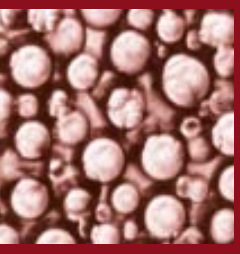
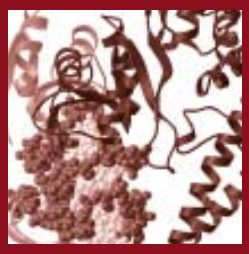
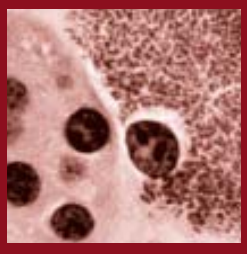
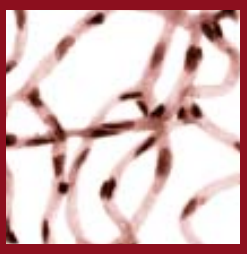
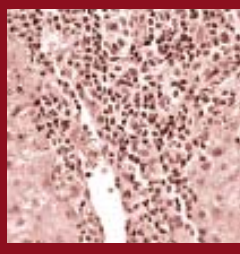
NIDDK

Recent Advances & Emerging Opportunities

February 2004

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

CONTENTS

Message from the Director

The research mission of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) encompasses a wide array of diseases and conditions affecting tens of millions of Americans. Our overarching goal is to conduct and support research that will uncover the biological bases for health and disease, and to encourage rapid translation of this knowledge into clinical interventions to treat, prevent, or cure diseases within the NIDDK's purview. This document is our fourth annual compendium of highlights of NIDDK-supported basic and clinical research. It provides an overview of the strides we have made over the past year toward reaching our goals.

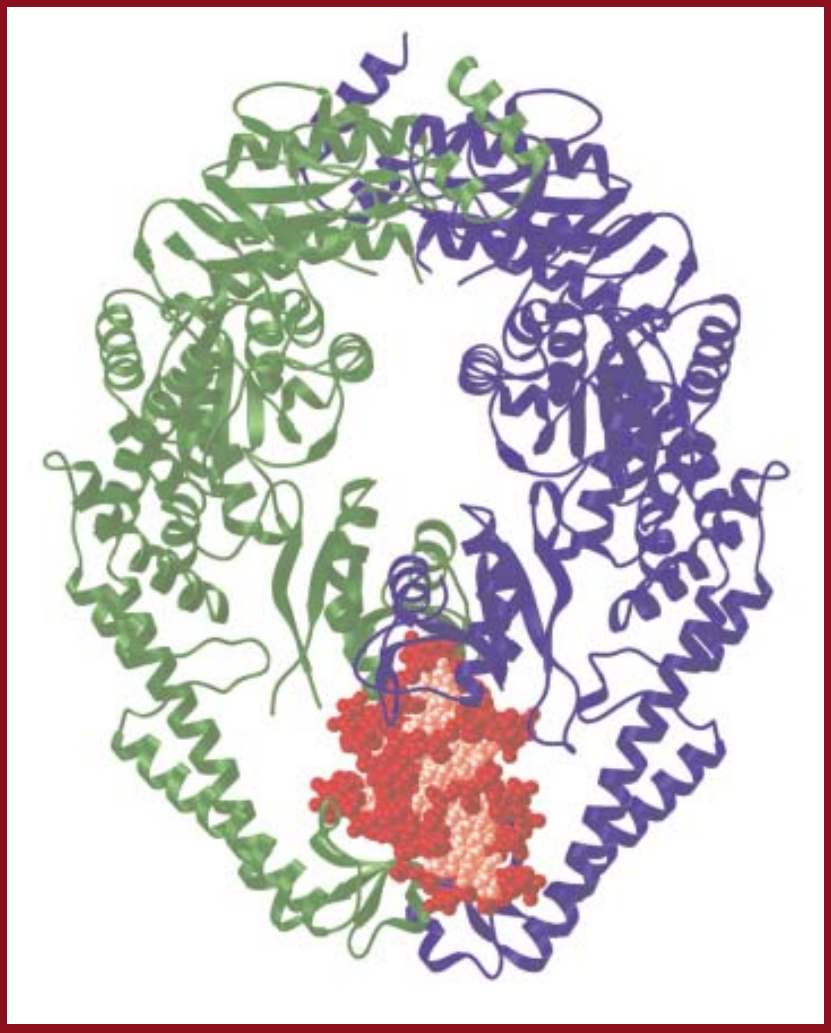
The examples of research advances collected in this booklet represent a substantial return on the significant investment that has been made in research over the past several years. In 2003, public policy makers completed the effort to double the NIH budget over five years. For the NIDDK, this expanded NIH support has meant greater opportunities to enable young scientists to pursue bold new ideas, as well as to launch many new initiatives. As we move forward into the "post-doubling" period, we will carefully continue our scientific stewardship for the American people.

