, NIH Publication Number 07-5512, 2007.*

of IgAN is unknown, although there is evidence that it runs in families. Over time, IgA deposits can damage the kidneys, and in severe cases patients require dialysis or a kidney transplant to live.

In most patients with IgAN, the sugar molecules that are normally attached to the IgA antibodies are aberrantly-formed, and this is thought to lead to IgA accumulation in the kidneys. Basic research on IgAN has been hampered by a dearth of experimental models. To advance research progress, scientists recently used a blood sample from a patient with IgAN to establish IgA-producing cells that can be grown in the laboratory. By analyzing these cells, they identified the specific step in the biologic pathway at which the addition of sugar molecules to the IgA antibodies goes awry. Such studies may identify new targets for future therapies.

In a second study, researchers measured aberrant IgA levels in patients with IgAN, their relatives, and other volunteers as controls. High levels of aberrant IgA were detected in blood from patients with IgAN compared to controls. Somewhat surprisingly, approximately half of the family members of IgAN patients had elevated levels of the aberrant IgA but did not display symptoms of IgAN. The study suggested that the defect in sugar addition to IgA antibodies is an inherited trait, but that additional factors—either genetic or environmental—are required for kidney disease to develop. The study also showed clustering of abnormal IgA within some of the families, a result that suggested that there may be different subtypes of IgAN.

The new cultured cell line will facilitate future studies of the mechanism of the disease, may identify new targets for therapy, and could help scientists test possible approaches to treatment. The discovery that IgAN arises at least in part due to a genetic component helps scientists understand how the disease is transmitted, and the observation that some people with elevated abnormal IgA do not display symptoms suggests that additional, unknown factors may be contributing to the disease. These and future studies may allow physicians to predict which at-risk patients are likely to develop IgAN, and to personalize treatment depending on an individual's disease subtype.

*Gharavi AG, Moldoveanu Z, Wyatt RJ, Barker CV, Woodford SY, Lifton RP, Mestecky J, Novak J, and Julian BA: Aberrant IgA1 glycosylation is inherited in familial and sporadic IgA nephropathy. J Am Soc Nephrol 19: 1008-1014, 2008.*

*Suzuki H, Moldoveanu Z, Hall S, Brown R, Vu HL, Novak L, Julian BA, Tomana M, Wyatt RJ, Edberg JC, Alarcón GS, Kimberly RP, Tomino Y, Mestecky J, and Novak J: IgA1-secreting cell lines from patients with IgA nephropathy produce aberrantly glycosylated IgA1. J Clin Invest 118: 629-639, 2008.*

**Rare Mutations in Kidney Salt Handling Genes Confer Reduced Blood Pressure:** While studying three genes (*SLC12A3*, *SLC12A1*, and *KCNJ1*) known to cause severe inherited blood pressure disorders, scientists identified rare mutations which clinically lower blood pressure and protect individuals from developing hypertension (high blood pressure). While there is known inherited variability in blood pressure, it has been difficult to identify the genes involved.

For this study, researchers analyzed DNA from 3,125 participants in the well-characterized Framingham Heart Study offspring cohort. People have two copies of most genes, and either copy can be normal or contain a mutation that affects the function of the gene (or the protein it encodes). The blood pressure disorders caused by mutations in *SLC12A3*, *SLC12A1*, or *KCNJ1*, genes that affect salt reabsorption by the kidneys, were known to result from inheritance of two mutant copies of any of these genes. For example, two mutant copies of *SLC12A1* results in severe low blood pressure and a greatly enhanced risk of death at a young age.

In the present study, researchers sought to investigate whether there is an effect on blood pressure in people who have only one mutant copy of any of these genes, with the other copy being normal. Approximately 1.6 percent of the tested participants had both a mutation that led to a single defective copy of one of the three salt handling genes, and also a normal copy of the gene. Researchers studied the 49 people with only one mutation. When present in only one copy, these mutations were found to be associated with significantly reduced blood pressure compared to the blood pressure of control participants who had two copies of the more common gene sequence. Of the mutations, 10 had been previously proven to be mutations that cause the protein to malfunction, with the remaining 20 suspected to cause similar loss of protein function. While mutations in both of a person's copies of any of these genes

result in clinically significant lower blood pressure, this new research interestingly identified a beneficial effect—protection from hypertension—conferred by rare mutations when present in only one copy of the gene. The scientists hypothesized that in such cases, the mutations may lower blood pressure by affecting salt reabsorption, although perhaps not to the extent seen with two mutant gene copies. This study contributes to overall understanding of the genetic basis of blood pressure variation in the general population.

*Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, and Lifton RP: Rare independent mutations in renal salt handling genes contribute to blood pressure variation. Nat Genet 40: 592-599, 2008.*

**Genome-wide Scan Identifies Genetic Regions Linked to Diabetic Kidney Disease:** Researchers have recently reported the identification of four chromosomal regions associated with the development of diabetes-related kidney disease and three chromosomal regions linked to the presence of protein in the urine, a sign of impaired kidney function. These results confirm and extend previous searches for genetic links to kidney disease in people with diabetes, and will allow for more detailed studies that may identify individual genes linked to this serious health problem.

The NIDDK established the Family Investigation of Nephropathy and Diabetes (FIND) consortium to identify genes that confer susceptibility to diabetes-related kidney disease, a life-threatening complication of diabetes. The consortium has focused its research efforts on four ethnic groups: European Americans, African Americans, Mexican Americans, and American Indians. Using genome-wide scans of samples collected from over 1,200 people with diabetes-related kidney disease and their relatives, the FIND researchers identified four regions on chromosomes 7, 10, 14, and 18 where subtle variations correlated with an increased risk of diabetic kidney disease. Similar scans identified three regions on chromosomes 2 and 15 and a different part of 7 associated with elevated protein in the urine. The strength of the linkages varied with the ethnic background of participants. For diabetic kidney disease, the linkage to chromosome 7 was strongest in African American families, while the linkage to chromosomes 10 and 14 was driven primarily by American Indians. For protein in the urine, the linkage on chromosome 2 was strongest in American Indians, on 7 for European Americans, and on 15 for African Americans.

These findings confirm earlier studies implicating regions of chromosomes 7, 10, and 18 in increased risk of diabetic kidney disease, and identify a new region of interest on chromosome 14. The next step is to perform more detailed analyses of these chromosomal regions to identify candidate genes that may confer susceptibility to diabetic kidney disease. Identification of such genes could greatly improve understanding of the disease process as well as provide targets for novel therapeutic strategies.

*Iyengar SK, Abboud HE, Goddard KA, Saad MF, Adler SG, Arar NH, Bowden DW, Duggirala R, Elston RC, Hanson RL, Ipp E, Kao WH, Kimmel PL, Klag MJ, Knowler WC, Meoni LA, Nelson RG, Nicholas SB, Pahl MV, Parekh RS, Quade SR, Rich SS, Rotter JI, Scavini M, Schelling JR, Sedor JR, Sehgal AR, Shah VO, Smith MW, Taylor KD, Winkler CA, Zager PG, and Freedman BI on behalf of the Family Investigation of Nephropathy and Diabetes Research Group: Genome-wide scans for diabetic nephropathy and albuminuria in multiethnic populations: the Family Investigation of Nephropathy and Diabetes (FIND). Diabetes 56: 1577-1585, 2007.*

**Genetic Link to Kidney and Eye Complications of Diabetes:** Scientists have identified a gene associated with risk of kidney and eye complications of diabetes. Proliferative diabetic retinopathy, or PDR, is a serious form of diabetic eye disease and the most common cause of new cases of legal blindness in working age adults in the U.S. Similarly, diabetes is the leading cause of irreversible kidney failure, also called end-stage renal disease (ESRD), in this country. People who develop PDR and ESRD usually develop them both, rather than developing one condition or the other. This observation suggests that there is a shared genetic factor(s) underlying susceptibility or resistance to developing these complications, but until recently, no causal gene had been conclusively identified.

Researchers sought to identify possible genetic factors contributing to these severe diabetes complications by comparing 11 genes in people with type 2 diabetes who either had or did not have PDR and ESRD. These genes were chosen because they were involved in regulating new blood vessel growth. Although new

blood vessel growth is an important part of healthy development, problems with this process are known to play a role in the development of diabetic eye and possibly diabetic kidney disease. One of the genes the researchers examined codes for the hormone erythropoietin, a potent stimulator of new blood vessel growth. The researchers found that variation in a region of DNA near the erythropoietin gene—a region that influences how much of the hormone is made—was associated with PDR and ESRD. They also analyzed genes of people with type 1 diabetes and found the same result, suggesting even more strongly a link between this genetic variation and diabetic eye and kidney disease in people of European American ancestry, who comprised the study groups. Additional analysis suggested that the risk variant may lead to the production of too much erythropoietin, which in turn could promote excessive new blood vessel growth and contribute to the development of PDR and ESRD in people with diabetes. This study identifies a genetic region influencing susceptibility to serious kidney and eye complications of diabetes, and also points to new targets for prevention and therapy. Further study is needed to determine if this genetic variant raises the risk of these complications across racial and ethnic groups. Identifying people at high risk for developing PDR and ESRD could also lead to personalized therapies.

*Tong Z, Yang Z, Patel S, Chen H, Gibbs D, Yang X, Hau VS, Kaminoh Y, Harmon J, Pearson E, Buehler J, Chen Y, Yu B, Tinkham NH, Zabriskie NA, Zeng J, Luo L, Sun JK, Prakash M, Hamam RN, Tonna S, Constantine R, Ronquillo CC, Sadda S, Avery RL, Brand JM, London N, Anduze AL, King GL, Bernstein PS, Watkins S; Genetics of Diabetes and Diabetic Complication Study Group, Jorde LB, Li DY, Aiello LP, Pollak MR, and Zhang K: Promoter polymorphism of the erythropoietin gene in severe diabetic eye and kidney complications. Proc Natl Acad Sci USA 105: 6998-7003, 2008.*

## DEVELOPING NEW TREATMENTS FOR KIDNEY DISEASE

**Novel Mechanism of Action of a Drug Used To Treat Kidney Disease:** A recent report describes a previously unknown direct effect on kidney cells of a drug used to treat various forms of kidney disease. Physicians use cyclosporine A (CsA) to treat people with a variety of kidney diseases, including focal segmental glomerulosclerosis, that are characterized by proteinuria (large amounts of protein in the urine). One cause of proteinuria is injury and damage to specialized cells in the kidneys called podocytes. Damaged podocytes cannot maintain close contact with each other and surrounding membranes, and this disruption allows protein to leak from the bloodstream into the urine. CsA has generally been effective at reducing proteinuria in patients, though its precise mechanism of action was previously unknown. It has been widely assumed that CsA's beneficial effect in kidney disease was due to its suppression of the immune system. However, researchers have recently discovered a direct effect of CsA in the kidney: the drug acts on the podocyte's cytoskeleton, a three-dimensional structure within the cell that is important in cell adhesion and motility and that helps the cell maintain or change its shape. CsA inhibits the degradation of synaptopodin, a podocyte cytoskeletal protein, thereby stabilizing the podocyte's three-dimensional structure and helping to maintain tight cell-cell contact. Experiments in mice indicate that the introduction of genetically-modified forms of synaptopodin that are resistant to degradation protects the animals against experimentally-induced proteinuria. Conversely, activation in podocytes of the enzyme that breaks down synaptopodin causes proteinuria. Together, these results establish a key role for cytoskeletal stability in proper podocyte function, and have important clinical implications. The findings may open new opportunities for the development of drugs to treat proteinuria that directly and selectively act on the podocyte. This avenue of research is particularly important because long-term CsA treatment is associated with serious side effects, which might be avoided with specific podocyte-targeting therapies.

*Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, Chang J-M, Choi HY, Campbell KN, Kim K, Reiser J, and Mundel P: The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. Nat Med 14: 931-938, 2008.*

*(For more information about how possible changes in cellular cytoskeletal components might play a role in kidney disease, see the accompanying Story of Discovery, "Newly-identified Genetic Variations Account for Much of the Increased Burden of Kidney Disease among African Americans.")*

**Reducing Blockage Fails To Improve Access to the Bloodstream for Kidney Dialysis:** Reducing early blockages in bloodstream access in patients with kidney failure (also called end-stage renal disease, or ESRD) by using a drug that inhibits blood clotting does not increase the likelihood that the access will function adequately for long-term treatments, an NIDDK-funded study has found.

Most Americans with ESRD depend on hemodialysis for survival. Hemodialysis, which removes waste products and excess fluid from the bloodstream, requires a vascular access—a surgically-created site that allows blood to be removed from and returned to the body. A fistula is a type of access that is preferred by many physicians because it is less likely to clot or become infected and is less expensive than other types of vascular access sites. However, maintaining an access site represents a significant clinical challenge, and blood clots at the fistula are the most frequent cause of early fistula failure. The Dialysis Access Consortium (DAC) enrolled almost 900 patients with ESRD and fistulas for vascular access, and randomly assigned them to receive either placebo or the anti-platelet drug clopidogrel, which inhibits blood clotting. After 6 weeks, only 12 percent of patients developed blood clots in the fistula when treated with clopidogrel, compared to nearly 20 percent of patients treated with placebo. In order for a fistula to be used for dialysis, however, it must mature and undergo remodeling in order to accommodate increased blood flow. Despite the improvement in short-term outcomes in the group receiving clopidogrel, about 60 percent of the new fistulas in both groups were unsuitable for long-term dialysis.

The DAC Fistula Trial was the largest multi-center clinical trial to examine the effectiveness of approaches to prevent blood clots in new fistulas, and was the first to test whether a prevention strategy would allow more fistulas to function for long-term dialysis. Because vascular access is critical for delivering life-sustaining care to people with ESRD, these results highlight the compelling need for research into novel therapies to reduce or prevent access failure in these patients. The results also underscore the continuing importance of preventing kidney disease in people at risk, preempting the need for hemodialysis in the first place.

*Dember LM, Beck GJ, Allon M, Delmez JA, Dixon BS, Greenberg A, Himmelfarb J, Vazquez MA, Gassman JJ, Greene T, Radeva MK, Braden GL, Ikizler TA, Rocco MV, Davidson IJ, Kaufman JS, Meyers CM, Kusek JW, and Feldman HI for the Dialysis Access Consortium Study Group: Effect of clopidogrel on early failure of arteriovenous fistulas for hemodialysis: a randomized controlled trial. JAMA 299: 2164-2171, 2008.*

**Chronic Kidney Disease Substantially Worsens in a Fourth of African Americans Despite Recommended Therapy:** Recent findings from a follow-up study to the landmark African American Study of Kidney Disease and Hypertension (AASK) clinical trial show that recommended treatment for chronic kidney disease (CKD) due to high blood pressure did not keep the disease from substantially worsening in about a quarter of the study participants. On a positive note, however, about one-third of participants in the study experienced only a slow decline in kidney function, about what is generally observed with aging.