The past year has also been marked by far-reaching scientific achievements and new opportunities that will enhance our ability to advance the NIDDK mission. Through the tremendous achievement of an international, NIH-led consortium, the complete sequence of the human genome is now known. Widely available access to the information from the Human Genome Project and its databases will accelerate and facilitate efforts by NIDDK-supported researchers to find the genes involved in many chronic and acute disorders, including complex diseases–such as type 1 and type 2 diabetes, and inflammatory bowel disease–and single gene disorders with secondary modifiers–such as polycystic kidney disease and Cooley's anemia. At the same time, we foresee benefits from a new, NIH-wide strategic effort, spearheaded by the NIH Director–the NIH Roadmap for Biomedical Research. New initiatives created through the NIH Roadmap will strive to develop new technologies, build new research teams and disciplines, and re-engineer the clinical research enterprise–both in the translation of basic advances into clinical research, and the translation of clinical research into widespread application. Our participation in these cross-cutting initiatives, and our leadership of those on "metabolomics," interdisciplinary training, and translational research cores, will help to advance research progress in the diseases within the NIDDK mission.

The health problems within the NIDDK's purview exact a significant toll on the Nation. We have included in this year's document a new chapter devoted to obesity. This chapter highlights both new research findings and recent strategic planning processes and administrative changes at the NIDDK and at the NIH. Similarly, we have highlighted recent strategic efforts in liver disease that are meant to strengthen and facilitate research in this area. Two essays in this booklet capture the essence of scientific talks by NIDDK-supported scientists who are leaders in the fields of genomics and adult stem cell biology. As in past years, we also feature several "Stories of Discovery," which are intended to illustrate how today's science advances are built on a strong foundation of past research accomplishments. Within these pages you will also find profiles of several people affected by diseases for which the NIDDK bears research responsibility, such as diabetes, celiac disease, Cooley's anemia, and polycystic kidney disease. These personal accounts serve to remind us that the ultimate purpose of biomedical research is to preserve health and to benefit people touched by disease.

Our highlights of recent advances and emerging opportunities provide just a glimpse of the work being carried out by an immense network of basic scientists, clinical researchers, and patient volunteers. It is our hope that you will find these advances an exciting and promising reflection of the NIDDK's many contributions to the national biomedical research enterprise.

Allen M. Spiegel, MD
Director
National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health
Department of Health and Human Services



This three dimensional structure shows how the cellular protein MutS (blue and green ribbons) contacts a DNA double helix (red and pink) to repair mismatches in the two strands and prevent mutations. Maintaining the accuracy of a DNA sequence is crucial for all organisms, from bacteria to humans. Photo: Dr. Wei Yang. Reprinted with permission from Obmolova G *et al, Nature* 407: 703-10, 2000. © 2000 Nature Publishing Group *(http://www.nature.com)*

Cross-Cutting Science: Paving the Way to Discovery

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. While the ultimate application of such basic research is not always obvious, 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 of fundamental processes, ranging from the development of cells to the development of organisms, and new approaches and 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. Investment in such cross-cutting scientific research today will have future applications that we cannot now describe with certainty, but which we know will surely be realized.

THE NIH ROADMAP

In October 2003, the NIH Director announced the first initiatives to be launched through a new, far-reaching strategic effort to identify opportunities and gaps in biomedical research that no single institute at NIH could address alone, but that the NIH as a whole should address in order to accelerate biomedical research. That effort is called the "NIH Roadmap for Medical Research." Designing the NIH Roadmap has been a collaborative process, which involved NIH senior scientific staff, with input from over 300 nationally recognized leaders in academia, industry, government, and the public.

The NIH Roadmap is a new vision for research that provides a framework for making strategic NIH investments to optimize the agency's entire research portfolio. The NIH Roadmap is divided into three major themes: (1) New Pathways to Discovery; (2) Research Teams of the Future; and (3) Re-engineering the Clinical Research Enterprise. Through the Roadmap effort, scientific initiatives were developed to propel research under these three themes. To be part of the NIH Roadmap, initiatives had to be deemed of high potential impact, had to enhance the disease and mission-specific activities of all of the NIH Institutes and Centers, and had to respond to the needs and concerns of the public. These initiatives were refined and are being carried out by nine focused "Implementation Groups" chaired by Directors of the NIH Institutes and Centers. To facilitate implementation, each initiative is being "led" by an Institute whose interests and expertise are closely aligned with the stated goals.

The NIDDK Director co-chaired the "Building Blocks, Biological Pathways, and Networks" Implementation Group, which is under the "New Pathways to Discovery" theme. This group focused on "systems biology," an integrative study of how all biological components work together in an organism to promote normal development and to sustain health. Further research is needed in this

area to understand how biological pathways are integrated in humans and in other complex organisms, to determine how disturbances in these pathways may lead to disease, and to discover how to restore disturbed pathways to their normal functions. Many of the diseases within the NIDDK mission are chronic conditions that result from disturbances in biological systems either at the outset of life or over the course of life. Thus, initiatives in this area are particularly relevant to NIDDK-supported research.

Roadmap Initiatives–New Technology Development: In order to increase knowledge in systems biology, new experimental technologies are needed to study cellular components more accurately, quickly, and on a very small scale. The impact of new technologies is underscored by the recent completion of the Human Genome Project, in which an emerging technology–high-throughput DNA sequencing–enabled scientists to achieve this momentous accomplishment. The data generated from this project can be used to understand the underlying genetics of healthy and disease states. It is an ongoing challenge for scientists to take large amounts of data and apply them to achieve greater knowledge about biology. Under the "New Pathways to Discovery" theme, Roadmap initiatives are encouraging development of new technology to increase understanding about cellular pathways. Such general technology development is beneficial to the NIDDK because the technologies can ultimately be used to study specific diseases within the Institute's mission.