The AASK clinical trial found that initial drug therapy with an angiotensin converting enzyme (ACE) inhibitor—which targets the renin-angiotensin hormone system that regulates blood pressure and fluid balance—was more effective than either initial therapy with either a calcium channel blocker or beta-blocker in slowing kidney disease progression in African Americans with CKD attributed to high blood pressure. The ACE inhibitor also significantly reduced the risk of kidney failure and death in these patients. No beneficial effect was observed in the trial of a lower than usual blood pressure goal (less than 130/80 mm Hg). However, little data were available regarding the long-term effects of an ACE inhibitor and a low blood pressure goal. Therefore, upon completion of the study in 2001, participants were invited to enroll in the follow-up cohort study, which examined whether a low blood pressure level (less than 130/80) and the use of an ACE or a similar drug, an angiotensin receptor blocker (ARB), would confer long-term benefits. About one-fourth of the AASK Cohort Study had substantial loss of kidney function or developed end-stage renal disease.

The results of the AASK Cohort Study highlight the continuing importance of research into better ways to treat CKD once it occurs, in order to preserve kidney function and prevent progression to end-stage renal

disease. These findings are particularly important because uncontrolled high blood pressure, an increase in the number of people with diabetes, and the aging of the U.S. population—all risk factors for impaired kidney function—will result in more people with CKD. These results also underscore the importance of preventing kidney disease through education of patients and health care providers, increased awareness of the seriousness of the problem, and careful monitoring of people at risk.

*Appel LJ, Wright JT Jr, Greene T, Kusek JW, Lewis JB, Wang X, Lipkowitz MS, Norris KC, Bakris GL, Rahman M, Contreras G, Rostand SG, Kopple JD, Gabbai FB, Schulman GI, Gassman JJ, Charleston J, and Agodoa LY for the African American Study of Kidney Disease and Hypertension Collaborative Research Group: Long-term effects of renin-angiotensin system-blocking therapy and a low blood pressure goal on progression of hypertensive chronic kidney disease in African Americans. Arch Intern Med 168: 832-839, 2008.*

## PREVALENCE OF CHRONIC KIDNEY DISEASE

**Ominous Findings in Study of Chronic Kidney Disease:** A recent study found that a growing number of Americans have chronic kidney disease, but that most of them are unaware of their condition. An estimated 26 million people—about 13 percent of the U.S. population—now have chronic kidney disease, a 30 percent increase in prevalence since 1994. A rather alarming finding of this study was that most people who have impaired kidney function are not aware of their condition and are therefore not receiving treatment. Only 11.6 percent of men and 5.5 percent of women with moderate (stage 3) kidney disease knew it. Awareness was highest among people with severe (stage 4) kidney disease, but even in this group only 42 percent of people knew of their condition. (Stage 5 disease is complete kidney failure, in which patients require either dialysis or a transplant to live.)

People with kidney disease have an increased risk of heart attack, stroke, high blood pressure, and early death. Kidney disease is often silent until its late stages. Once kidney function is lost, patients must undergo kidney dialysis or receive a kidney transplant. But if detected early there are interventions that can preserve kidney function or slow its decline. Patients at risk for kidney disease—those with diabetes, high blood pressure, and/or a family history of kidney problems—should be screened for kidney damage with routine blood and urine tests.

*Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, and Levey AS: Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047, 2007.*

## POLYCYSTIC KIDNEY DISEASE RESEARCH

**Genetic Predisposition, Cell-cell Signaling, and Cyst Development in Polycystic Kidney Disease:** Polycystic kidney disease (PKD) is a genetic disorder characterized by the growth of numerous fluid-filled cysts, primarily in the kidneys. PKD cysts can profoundly enlarge the kidneys while replacing much of the normal structure, resulting in reduced kidney function and leading to kidney failure. Mutations in two genes, *PKD1* and *PKD2*, are associated with the most common form of the disease, autosomal dominant PKD (ADPKD). The proteins encoded by these genes, polycystin-1 and polycystin-2, form an ion channel on the surface of kidney cells. This channel regulates the flow of calcium into and out of the cell. Mutation of either gene inhibits the activity of the channel, thus disrupting calcium-dependent intracellular signaling pathways. Evidence suggests, however, that additional factors play a role in cyst development.

Scientists have reported that the inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) may play a role in cyst development seen in ADPKD. Expression of this protein is increased in patients with high blood pressure and kidney injury, two conditions often seen in patients with ADPKD. Treatment of cultured mouse kidney cells with TNF-alpha disrupted the ability of polycystin-2 to properly localize to the cell surface. TNF-alpha treatment of cultured whole mouse kidneys led to cyst formation, and this effect was greater in kidneys taken from mice lacking one of the normal two copies of the *PKD2* gene ($PKD2^{+/-}$). Furthermore, in contrast to $PKD2^{+/-}$ mice, which have been shown to develop cysts, $PKD2^{+/-}$ mice treated with an inhibitor of TNF-alpha did not develop cysts in their kidneys. These results connect an inflammatory

response, mediated through TNF-alpha signaling, and a reduction in polycystin-2 function as part of a critical pathway toward cyst formation. Unraveling of both the genetic and nongenetic factors that contribute to cyst formation may identify new targets for therapies to treat ADPKD.

*Li X, Magenheimer BS, Xia S, Johnson T, Wallace DP, Calvet JP, and Li R: A tumor necrosis factor-alpha-mediated pathway promoting autosomal dominant polycystic kidney disease. Nat Med 14: 863-868, 2008.*

## UROLOGY RESEARCH

**"Intracellular Bacterial Communities" Detected in Urinary Tract Infections in Women:** Scientists have shown that some human urinary tract infections (UTIs) are associated with intracellular bacterial communities (IBCs). Infections of the urinary tract are common in women—about one-third of all women in the U.S. are diagnosed with a UTI by the time they reach 24 years of age—and many women suffer repeated UTIs. While antibiotic treatments are available, better prevention and treatment strategies are needed. Most UTIs are caused by a common type of *Escherichia coli* (*E. coli*) bacterium. An acute UTI begins when bacteria attach to cells lining the inside of the bladder. This provokes a defense response in the infected individual including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the body of offending bacteria.

Over the past several years, scientists studying UTIs in a mouse model found that UTI-causing *E. coli* are able to invade cells lining the bladder and form so-called IBCs, which appear to help promote sustained infection. Following up on these intriguing findings in mice, the researchers recently turned their attention to determining whether IBCs play a role in human UTIs. To do so, they looked for evidence of IBCs in urine samples from 100 women, 80 of whom had been recently diagnosed with an acute UTI, and 20 of whom had a history of UTI but did not have symptoms of an active infection. Using a variety of microscopic techniques, the investigators found that urine samples from 14 of the 80 women with UTIs (18 percent) contained IBCs. None of the asymptomatic women with a history of UTI showed evidence of IBCs.

Urine samples were also analyzed for the presence of filamentous bacteria—a form of the UTI-causing *E. coli* bacteria that can evade the host immune response. While nearly half of the urine samples from women with a UTI had filamentous bacteria, none were detected in the asymptomatic group. Filamentous bacteria were detected in all of the IBC-containing urines, compared to 29 percent of samples with no detectable IBCs. This study demonstrates, for the first time, that filamentous bacteria and IBCs do occur in some women with UTIs. Additional studies need to be done to determine whether IBCs contribute to recurrent infections in women as they do in mice. However, these results already suggest that new treatment strategies that address the host-evading nature of IBCs and filamentous bacteria may be beneficial for women who test positive for UTIs.

*Rosen DA, Hooton TM, Stamm WE, Humphrey PA, and Hultgren SJ: Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med 4: 1949-1957, 2007.*

**Common Gut Bacteria May Reduce Risk of Kidney Stone Formation:** The presence of a particular kind of bacteria in the intestinal tract may protect against the formation of the most common kind of kidney stone, according to a recent report. Kidney stone disease is a common and painful health problem in the U.S. It is estimated that between 5 and 15 percent of people will form a stone at some point during their lives, and that 30 to 50 percent of these people will suffer from recurrent stones over the ensuing 5 years. Most kidney stones mainly consist of crystallized calcium oxalate and small amounts of other compounds. Both calcium and oxalate are components of a normal diet, and high levels of oxalate in the urine correlate with increased risk of stone formation. However, decreasing dietary intake of oxalate has not been demonstrated to be effective in preventing kidney stone formation.

A recent report suggests that the common gut bacterium, *Oxalobacter formigenes* (*O. formigenes*), can metabolize and break down oxalate in the digestive tract, thereby reducing the likelihood of oxalate entering the body and forming a kidney stone. Researchers studied nearly 250 people who suffered from recurrent calcium oxalate stones and compared them to 250 people who did not form stones. They found that, among patients who formed stones, 17 percent had the bacterium *O. formigenes* in their intestinal tracts;

among those who did not form stones, the fraction with *O. formigenes* was 38 percent. *O. formigenes* presence in the intestinal tract was associated with a 70 percent reduction in risk for being a recurrent calcium oxalate stone former. Surprisingly, urinary oxalate levels did not differ with the presence or absence of *O. formigenes* colonization. Nevertheless, these results suggest that future therapies could involve introduction of *O. formigenes* or similar agents into the intestinal tracts of individuals who are at risk of recurrent stone formation. Such approaches may significantly reduce the likelihood of calcium oxalate stone formation in such individuals.

*Kaufman DW, Kelly JP, Curhan GC, Anderson TE, Dretler SP, Preminger GM, and Cave DR: Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. J Am Soc Nephrol 19: 1197-1203, 2008.*

**Brain Reacts to Bladder Disease:** Overactive bladder is a prevalent condition which negatively impacts the quality of life. Partial bladder obstruction is the common cause of overactive bladder in males. Although many studies have characterized the structural and functional changes associated with the partially obstructed bladder, little is known regarding the impact of overactive bladder on brain function. The researchers determined that a portion of the brain, including the locus ceruleus, displayed altered activity as a result of the partial bladder obstruction. These findings might have potential implications for overactive bladder in people, in that altered activity in this region of the brain might cause disordered sleep, anxiety, and difficulty in concentrating. The findings of this study suggest that pharmacological interventions which target the locus ceruleus may be beneficial in patients with bladder dysfunctions.

*Rickenbacher E, Baez MA, Hale L, Leiser SC, Zderic SA, and Valentino RJ: Impact of overactive bladder on the brain: Central sequelae of a visceral pathology. Proc Natl Acad Sci USA 105: 10589–10594, 2008.*

## HEMATOLOGY RESEARCH

**New Insights into Gene Regulation During Blood Cell Development:** A recent study shows that there is an inherent spatial organization that relates to how genes are regulated within the cell. Different types of cells require different proteins in order to perform their specialized functions, and thus must activate different arrays of genes. For example, red blood cells, infection-fighting white blood cells, and early-stage progenitor cells (that can develop into multiple types of blood cells) all need to turn on different sets of genes as they mature. But how are genes and chromosomes organized in the cell to achieve the co-regulation of specific sets of genes required for the development of specific types of blood cells?

In a recent report, scientists analyzed the arrangement of co-regulated genes along chromosomes and determined their organization during blood cell development in the mouse. By assessing the extent to which different genes are expressed (active or inactive) during blood cell development, as well as the chromosome locations of the genes, the researchers were able to demonstrate that coordinately-regulated genes, or gene sets, are clustered within individual chromosomes and that chromosomes are positioned in a cell's nucleus such that co-regulated gene clusters on separate chromosomes touch one another. These experiments determined that active genes associated with blood cell development are found within a particular part of the cell nucleus, the cellular compartment which houses most of the genes. The results indicated that active genes are localized to the inner portion of the nucleus and also that each duplicate pair of chromosomes—one from each parent—tend to associate and that this organization is related to the distribution of co-regulated genes along chromosomes. Thus, this study indicates that the proximity of chromosomes to the inner portion of the nucleus and the positioning of duplicate pairs of chromosomes in the cell nucleus has an important role in coordinating gene regulation during blood cell development.

*Kosak ST, Scalzo D, Alworth SV, Li F, Palmer S, Enver T, Lee JSJ, and Groudine M: Coordinate gene regulation during hematopoiesis is related to genomic organization. PLoS Biol 5: 2602-2613, 2007.*

**Genetic Cause Identified for Iron Deficiency in Individuals Unresponsive to Oral Iron Supplementation:** A recent study implicates mutations of the gene *TMPRSS6* as causing a particular form of iron deficiency anemia. *TMPRSS6* encodes a cell membrane-bound protein produced in the liver that controls levels of a critically important iron-regulatory protein called

hepcidin. In the U.S., most people with iron deficiency are easily treated with oral iron therapy; however there exists a small subset of children who don't respond to oral iron therapy—a condition termed iron-refractory iron-deficiency anemia (IRIDA). Investigators identified five families in which IRIDA was present in siblings. Because the siblings' parents did not have iron deficiency, the scientists thought that each parent may have one mutant and one normal copy of the causative gene, and that perhaps the children inherited only mutant copies of the gene, resulting in the disorder. Once identified as a likely candidate gene for this form of iron deficiency, analysis of this gene derived from the five families revealed several different types of genetic mutations.

Although not fully understood, mutations in the TMPRSS6 protein cause the body to over-produce hepcidin. Hepcidin controls iron concentrations in the body by regulating the recycling of iron from old red blood cells, and also by controlling intestinal iron absorption. The over-production of hepcidin effectively shuts down absorption of dietary iron from the intestine and traps the body's existing iron within the cells (called macrophages) that attempt to recycle it, thereby limiting the availability of iron to be used for new red blood cell production. These findings demonstrate the importance of TMPRSS6 to normal iron regulation in humans. Additionally, this study raises the possibility that delivery of functional TMPRSS6 into patients with IRIDA may reduce hepcidin levels and timely improve iron absorption from the intestine and release of iron from internal iron stores. Because other iron metabolism disorders are also associated with abnormal hepcidin levels, the development of strategies to modulate TMPRSS6 activity could potentially have even broader clinical implications in the future.

*Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, Mayo MM, Samuel SM, Strouse JJ, Markianos K, Andrews NC, and Fleming MD: Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet 40: 569-571, 2008.*

**"Nix"-ing Mitochondria as Part of Red Blood Cell Maturation:** NIDDK-supported researchers have determined that a protein called "Nix" plays a vital role in the normal maturation of red blood cells by targeting the cells' mitochondria for destruction. While mitochondria are cell components typically used for energy production, they can also be detrimental to cell survival by producing reactive oxygen species that can lead to cell death. In red blood cells, the removal of mitochondria through autophagy (self digestion) within so-called "autophagosomes" is a natural part of maturation, which also helps to prolong cell life. In people with defects in this process, disorders such as anemia can result. The Nix protein is a member of a family of proteins that regulate a pathway the body can use to get rid of cells—a cell death pathway. During differentiation of cells into mature red blood cells, the Nix protein is increased and is thus well positioned to influence this process.

In this series of studies, researchers studied mice that were unable to produce Nix in order to evaluate the role that this protein plays in a cell's digestion of its mitochondria as part of normal red blood cell maturation. In the absence of Nix, the mice developed both anemia associated with a reduced number of circulating red blood cells, and an expanded population of immature red blood cell precursors. The circulating red blood cells that were present retained mitochondria and had a shorter lifespan which was associated with an inability of the mitochondria to undergo autophagy. These studies enrich understanding of key molecular events in red blood cell maturation and how these events can be disturbed to result in anemia and other disorders. Based on these findings, novel therapeutic approaches could be developed in the future to treat certain forms of anemia.