The NIDDK will be the lead Institute in implementing an initiative on "Metabolomics Technology Development." The "metabolome" is the complete set of metabolites in an organism; examples of metabolites include amino acids, peptides, and lipids. "Metabolomics" is the study of these low-molecular weight molecules. The purpose of this initiative is to promote the development of highly innovative and sensitive tools for studying metabolomics. The development of novel technologies can directly benefit the study of diseases within the NIDDK mission. For example, such technologies can be used to develop surrogate markers to predict risk, aid in diagnosis, and assess progression of the complications of diabetes. Metabolomics technologies can also be applied to advance understanding of the metabolic changes that occur with obesity and its co-morbidities. The ability to study metabolites at the single-cell level would also aid in characterizing tissues and organs that contain a variety of specialized cell types, such as the kidney.

Another initiative will develop a network of research Centers to create new tools for "proteomics," a high-throughput approach to studying the dynamics of protein interactions. The Centers will develop instruments, methods, and reagents for quantitative measurements of proteins at sub-cellular resolution and with very short timescales. The NIDDK plans to complement this Roadmap initiative with proteomics studies specifically geared toward diseases within the NIDDK mission. As the technology advances, NIDDK researchers can apply these new technologies to examine mechanisms regulating the pathology of many different diseases and conditions within the NIDDK mission.

Roadmap Initiatives–Molecular Libraries: Also under the "New Pathways to Discovery" theme, this initiative focuses on the use of "small molecules" to study biological processes. Small molecules have proved to be important tools to manipulate biological processes, many times leading toward important therapeutic advances. For example, small molecules can act to regulate the natural activities of important proteins to achieve a desired biological effect. With the emergence of large amounts of genomic information, it has become possible to use libraries of small molecules to screen for and identify compounds useful for study of the regulation of important

proteins. In order to find an appropriate molecule to interact with the protein of interest, scientists may screen a large number of small molecules from a "molecular library" of thousands of molecules. Because a very small number of molecules will interact with a target protein in the precise way needed to observe a biological effect, a molecular library screening approach increases the chances that an appropriate molecule will be discovered.

The importance and biological significance of using a molecular library approach was demonstrated in recent studies by NIDDK-supported researchers in the areas of diabetes and cystic fibrosis (see next chapter on "Diabetes, Endocrinology, and Metabolic Diseases"). NIDDK-supported researchers also used this approach to study a protein called "FXR." FXR is a key protein that functions as a receptor to regulate bile acid and cholesterol metabolism. Researchers hypothesized that it might be possible to test the biological function of FXR through the use of small molecules. Therefore, they used a molecular library of over 10,000 small molecules in a high throughput screening assay to identify any that would turn on, or activate, FXR. The result was identification of small molecules that had specific effects on FXR. This enabled more refined screening to finally arrive at a class of small molecules with better effects on FXR than natural compounds. These optimized molecules potently activated FXR, but, importantly, did not activate most other proteins that were similar to FXR, and facilitated studies to understand the underlying mechanism of action of FXR. Because disorders of bile and cholesterol metabolism are often interconnected, these findings now open the door for future research to develop additional small molecules that can have a bearing on diseases that these processes may regulate.

The Molecular Libraries Roadmap initiative will give researchers tools to study a wide range of diseases within the NIDDK mission. In addition, a new NIDDK initiative will develop assays for small molecule screening for potential mission-specific therapeutics. The development and application of molecular libraries can provide insights into important biological and disease-related processes, and serve as the basis for the generation of new drugs.

The Future: The Roadmap initiatives will address areas of major scientific opportunity and need for all of NIH, including the NIDDK. Exploiting new pathways to discovery, fostering interdisciplinary research, and re-engineering the clinical research enterprise will speed discoveries that can ultimately lead to improved prevention and treatment of obesity, diabetes, and digestive, kidney, urologic, and blood diseases. The Roadmap initiatives tackle areas that the NIDDK is not able to do on its own, even though advances in these areas would greatly help the study of the Institute's categorical disease. The NIDDK also plans to issue Institute-specific initiatives that will complement and enhance Roadmap initiatives. This approach will take full advantage of the Roadmap process to ensure that the NIDDK reaps maximum benefits from this exciting and innovative process for years to come.

Nicolaou KC, Evans RM, Roecker AJ, Hughes R, Downes M and Pfefferkorn JA: Discovery and optimization of non-steroidal FXR agonists from natural product-like libraries. *Org Biomol Chem* 1: 908-20, 2003.

VISUALIZING LIFE'S PROOFREADERS: STRUCTURAL STUDIES OF DNA REPAIR PROTEINS

Cell division is a basic biological process in which one cell becomes two. The new cell must contain all of the material in the original parent cell, in particular the genetic blueprint known as DNA, in order for the new cell to be functional and continue to divide. Making a copy of the DNA requires a process called "DNA replication," which uses the parent cell's DNA as a template to

make a new copy. DNA consists of "coding letters," also called "bases," which are used as a blueprint to make proteins that carry out biological processes. The DNA code for making a protein is analogous to using letters to make sentences. Specifically, there are four DNA bases (letters); three letters in combination code for an "amino acid" (word), and many amino acids string together to form a "polypeptide chain" (sentence). A functional protein may contain single or multiple polypeptide chains.