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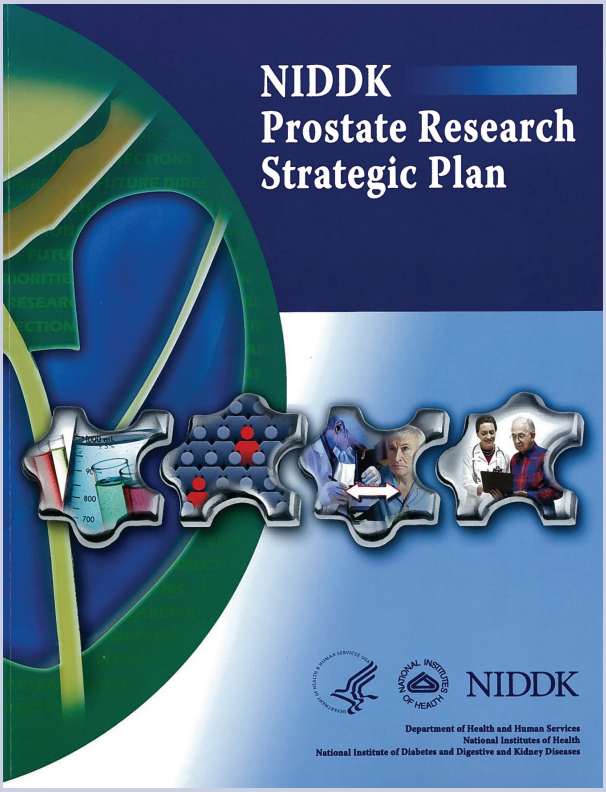
# *A Strategic Plan for Prostate Research*




**The *NIDDK Prostate Research Strategic Plan* will guide the development of future research efforts targeting benign prostate disease cause, prevention, and treatment.**

The *NIDDK Prostate Research Strategic Plan*, published in May 2008, was developed under the Institute's auspices with the contributions of external experts in benign urologic disease. The Plan identifies questions of highest significance and provides recommendations for research to address them. The NIDDK will use the recommendations and insights in the *Plan* to guide the development of future research efforts targeting benign prostate disease cause, prevention, and treatment.

The research area of benign prostate disease includes two of the most significant non-cancerous disorders affecting men—benign prostatic hyperplasia (BPH) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). BPH, an enlargement of the prostate gland, is often associated with lower urinary tract symptoms (LUTS). LUTS, which can include symptoms such as overactive bladder, restricted or excessive urination, and sensations of urgency, affects men of all races and ethnic groups and can become severe over time. An estimated 50 percent of men in their 50s have BPH, and 26 to 46 percent of men between the ages of 40 and 79 have moderate to severe symptoms. CP/CPPS is generally described as inflammation of the prostate gland and is sometimes associated with urinary symptoms, pain, and sexual dysfunction. The source of the pain in this syndrome is unknown and there are no generally effective methods for preventing or treating the condition.

*The NIDDK Prostate Research Strategic Plan* addresses four major research areas judged critical for advancing the field. These include basic science, epidemiology and population-based studies, translational research, and clinical sciences. Selected high priority recommendations from the *Plan* include:

- Promote interdisciplinary research that focuses on how benign prostate diseases are influenced by other organ-specific diseases and systemic conditions, such as obesity, high blood pressure, high cholesterol, cardiovascular disease, diabetes, and erectile dysfunction. For example, the possible influence of high blood pressure on BPH/LUTS is a previously unexplored area of research.
- Study the primary prevention of benign prostate diseases, including possible benefits of lifestyle changes such as avoidance of alcohol and caffeine, frequency of sexual practice, pelvic massage therapy, stress reduction, and diet modulation for relief of CP/CPPS.
- Develop data and human tissue resources from patients of various ages to derive information useful in investigating risk factors, underlying causes and natural history of disease progression, quality of life, quality of care, and decision making regarding treatment of benign prostate disease.
- Develop imaging approaches and other biomarker studies to assess severity and risk of progression based on physical and cellular findings.
- Develop targeted medical therapies based on new insights into disease-relevant cellular pathways and physiological events.

- Develop standardized, clinically significant benign prostate disease syndrome definitions and classifications based on measurable phenotypic features.
- Train and mentor epidemiologists, health services researchers, clinical investigators, and students interested in the study of benign prostate disease.

The *Plan* is designed for researchers, clinicians, professional organizations, and patients. Each major section includes a mission statement, a lay summary, an overview of current knowledge, and high-priority recommendations for future research.

The Plan is online at *http://www2.niddk.nih.gov/NR/rdonlyres/318606D2-A9D1-4CAD-B9BF-8EB3009C83BE/0/NIDDKProstateStrategicPlan.pdf* and can be purchased online in print or compact disc format at *http://catalog.niddk.nih.gov/PubType.cfm?Type=182&CH=NKUDIC*

## STORY OF DISCOVERY

# *Newly-identified Genetic Variations Account for Much of the Increased Burden of Kidney Disease among African Americans*

For the first time, researchers have identified variations near a single genetic locus that are strongly associated with kidney diseases disproportionately affecting African Americans. Two research teams independently studied kidney diseases arising from causes other than diabetes. Kidney disease can lead to kidney failure, requiring long-term dialysis or a kidney transplant to sustain life. Using a type of genome-wide association technique that relies on differences in the frequency of genetic variations between populations, the researchers identified several variations in the region of the *MYH9* gene on chromosome 22 as major contributors to excess risk of non-diabetic kidney disease among African Americans. Somewhat surprisingly, both research teams found no association between the *MYH9*-area variants and diabetes-related kidney failure in this population, a finding that suggests the mechanisms leading to chronic kidney disease and then to kidney failure may be different depending on the underlying cause. This insight may have important implications for the treatment of the very large number of individuals with kidney disease.

**Kidney Disease: A Heavy Burden for Some Populations**

Early-stage kidney disease often has no symptoms. Left unchecked, however, it can silently progress to kidney failure, a condition in which the kidneys are no longer able to filter waste and excess fluids from the blood. As many as 26 million U.S. adults over the age of 20 are estimated to 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 2006[2] (the most recent year for which complete data are available). 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 full-blown kidney failure.[3]

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 nearly 3 times as likely as whites to develop kidney failure from any cause.[4] One such cause is a form of kidney disease called focal segmental glomerulosclerosis (FSGS), in which the glomeruli—the tiny filtering units of the kidneys—are damaged and scarred.[5] Most FSGS arises from unknown causes and is termed "idiopathic" FSGS. African Americans are approximately 5 times more likely to develop idiopathic FSGS compared to individuals of other racial backgrounds. The health disparity increases with HIV infection: African Americans are 18 to 50 times more likely than whites to develop FSGS related to infection with HIV, the virus that causes AIDS.[6,7] These rather striking disparities represent a serious public health problem, not only because of the kidney disease itself, but also because people who have even mild- to moderately-severe kidney disease typically have high blood pressure and other risk factors for serious complications such as cardiovascular disease.[2]

What accounts for this dramatically increased risk of severe kidney disease in African Americans? Scientists and physicians have long known that kidney disease tends to run in families and cluster in ethnic groups. These observations indicate that

kidney disease is likely to have a genetic component. It is also almost certain that environmental factors play a role in disease susceptibility as well. However, studies that have attempted to identify genes that confer susceptibility to kidney disease and kidney failure have not generally been successful.

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

**New Techniques Allow Researchers To Ask New Questions**

For some conditions, mutations in a single gene are sufficient to cause disease, and careful analysis of inheritance patterns in families can often readily identify the gene responsible. These diseases are termed "simple" genetic diseases, because their underlying cause, while not always easy to uncover, tends to lead to disease in a straightforward way.

However, many diseases likely arise not from mutations in a single gene but from the interplay of complex genetic susceptibility—resulting potentially from multiple genes, each of which may have only modest effects—and environmental influences. In the case of these "complex" genetic diseases, identifying the genetic contribution of multiple, widely-spaced chromosomal regions to disease development and progression can be quite difficult.