The process of DNA replication is very efficient; however, there are approximately 3,200 million bases that must be copied, so it is inevitable that some errors will occur. Approximately 30-3,000 errors are made each time a cell divides. This is analogous to typing on a computer–some words will contain typing errors. Luckily, a convenient tool–the spellchecker–finds the mistakes so they can be corrected. Likewise, the cell has different types of tools to "proofread" the DNA for mistakes so they can be repaired before the cell divides. Interestingly, these tools are found in all species, from bacteria to humans. Their presence demonstrates that maintaining the accuracy of this fundamental process is extremely important and has been conserved throughout evolution.

Why is it so important to correct errors in copying the DNA? When there is a mistake in the DNA, there can subsequently be a mistake in the protein whose production it controls. Many times, even one mistake prevents the protein from functioning properly and can lead to serious health problems. For example, certain types of cancer are caused because of mistakes in proteins that are important in regulating cell division. In addition, an increase in overall instability of DNA has been implicated in the aging process. Thus, maintaining the integrity of the DNA is crucial to the cell.

DNA Mismatch Repair: A type of mechanism, called "mismatch repair" (MMR), is one component of the cell's repair system to correct errors in DNA that may arise during replication or damage (such as damage to DNA bases by chemical agents). The importance of this process is underscored by the fact that people with defective MMR proteins are at increased risk for developing a type of colorectal cancer, called "hereditary nonpolyposis colorectal cancer" (HNPCC). Errors in MMR proteins are also found in some sporadic cancers, which are cancers that do not appear to be inherited.

In bacteria, an important protein that is involved in MMR is called "MutS." MutS can be called a "sensor" that sends signals to many other cellular proteins when there is an error in the DNA. MutS can recognize DNA bases that are improperly matched, as well as small loops of unpaired bases; MutS can also recognize a limited repertoire of chemically altered bases. When MutS finds an error, it can communicate to other MMR proteins to begin the DNA repair process. In some instances, MutS can initiate a cell death pathway that kills the cell when the errors are irreparable. The role of MMR proteins, such as MutS, in regulating cell death pathways comes into play in the treatment of cancer patients. Cisplatin, a chemotherapy drug, is not capable of "killing" the cancer cell on its own. Cisplatin damages DNA and then requires MutS to "sense" the damage and activate the cell death pathway. However, many cancer patients become resistant to the chemotherapy because the cancer cells have found a way to inactivate the MMR pathway. The resistance to chemotherapy is a serious barrier that prevents successful treatment of cancer patients.

Because the DNA replication process itself is so efficient, there are generally not many mistakes. Thus, how does MutS find the few errors embedded among the correctly formed DNA? How does MutS recognize different types of errors? These

questions are important to begin to unravel the underlying molecular mechanisms of this fundamental biological process. Recent studies by NIDDK scientists have begun to provide many answers.

Determining the Three-Dimensional Structure of Proteins: Proteins are three-dimensional structures that have characteristics such as loops, curves, and grooves. It is impossible to determine the three-dimensional structure of a protein by only knowing the sequence of individual building blocks (amino acids) that make up the protein. Therefore, researchers use special tools and techniques to determine this structure. One experimental method used by scientists is called "X-ray crystallography," in which proteins in liquid solution are subjected to conditions to make them form crystals. Once high-quality crystals are formed, they diffract X-rays and the patterns of diffraction are analyzed to determine the three-dimensional structure of the protein.

Solving a structure of a protein gives scientists considerable insight into how it may function in the cell. This approach permits scientists to actually "see" what the protein looks like and how it may be interacting with other molecules. This visualization can bridge the gap between genetic studies and understanding disease states. For example, genetic studies can identify mutations in proteins that cause disease; however, it is often not known how the mutations may alter the protein's function. With a protein's three-dimensional structure, scientists may be able to see how the specific mutations that cause disease may be detrimental to the protein's function–such as changing the structure of a protein or preventing the protein from interacting with other proteins or DNA. This knowledge can greatly improve the ability of scientists to hypothesize about ways to "fix" the protein–instead of making blind guesses about how the mutation may be harmful.

Three-Dimensional Structural Studies of MutS: NIDDK scientists have recently performed important structural and biological studies on a bacterial MutS protein that have given insight into the role of MutS in MMR. MutS was very difficult to crystallize because a portion of the protein was mobile. To overcome this barrier, the scientists removed the mobile portion, which did not affect the protein's function, but did enable the researchers to determine its structure.

The NIDDK researchers determined that it is necessary for two MutS polypeptide chains to come together–in an asymmetric manner–to bind the DNA. Interestingly, they determined that MutS binding caused the DNA to severely bend. This observation helps to explain why MutS is able to recognize many types of DNA damage. DNA is similar to a wooden ladder that has many rungs. When the rungs are constructed properly, a person is able to step on them and be supported. However, if there is a crack in the wood of a single rung, that rung is much weaker and will easily bend or break when a person steps on it. The structural studies suggest that MutS recognizes and then can help to fix errors in DNA by finding the "weak rung," or the area of DNA that can bend.