Recently, a new technique, termed admixture mapping, has been developed to search for genes that cause complex genetic diseases. 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. Using admixture mapping, scientists examine haplotypes—groups of genes spanning multiple chromosomal loci that are transmitted together. These haplotypes are inherited; therefore, haplotypes tend to be similar among members of the same population but to differ between members of different populations. Admixture mapping 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. The relatively recent (anthropologically speaking) mixing of European and African populations is referred to as "admixture": the formation of a new population with a heterogeneous mixture of African- and European-derived haplotypes.

**A New Window into the Genetics of Kidney Disease**

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. The rationale behind these experiments was that chromosomal regions that confer an increased risk of kidney disease would be more common in individuals of African ancestry than in those of European ancestry. At least two groups of scientists hypothesized that, by using admixture mapping, they could identify genetic variants that tracked closely with disease development.
In the fall of 2008, the two research teams reported

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the identification of genetic variants more common in African Americans that seemed to explain a large proportion of the excess burden of FSGS and HIV-associated and other non-diabetic kidney disease in African Americans. In addition, the contribution of this genetic variation to an individual's risk of developing kidney disease is higher than that observed for nearly all previously described genetic factors discovered by genome-wide scans, including those for prostate cancer, diabetes, cardiovascular disease, breast cancer, and hypertension.

One research team, which included members of the NIDDK Intramural Research Program's Kidney Disease Branch and other researchers, studied individuals with FSGS, HIV-associated FSGS, and hypertensive end-stage kidney disease. The other team, consisting of researchers working as part of the NIDDK-funded Family Investigation of Nephropathy and Diabetes Consortium, was led by researchers at The Johns Hopkins University and included collaborating scientists at other institutions. They examined patients with kidney failure arising from multiple causes, including diabetes, hypertension, FSGS, and HIV infection. Using admixture mapping, both groups of scientists identified a genetic variant in a region of chromosome 22 that correlated strongly with susceptibility to certain kidney diseases.

Fine mapping of this chromosomal region revealed that the gene *MYH9* was located in the identified area. *MYH9* encodes the protein "non-muscle myosin heavy chain 9," which is part of non-muscle myosin IIA. Myosin is a protein made up of several subunits and serves as a cellular motor, providing the force for cell movement, cell tension, and cell division. The most common form of myosin is found in skeletal muscle and is involved in muscle contraction. Non-muscle myosin IIA is a form of myosin found in many tissues, including—despite its name—muscle. The *MYH9* gene is expressed in podocytes, specialized cells within kidney glomeruli that play an important role in the filtering of waste and excess fluid. Podocyte damage is a hallmark of FSGS and other kidney diseases that can lead to reduced kidney function and/or kidney failure. However, it is not known how variations in the *MYH9* region might impact podocyte function.

The degree to which these genetic variants increase risk of developing kidney disease in African Americans from certain causes is truly striking. *MYH9* risk variants account for nearly all of the increased risk for idiopathic FSGS and HIV-associated FSGS among African Americans compared to European Americans and a portion of the increased risk for hypertensive kidney disease. Surprisingly, however, these variants were not associated with kidney failure arising from diabetes.

The risk of developing kidney disease is strongest when an individual has two copies of the risk variant. Nonetheless, even among individuals with two risk variants, kidney disease is uncommon. Thus 36 percent of African Americans have two copies of the risk variant but only approximately 1 in 50 of these individuals will develop FSGS during the course of a lifetime. It is likely other factors, possibly additional genes or environmental influences, are important in triggering FSGS. Future research efforts will focus on the identification and characterization of these additional factors.

It is important that it is the presence of the variant that confers the increased risk of kidney failure, not African ancestry *per se*. However, these variants were much more frequently seen among people of African ancestry than among those of European ancestry—60 percent of alleles among African Americans are the risk variant (84 percent of African Americans carry one or two copies of the risk allele), while only 4 percent of alleles among European Americans are the risk variant (8 percent of European Americans carry one or two copies of the risk allele).

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**Implications and Future Directions**

Although both studies described here implicate variations in the chromosomal region surrounding *MYH9* as important risk factors for kidney disease, scientists have not identified specific mutations in the *MYH9* gene that might suggest a causal mechanism. One possibility is that the critical genetic variations lie not within the coding sequence of the *MYH9* gene, but in the surrounding chromosomal regions. The nature of these hypothetical variations, and the ways they might alter cellular metabolism or function so as to confer greater risk of non-diabetic kidney disease, are the subject of ongoing investigations. Future studies will aim to characterize the exact nature of the variations in the *MYH9* region and how these variations may influence susceptibility to non-diabetic kidney disease. Additional future studies will focus on the pattern of *MYH9* expression across tissues, and investigation into the role played by *MYH9* in podocyte function, and how this might be disrupted in individuals carrying the risk variant.

One of the central questions facing researchers who study kidney disease is whether all kidney disease is created equal: although many different conditions—diabetes, hypertension, and FSGS were among the ones studied by these investigators—put people at increased risk for chronic kidney disease and kidney failure, it is not known whether these conditions share a common disease pathway or each have unique characteristics that define them. This distinction is important, because current approaches to therapy are aimed at preserving kidney function and addressing the underlying health problem, not at addressing specific processes that may damage the kidneys. The discovery that a particular genetic variation confers susceptibility to kidney failure by some mechanisms—such as hypertension and FSGS—and not by others—such as diabetes—indicates that there are likely at least two pathways to kidney failure.

These findings also validate the use of admixture mapping to perform genome-wide scans to identify susceptibility genes for complex diseases. Insights gained from the studies have important implications for improved patient care and for understanding the basic biology of kidney disease and kidney failure.

Finally, this story highlights the importance of collaborations between scientists at the NIH and NIH-funded investigators at outside research institutions. Government-academic collaborations of this kind are one way to move translational research forward, from the bench to the bedside and beyond, and provide the knowledge base for developing new therapies for chronic health disorders such as kidney disease and kidney failure.

*The investigators in the NIDDK Intramural Research Program, who first identified the* MYH9 *gene as contributing to kidney disease, have been conducting basic and clinical research studies of kidney disease, focusing on focal segmental glomerulosclerosis, at the NIDDK since 1995. Scientists at the National Cancer Institute's Center for Cancer Research also contributed to this study. The Johns Hopkins-led research team, that confirmed and extended the MYH9 findings, is part of the NIDDK-funded Family Investigation of Nephropathy and Diabetes (FIND) Consortium. First funded in 1999, the Consortium was established to identify genetic pathways that may be critical for the development of diabetic kidney disease as well as to identify candidate genes and/or pathways that may be amenable to therapeutic strategies to prevent the onset or progression of kidney disease. Though originally conceived as an effort to identify genes associated with diabetes-related kidney disease, FIND investigators discovered an important clue regarding non-diabetic kidney disease. The two studies were published in the journal* Nature Genetics *in October 2008; the citations are* Nat Genet *40: 1175-1184, 2008 and* Nat Genet *40: 1185-1192, 2008.*

## STORY OF DISCOVERY


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# More Intensive Dialysis Does Not Improve Outcomes among Patients with Acute Kidney Injury

*Dr. Paul M. Palevsky*

*Dr. Paul M. Palevsky is a Professor of Medicine in the Renal-Electrolyte Division, Department of Medicine, University of Pittsburgh School of Medicine, and is Section Chief of the Renal Section at the VA Pittsburgh Healthcare System. His research interests focus on the prevention and treatment of acute kidney injury and the management of kidney replacement therapy in acute and chronic kidney disease. In May of 2008, Dr. Palevsky described the findings of the Veterans Affairs/ National Institutes of Health Acute Renal Failure Trial Network Study in a featured presentation at the annual American Thoracic Society International Conference in Toronto, Canada. The following summary is based on that presentation. The results of the study were subsequently published in the July 3, 2008, issue of the* New England Journal of Medicine.

Acute kidney injury (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. The resulting inability to excrete nitrogenous waste products and maintain fluid and electrolyte balance poses urgent health problems for patients and their physicians. Acute kidney injury 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 drugs or other toxins. It is a relatively common complication among hospitalized patients; it affects between 2 and 7 percent of all hospitalized patients.[1] Even though a significant fraction of patients with acute kidney injury 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.[1]

There is no effective drug therapy to reverse acute kidney injury. The 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 keeps the proper electrolyte balance.

Although dialysis has been used to treat acute kidney injury 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. Several recent, small studies had suggested that increased frequency or intensity of dialysis might improve survival in patients with acute kidney injury. However, the results of these studies have not been definitive. This uncertainty raises the possibility that some patients may be receiving a sub-optimal dose or frequency of dialysis, or that other patients may be receiving excessive dialysis that may carry no clinical benefit and may, in fact, expose them to unnecessary risk. In order to investigate this issue, the NIDDK partnered with the U.S. Department of Veterans Affairs to launch a clinical trial comparing "standard" with "intensive" dialysis in patients with acute kidney injury.

**Design of the ATN Study**

The VA/NIH Acute Renal Failure Trial Network (ATN) Study was designed to determine whether higher-dose (intensive) dialysis would reduce the death rate, shorten the duration of the illness, and decrease the number of complications in other organs among patients with acute kidney injury, as compared to standard-dose dialysis. It enrolled over 1,100 critically

ill patients—defined as patients with acute kidney injury as well as either sepsis or the failure of at least one other organ. Notably, the trial did not enroll patients with chronic kidney disease. These patients were not studied in this trial because the causes and progression of their acute kidney injury are different from that seen in people without underlying chronic kidney disease.

Patients were randomly assigned to receive intensive- or standard-dose dialysis. Patients who did not require medications to maintain their blood pressure were treated with conventional dialysis, either three times per week in the standard arm of the study or six times per week in the intensive arm. Patients with very low blood pressure who required medications to increase their blood pressure were treated with more gentle forms of dialysis, either a slower form of hemodialysis, three or six times per week, or a continuous form of dialysis, at a lower or higher dose, as randomly assigned. One important element in the design of the study was that patients were able to switch between forms of therapy as their clinical condition changed, while remaining within the lower or higher intensity treatment arms of the study. This approach reflects typical clinical practice in that it allowed physicians to adjust the method of dialysis as the patient's condition changed, and was chosen so that the results of the trial would be more relevant to actual patient care.

**Results of the ATN Study: Is More Better?**

The primary question the trial was designed to answer was whether more intensive dialysis provided a clinical benefit. The first, and perhaps most important, clinical endpoint was patient survival. After 60 days, no significant difference in rates of death by any cause was found between the two groups of patients. Over this period, 289 of 561 patients (51.5 percent) in the standard-dose treatment group died, compared to 302 of 563 patients (53.6 percent) in the intensive treatment group. Mortality rates were similar in men and women and across racial and ethnic subgroups.

When the researchers assessed kidney function and other medical conditions, similar patterns were seen. A total of 102 patients (18.4 percent) in the standard-dose group had complete recovery of kidney function after 28 days, and 50 patients (9.0 percent) had partial recovery. By comparison, 85 patients (15.4 percent) in the intensive-treatment group had complete recovery of kidney function over the same time period, and 49 patients (8.9 percent) had partial recovery. A total of 92 patients (16.4 percent) undergoing less-intensive therapy were able to return home without requiring continued dialysis after 60 days, compared to 88 patients (15.7 percent) who underwent intensive therapy. None of these differences between groups was statistically significant. Rates of treatment-related complications across all groups were also similar.

In summary, the ATN Study found no significant differences between the two groups in recovery of kidney function, the rate of failure of organs other than kidneys, or the number of patients able to return home after recovery. In patients enrolled in this trial, there was no benefit to intensive dialysis.

**Implications of the ATN Study**

Although a few studies have suggested that increased frequency or intensity of hemodialysis might improve survival in patients with acute kidney injury, they have been small and conducted at single sites. In contrast, the ATN study enrolled over 1,100 patients from 17 Veterans Affairs medical centers and 10 university-affiliated medical centers across the U.S. The results of the larger ATN Study show that when it comes to dialysis in acute kidney injury, more is not better.

The results of the ATN study, however, should be interpreted carefully. One limitation of the ATN study concerns the exclusion from the trial of patients with advanced chronic kidney disease. Such patients make up a substantial proportion of people who develop acute kidney injury. Therefore, it may be inappropriate to extrapolate the ATN results to persons in whom acute kidney injury develops in

the context of pre-existing chronic kidney disease. Further study will be necessary to resolve this longstanding question and address the optimal treatment of acute kidney injury in this population.

**Conclusion**

The results of the ATN study indicate that increasing dialysis treatments to five to six times per week does not confer an additional benefit beyond a standard three times per week regimen. However, this does not mean that dose of dialysis does not matter. The dose of dialysis targeted in the standard-treatment group was greater than what is often achieved in a typical clinical setting. The results also do not mean that higher doses of continuous therapies are never beneficial, only that routine use of higher-dose dialysis is unnecessary. Nevertheless, the findings of this study may spare patients from unnecessarily-intensive medical interventions. They also underscore the importance of continued research into other approaches to treating acute kidney injury. Future research efforts may include studies to identify biomarkers of kidney injury prior to renal failure, which could enable physicians to predict who is likely to develop acute kidney injury, to lessen its severity through earlier intervention, or to preempt this life-threatening condition altogether.

*The NIDDK has begun a new initiative entitled "Identification and Evaluation of Biomarkers and Risk Assessment Tools for Chronic Kidney Disease and Acute Kidney Injury." The goal of this initiative is to identify and validate biomarkers and risk assessment tools for kidney function, injury, and progression. Both existing and new biomarkers and risk assessment tools will be rigorously evaluated for clinical utility under this initiative. In addition to seeking new molecular markers in chronic kidney disease and acute kidney injury, the initiative will also examine whether these two conditions share common biomarkers.*

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## PATIENT PROFILE

# James Willingham

## *Vascular Access—A Major Component To Treating Kidney Failure with Hemodialysis*

**James Willingham**

To listen to the lilt and laughter in James Willingham's voice one would be hard-pressed to believe that this 66-year-old was diagnosed with kidney failure, referred to as end-stage renal disease (ESRD), 5 years ago while in the hospital for congestive heart failure and cardiac asthma, and that he has been undergoing hemodialysis treatments three times a week ever since.

"I was in the hospital being treated for my heart and asthma conditions when they checked my kidneys and found that they were functioning at only 10 to 15 percent of normal. They immediately put me on dialysis," says James. Dialysis is a treatment for kidney failure; the dialysis machine cleanses the blood—a vital process that would normally be done by working kidneys. Patients with ESRD need either dialysis or a kidney transplant to live.

A couple of months after his ESRD diagnosis, James was asked if he would like to take part in one of the clinical studies being conducted by the Dialysis Access Consortium, or DAC, sponsored by the NIDDK. The DAC Study was testing the impact of anti-clotting reagents in preventing early failure in "vascular access," which is required for dialysis. A vascular access is the site on the body where blood is removed and returned during dialysis treatments.

James responded in the affirmative. "I figured that even if I couldn't help myself, maybe I could help someone else" as a result of participating in the study.

*"I have my good days and bad," says James, "but if I exercise a bit, and don't go overboard with what I eat and drink, I can live a pretty good life on dialysis."*

**Family History**

According to James, there's been a long history of high blood pressure and type 2 diabetes in his family. Yet, none of his four siblings has ESRD. However, three of his first cousins, the children of his mother's sister, are on dialysis as well.