Once MutS finds the DNA error, how does it communicate with other proteins to activate a correction through mismatch repair (MMR)? When the researchers compared the three-dimensional structures of MutS alone to MutS bound to DNA, they discovered that MutS itself undergoes a change in structure when it binds DNA. It is in this DNA-bound conformation that MutS interacts with other MMR proteins to begin repair. Thus, researchers hypothesize that MutS must be bound to a DNA mismatch in order to initiate MMR.

An important portion of MutS is able to convert a high energy metabolite, called "ATP," into a lower energy form called "ADP." Previous studies had shown that this conversion was necessary for

MMR. To further examine the importance of this portion of MutS, the NIDDK researchers analyzed the three-dimensional structure of MutS bound simultaneously to DNA and ADP. The structure, as well as insights from additional experiments, led them to conclude that the overall role of ATP to ADP conversion was to increase the sensitivity and specificity of MutS in finding the error in the DNA. ATP was necessary for the MutS to recognize the error in the DNA; it was also necessary to "authorize" the repair process to start, thus preventing repair from being performed on a "correct" DNA molecule. For example, if MutS bound to a piece of DNA that had no errors, the ATP would cause the MutS to dissociate from the DNA so that it did not repair something that was already correct. If MutS were bound to DNA containing an error, the ATP was the signal that gave the "green light" for repair to occur. Additional genetic and biochemical studies showed that MutS must bind both ATP and the incorrect DNA in order for repair to begin.

Researchers would also like to study the structure of MutS bound to both DNA and ATP. However, this MutS-DNA-ATP complex is very unstable, because the ATP is rapidly converted to ADP. Thus, to date, it has not been possible to perform structural studies of MutS with ATP. NIDDK researchers therefore looked for other molecules that were similar to ATP to see if they could use an alternate molecule to determine the three-dimensional structure. They found a molecule, called "ADP-berillium fluoride," or "ABF," which they have used in subsequent X-ray crystallography studies. ABF is similar to ATP and binds to MutS, but is not converted to ADP. In addition to making it useful for three-dimensional studies of MutS, these properties make ABF an invaluable tool to further study the DNA repair process.

The structural biology studies have also enabled the researchers to make predictions about the regions of MutS that may be critical for its biological function. This capability has led to biochemical studies of the protein to understand the importance of different regions in regulating repair. For example, the researchers have determined that a region of MutS, called the "HuH" motif, is necessary for function. These types of studies demonstrate that a combination of structural biology, biochemical, and genetic approaches is an efficient and productive way to begin to understand a very complex process.

Implications: Repairing damaged or incorrectly replicated DNA is a critical process for all living things. When this process is not faithfully maintained, subsequent mistakes in proteins can occur, which may lead to serious health problems, such as cancer. The previously described studies using bacterial proteins can be expanded to studying mismatch repair in other organisms, including humans. Because the processes are similar, much of the knowledge gained from the bacterial studies can be directly applied to higher organisms.

Understanding the molecular basis and regulation of DNA repair processes can help researchers determine what may go wrong biologically that can lead to a disease state. For example, the structure of MutS can be used to map mutations that cause diseases. It is known that errors in the MMR proteins can cause an inherited form of colorectal cancer. NIDDK researchers have used MutS structural studies to determine that many of the mutations involved in this type of cancer are located in regions of MutS that are critical for its function. This may help researchers understand why the mutated protein does not function properly in disease. Information about a fundamental biological process such as DNA repair can also be used to increase knowledge about cancer in general. This work can therefore be applied to other cancers within the NIDDK and NIH research mission, such as prostate and pancreatic cancers.

Although determination of a protein's three-dimensional structure may be a difficult process, it gives scientists a way to actually "see" what a protein looks like. The structural studies per-

formed by NIDDK researchers complement biochemical and genetic studies, and have directly led to a new model for the mechanism by which MutS regulates mismatch repair of defective DNA. This knowledge builds a framework for future studies on this process as it relates to cancer, aging, and resistance to chemotherapy.

Alani E, Lee JY, Schofield MJ, Kijas AW, Hsieh P, Yang W: Crystal structure and biochemical analysis of the MutS•ADP•beryllium fluoride complex suggests a conserved mechanism for ATP interactions in mismatch repair. *J Biol Chem.* 278: 16088-94, 2003.

Junop MS, Obmolova G, Rausch K, Hsieh P, Yang W: Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. *Molecular Cell* 7:1-12, 2001.

Biswas I, Obmolova G, Takahashi M, Herr A, Newman MA, Yang W, Hsieh P: Disruption of the helix-u-turn-helix motif of MutS protein: Loss of subunit dimerization, mismatch binding and ATP hydrolysis. *J Mol Biol.* 305: 805-16, 2001.

Obmolova G, Ban C, Hsieh P, Yang W: Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. *Nature* 407: 703-10, 2000.

POTENTIAL AND PLASTICITY OF STEM CELLS–AN EVOLVING PICTURE

Scientists are striving to understand the processes that occur during normal development, when a vast number of different cell types are generated from a single fertilized egg. If they can understand normal development, scientists will have a better chance of determining how to recapitulate development in an adult in order to replace cells damaged by disease. Even within adults, special cells known as stem cells retain the ability to divide, and the divisions can give rise either to more stem cells or to cells that will differentiate into specific cell types.

Currently, scientists are determining the usefulness of different types of stem cells for treating human disease. Until now, replacement of cells has only been possible *via* organ or cell transplantation. However, doctors are unable to treat every needy patient with transplantation, because there are limited supplies of donor cells and organs. Stem cells are heralded as a possible means for overcoming this treatment barrier. Furthermore, by studying how stem cells become specialized cells within organs and tissues, researchers are reaching a greater understanding of the processes underlying normal and abnormal tissue regeneration and repair. This, in turn, is enabling them to develop and test treatments that could augment the body's reparative processes and foil disease.

The various types of stem cells are believed to differ mainly in the limits of their "potential"–their ability to differentiate into other cell types. Embryonic stem (ES) cells arise early in development. Because they must give rise to all the different cell types and tissues of the body, embryonic stem cells are thought to have almost unlimited potential.* Adult stem cells, on the other hand, reside within a mature tissue or organ and have been traditionally viewed as able to differentiate into a more limited number of cell types, although numerous investigators have reported that some of these cells actually may have a much greater differentiation potential than previously thought–a phenomenon also known as "plasticity." The NIDDK is supporting research on both types of stem cells, within established policies for NIH funding.

Bone Marrow Adult Stem Cells Take Different Paths to Become Liver Cells and Islets: There may be several means whereby stem cells derived from one mature, adult tissue or organ can apparently turn into differentiated cell types characteristic of another organ upon transplantation–including the presence of highly potent adult stem cells in donor tissues, the presence of several types of adult stem cells in donor tissues, and the fusion of donor cells with recipient cells. Cell fusion occurs

* Consistent with the policy announced by President George W. Bush on August 9, 2001, NIH funding of research involving human stem cells is in accordance with specific criteria established by the Administration.

during normal mammalian development in the formation of bone and muscle, is a feature of certain types of cancer, and can occur in cultured stem cells. Recently, in a major discovery, researchers showed that adult bone marrow-derived cells were able to repair damaged liver tissue in mice by actually fusing with the host liver cells. Previously, it was shown that mice with a fatal metabolic liver disease, tyrosinemia type I, which is caused by loss of a vital enzyme, regain normal liver function through transplantation of hematopoietic stem cells purified from bone marrow. It turns out that these stem cells do not alone directly generate new liver cells, but instead spontaneously fuse with the diseased liver cells to form a healthy-functioning hybrid cell containing both stem cell and liver DNA, with the liver cell molecules dominating the hematopoietic factors. It also appears that the contribution of the hybrid stem-liver cells depends on both the normal regenerative capacity of the liver, and the space in the organ that is created by the degeneration of the diseased host liver. Generally, when cell fusion occurs, the cell that results contains a greater than normal number of chromosomes. Remarkably, however, some of the new, hybrid cells contained the normal two copies of each chromosome. It is possible that in these hybrid cells, "reduction division" occurs, whereby chromosome pairs are lost. These new findings are an example of research on the mechanisms underlying putative adult stem cell plasticity, and also have implications for treating certain genetic metabolic disorders, with the possibility of gene transfer through cell fusion.

Researchers are also testing the potential of adult bone marrow-derived stem cells to replace the insulin-producing beta cells of the pancreas, which are lost to autoimmune destruction in type 1 diabetes. A new report from studies in mice has revealed that these stem cells may be a potential source of beta cells. Unlike the stem-liver cell fusion hybrid described previously, however, researchers systematically ruled out cell fusion as the mechanism for producing beta cells. In this research, mouse bone marrow-derived cells that express the "enhanced green fluorescent protein" (EGFP) if the insulin gene is actively making insulin, were transplanted into mice whose bone marrow was destroyed. Four-to-six weeks later, EGFP-positive (insulin-producing) cells appeared in the host pancreatic islets. Detailed studies revealed these cells to have several beta cell markers–they expressed the glucose transporter 2 gene and transcription factors that are indicators of beta cell differentiation, and were responsive to factors that induce insulin secretion. Thus, bone marrow-derived cells represent a possible new source of cells for beta cell replacement therapy for type 1 diabetes, and some forms of type 2 diabetes. This therapeutic approach would not require co-administration of immunosuppressant drugs to keep the recipient's immune system from rejecting the cells because the bone marrow could be taken directly from the diabetic patient.

"Niche" Control of Adult Stem Cell Fate:
Specialized microenvironments (niches) must exert critical influences on stem cell fate, but the nature of these influences remains poorly understood. Researchers studying this phenomenon in mouse bone marrow cells have discovered that bone-forming cells, called osteoblasts, are a major regulatory component of the bone marrow microenvironment. A key ingredient is parathyroid hormone (PTH), which is an important regulator of bone growth and metabolism. Bone marrow from a mouse strain altered so that the receptor for PTH is continuously activated in osteoblasts produced twice the number of hematopoietic stem cells as normal. A molecular signaling system between the osteoblasts and stem cells was found to be responsible for governing the formation of stem cells. PTH both boosted the number of osteoblasts and supercharged the signaling system. Normal mice injected with PTH also showed increased stem cell production, as well as marked improvement in survival following bone marrow transplantation. These findings may

be important for lessening the health risk for bone marrow transplant recipients, who have a limited supply of stem cells. The identification of PTH, osteoblasts, and signaling pathways as important factors in the stem cell microenvironment provides pharmacological targets with therapeutic potential for stem cell-based therapies.