James has known that he's had high blood pressure since his high school days. He's not sure exactly when, but later in life he was also diagnosed with type 2 diabetes. "When I was young I never took it seriously, never treated it," he says. But by around age 50, it caught up with him, eventually resulting in his kidney failure. He began taking steps to improve his health.

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He no longer needs to take insulin for his diabetes. "I control it mostly by diet," he says. He also walks 2 to 3 miles a week.

"I have my good days and bad," says James, "but if I exercise a bit, and don't go overboard with what I eat and drink, I can live a pretty good life on dialysis."

**Dialysis and the Importance of Good Vascular Access**

When renal failure occurs, the kidneys lose their capacity to remove bodily waste from the blood. Hemodialysis is a method for removing waste products such as urea and creatinine, as well as extra water from the blood when the kidneys are no longer functioning properly.

An important first step before starting regular hemodialysis sessions is preparing a vascular access on the body that will be used at each dialysis session for inserting a needle and tubing, through which blood is circulated out of the body, to the dialysis machine for cleansing, and then back into the body. The vascular access site is usually placed in the forearm or the upper arm. To maximize the amount of blood cleansed during hemodialysis treatments, the vascular access should allow continuous high volumes of blood flow. For easier and more efficient removal and replacement of blood with fewer complications, the access should be prepared weeks or months before dialysis is required.

Because James' kidney disease was diagnosed at such a late stage, he needed dialysis immediately. Consequently, physicians temporarily outfitted him with a traditional catheter in his chest. Catheters are not ideal for permanent vascular access because they can clog, become infected, and cause narrowing of the veins in which they are placed. But if hemodialysis needs to start quickly, as it did in James' case, a catheter will work for several weeks or even months while a more permanent, surgically created access has time to develop. In this situation the catheter was left in James' chest for an entire year, as no infections or any other complications developed.

**Types of Vascular Access**

In addition to traditional catheters, which are recommended only for temporary use, the two other types of vascular access are arteriovenous fistulas (AV fistulas) and arteriovenous grafts (AV grafts).

A properly functioning AV fistula is considered the best long-term vascular access for hemodialysis because it provides adequate blood flow, lasts a long time, and has a lower complication rate than other types of access. A fistula is an opening or connection between any two parts of the body that are usually separate—for example, a hole in the tissue that normally separates the bladder from the bowel. While most kinds of fistulas are a problem, an AV fistula is useful for hemodialysis patients because it causes the vein to grow larger and stronger for easy access to the blood system.

A surgeon creates an AV fistula by connecting an artery directly to a vein. Usually placed in the forearm, the vein of a new AV fistula will grow thicker after 3-6 months so that it can take repeated needle insertions and allow blood to flow quickly to the dialyzer. Once AV fistulas are working well for dialysis treatments, they tend to last longer than other types of dialysis access like catheters and AV grafts. A good fistula can function up to 10 years or longer.

Because James has smaller, weaker veins that wouldn't develop properly into a fistula, he was given an AV graft, a vascular access that connects an artery to a vein using a synthetic tube, or graft, implanted under the skin in his arm. The graft becomes an artificial vein that can be used repeatedly for needle placement and blood access during hemodialysis. A graft doesn't need to develop as a fistula does, so it can be used sooner after placement, often within 2 to 3 weeks.

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Compared with properly formed fistulas, however, grafts tend to have more problems with clotting and infection and need replacement sooner. However, a well-cared-for graft can function for several years. Fortunately, that was James' case.

**The Dialysis Access Consortium (DAC) Study**

The NIDDK established the Dialysis Access Consortium, which consists of seven primary clinical centers and a data coordinating center, to undertake interventional clinical trials to improve outcomes in dialysis patients who received either a fistula or a graft.

Two randomized placebo-controlled clinical trials were designed. The first trial evaluated the effects of the antiplatelet agent clopidogrel (Plavix®) on prevention of early AV fistula failure. The AV fistula trial ended in 2007 and revealed that clopidogrel did not improve the likelihood that an AV fistula would develop into a useable fistula for dialysis. The second clinical trial, which James participated in, focused on AV grafts for dialysis access. The AV graft trial studied a drug that combines dipyridamole with aspirin, and had the goal of preventing the narrowing of the vascular access in hemodialysis patients with grafts.

James, who says he is grateful for having been able to take part in the study, took his medication every day, twice a day; once in the morning and once in the evening, and reports having had no complications with his graft.

"The study was a very good experience for me," James says. "I had help monitoring the graft to make sure it was open and that my blood pressure was good. And my outpatient dialysis nurse was terrific. She took very good care of me, talked with me and told me how I was doing every step of the way." He says he did his part by keeping the graft clean and not picking up heavy objects with the grafted arm.

Dialysis is not the most pleasant of processes. "I've been on dialysis every Monday, Wednesday, and Friday morning for 5 years now, and each session lasts about 4 hours. That's a long time to sit in that chair. And it's very painful. It's a 16-gauge needle, about the size of a plastic coffee stirrer that they stick in you. And you don't want the needle to come out or you have a problem." So to be able to maintain a free-flowing, uninfected vascular access without complications is a real plus for patients like James.

The AV graft trial that James was in ended early in 2008, and the results will soon be made public. From James' perspective, he believes in the process. "It is studies like these that help people like me," he says, with that lively, friendly tone in his voice that he employs so well.

*"The study was a very good experience for me," James says. "I had help monitoring the graft to make sure it was open and that my blood pressure was good. And my outpatient dialysis nurse was terrific. She took very good care of me, talked with me and told me how I was doing every step of the way."*

**Hope through Further Research**

To improve the quality of life of patients with end-stage renal disease, the NIDDK currently supports additional clinical and basic science research efforts. For example, the Frequent Hemodialysis Network is conducting two clinical trials: the Daily Trial is comparing conventional hemodialysis (2.5 hours, 3 days per week) to more frequent hemodialysis (1.5 - 2.75 hours, 6 days a week) and the Nocturnal Trial is comparing home conventional hemodialysis delivered 3 days per week to nocturnal home hemodialysis given 6 times per week. Another example of NIDDK-supported efforts for patients with ESRD is a new consortium that will pursue studies to understand the high rate of AV fistula failure seen in many patients that have an AV fistula placed for dialysis access.

## PATIENT PROFILE

The NIDDK is also supporting the Chronic Renal Insufficiency Cohort (CRIC) to better understand how chronic kidney disease progresses to ESRD. Another effort supported by NIDDK is the Animal Models of Diabetic Complications Consortium, which has the goal of improving or creating animal models of human diabetes complications, including diabetic kidney disease. Diabetic kidney disease is currently the leading cause of ESRD in the U.S. The animal models will help scientists to elucidate the causes of kidney disease and develop prevention and treatment approaches. Finally, the NIDDK distributes science-based information on dialysis and other aspects of kidney disease to patients, health care providers, and the general public through its National Kidney and Urologic Diseases Information Clearinghouse (*http://kidney.niddk.nih.gov/*) and its National Kidney Disease Education Program (*http://nkdep.nih.gov*).

The Dialysis Access Consortium Arteriovenous Graft study was carried out at seven NIDDK-funded research sites: Boston University (Dr. Laura Dember); Duke University (Dr. Arthur Greenberg); the University of Iowa (Dr. Bradley Dixon); the University of Maine (Dr. Jonathan Himmelfarb); the University of Texas, Southwestern (Dr. Miguel Vazquez); the University of Alabama (Dr. Michael Allon); and Washington University in St. Louis (Dr. James Delmez). The Data Coordinating Center was located at the Cleveland Clinic (Dr. Gerald Beck). Three satellite sites were supported by NIDDK and five satellite sites were supported by industry.

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