These studies have all advanced progress towards developing alternative sources of cells for transplantation, as well as other possible therapeutic approaches, to treat human diseases. The NIDDK is supporting several efforts designed to capitalize on and extend previous stem cell and developmental biology discoveries and to stimulate new discoveries.

Beta Cell Biology Consortium (BCBC): The BCBC initiative was established to facilitate interdisciplinary approaches that will advance the understanding of pancreatic islet development and function. The long-term scientific goal is to develop a cell-based therapy to restore normal insulin production and action to diabetic patients. To accomplish this goal, the BCBC supports a host of stem cell-related research efforts, including development of key reagents to permit the prospective isolation of stem/progenitor cells in both neonatal and adult animals, development of assays to test the efficacy of pancreatic stem/progenitor cells as a cure for type 1 diabetes, and development of protocols to efficiently differentiate embryonic and adult stem cells into pancreatic islet tissue. Important resources of the BCBC include (1) EPConDB, a searchable database that contains information about genes expressed in the mouse and human pancreas during development (*http://www.cbil.upenn.edu/EPConDB/*), (2) a Microarray Core that is producing and distributing mouse and human cDNA microarrays, (3) an Antibody Core that is developing antibodies to human cell surface antigens and important markers of progenitor cells, (4) a Mouse ES Core that is producing important transgenic animal models that will be essential in identifying and characterizing pancreatic progenitor cells, (5) a Human Stem Cell Subcommittee that is establishing protocols for expanding and isolating human pancreatic progenitor cells, (6) a BCBC Coordinating Center (Vanderbilt University) which oversees the BCBC website (*www.betacell.org*), and coordinates all activities of the BCBC including scientific cores, investigator retreats, and the "pilot and feasibility studies" program, and (7) a Stem Cell Training Core which will train members of the BCBC to work with both adult and embryonic human stem cells.

Progenitor Cell Genome Anatomy Projects (GAPs): The successful treatment of many chronic and debilitating diseases afflicting Americans today will depend on the ability to replace organs or to stimulate regeneration and recovery of damaged organs. To build upon the achievements of the Human Genome Project, the NIDDK and other NIH Institutes have established a range of Genome Anatomy Projects (GAPs) to map the complex network of cellular interactions in normal and diseased tissues. One NIDDK initiative is the Progenitor Cell Genome Anatomy Project, which is studying how progenitor cells develop into different types of cells that form the organs and tissues of the body. It is important to understand these developmental processes, and how progenitor cells maintain and regenerate tissues and organs in health and disease. Research approaches include developing biomarkers to detect and classify stem cells and progenitor cells; profiling the cells to catalogue genes that are active; and creating tools for characterizing the functions of these genes. This research will capitalize on the sequence data from the Human Genome Project. It is also a goal to distribute to the broad research community well-characterized cells, DNA, and specific tools for progenitor cell analysis developed by the GAPs. Furthermore, the development of bioinformatics systems, including databases, will ensure that data produced are available to researchers worldwide

soon after being generated in the laboratories. Multiple Progenitor Cell GAPs are supporting research to identify and describe stem cells located within specific tissues of the gastrointestinal lining, liver, pancreas, kidney, urinary tract, prostate, and bladder. Hematopoietic Cell Lineage GAPs are supporting work to describe gene expression in bone marrow-derived stem cells. Another NIDDK effort will sponsor studies to describe normal development and stem cells of the gastrointestinal tract, liver, and exocrine pancreas. The NIDDK hopes that knowledge gained from these studies will enable medical doctors to use stem cells and developmentally-regulated genes to repair and replace damaged and diseased tissue.

Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM and Scadden DT: Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425: 841-6, 2003.

Ianus A, Holz GG, Theise ND, and Hussain MA: In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 111: 843-50, 2003.

Vassilopoulos G, Wang PR, and Russell DW: Transplanted bone marrow regenerates liver by cell fusion. *Nature* 422: 901-4, 2003.

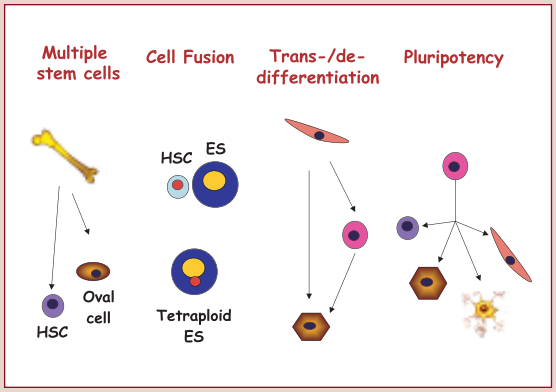
Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, and Grompe M: Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 422: 897-901, 2003.

Stem Cells: Promise and Reality

Dr. Catherine Verfaillie

The NIDDK National Advisory Council meets three times annually to provide advice to the Institute regarding its research portfolio and broad issues of science policy. These meetings are also an opportunity for the Council members to learn about recent scientific advances in different fields through presentations from NIDDK-supported extramural scientists. In 2003, the Council and NIDDK staff were privileged to hear from two leading scientists, Dr. Catherine Verfaillie and Dr. David Altshuler, who are conducting research studies in stem cell biology and human genetic variation in disease, respectively. The "Scientific Presentations" in this chapter are meant to capture the essence of their talks.

Catherine Verfaillie, M.D., is Professor of Medicine and Director of the Stem Cell Institute at the University of Minnesota. She received her M.D. from the Catholic University of Leuven in Belgium in 1982, and came to the University of Minnesota 1987 after an internal medicine residency and fellowship in hematology in Belgium. Dr. Verfaillie's recent research has focused on the plasticity of stem cells, the mechanisms controlling their differentiation, and evaluation of their therapeutic potential.



The recently observed capacity of adult stem cells from one tissue or organ to become cells from an unrelated tissue or organ may depend upon one or more different, biologically relevant pathways. Scientists are pursuing research to sort out the basis for this characteristic of stem cells, which is known as "plasticity."

Dr. Verfaillie explained that many researchers share the hope of one day treating diseases through cell-based approaches. To this end, scientists are exploring the capability of undifferentiated progenitor cells, called stem cells, to be induced experimentally to become specialized cells of the body. While stem cells are in the spotlight of research today, as recently as six years ago, scientists who worked on stem cells toiled in relative obscurity. This changed radically in 1998, when human embryonic stem cells were first identified. Furthermore, evidence accumulated since that time, in over 300 scientific publications, suggests that adult stem cells may have greater differentiation potential than previously thought, and that hematopoietic cells of the bone marrow might have the ability to differentiate into other cell types than blood cells. The field of stem cell biology moved to the forefront of scientific discussions, as well as discussions in the lay press. Although promising, this field requires extensive fundamental studies to understand how stem cells function and react to their environment, an exciting pursuit that will enlighten scientists about the steps stem cells take as they mature and commit themselves to different developmental fates whereby they become the myriad tissues and organs in the body.

SCIENTIFIC PRESENTATION

Stem Cell Basics

Stem cells have three fundamental properties: (1) they undergo self-renewing cell divisions; (2) they can differentiate into multiple different functional cell types; and (3) when administered to a human or an animal, they can functionally reconstitute an organ or tissue that has been destroyed.

Scientists are studying several types of stem cells. Adult stem cells are rare populations of undifferentiated cells found in the tissues of adult animals and humans. Research has shown that adult bone marrow cells, which feed the body's circulatory and immune systems, may be a good source of these cells. Another type of stem cells, called embryonic stem (ES) cells, may also be derived from either animal or human tissue.*

Because research on all of these types of cells is still in its early stages, scientists cannot now predict which of them may prove most effective and appropriate for therapeutic purposes. ES cells may turn out to be a better cell source for certain differentiated cell types, while adult stem cells might be a better source for other cell types. Research on both cell types, however, will have a synergistic effect in advancing knowledge in the field, and scientists are working diligently to characterize these cells and understand their workings.

There has been recent excitement about ES cells because they can differentiate into all of the body's cell types and maintain differentiation capacity when grown in the laboratory for long periods of time. Thus, ES cells might constitute an unlimited source of cells for a wide array of therapies. However, several difficulties are associated with potential clinical use of human ES cells. These cells would be obtained from a donor and might thus be rejected as foreign by the patient's immune system; the cells could form tumors (teratomas); and the generation of these cells from blastocysts (early embryos) is a controversial procedure.

Adult stem cells are already being used for therapies such as bone marrow transplantation. There are several positive aspects to using adult stem cells. One is that they can be obtained directly from the patient, eliminating, for some potential therapeutic applications, the problems associated with unrelated-donor-cell rejection. Another is that, because adults, not embryos, are the source of the cells, the research is not controversial. There also is growing evidence that adult stem cells appear to be more potent, or have greater "plasticity," than was previously believed. Numerous recent reports indicate that adult stem cells from one tissue can differentiate into specific cell types normally found in other tissues. For example, when bone marrow cells are injected into the hearts of animals damaged by a heart attack, these cells appear to differentiate into cells with the characteristics of heart muscle cells. Finally, findings suggest that bone marrow stem cells may be found in the pancreas, liver, and nervous system, kidneys, and other organs.

Potency of Adult Stem Cells

Dr. Verfaillie's research interest in certain components of the bone marrow led her to initiate studies on cells called mesenchymal stem cells. At the beginning, the goal was to purify such cells from patients with one of the inborn errors of metabolism–muccopolysaccharoidosis type 1, a lysosomal storage disease–in order to genetically-modify these cells for use in therapy for this disorder. However, in the course of these studies, her group identified so-called multipotent adult progenitor cells–opening up a new research avenue. Initially, the question was asked: Could adult mesenchymal cells within the bone marrow differentiate into other cell types? It was found that mesenchymal cells indeed had a much greater differentiation potential than one might expect. In addition to forming bone, cartilage, fat, and muscle cells, mesenchymal cells grown in the laboratory under the right conditions could be directed to form both liver cells and nerve cells. This discovery is significant because liver

and nerve cells normally arise from two different tissue layers during embryonic development, the embryonic endoderm and ectoderm, respectively, while mesenchymal cells arise from a third distinct layer, the mesoderm. Moreover, when adult mouse mesenchymal stem cells were placed into mouse blastocyst embryos, they were found to contribute to the subsequent development of every organ in the body. Because these adult mesenchymal stem cells were shown to be highly potent, possibly even as potent as ES cells (which are "pluripotent"), they were termed multipotent adult progenitor cells (MAPCs).

Many interesting questions arise from these studies, including, how can adult stem cells be endowed with properties of early embryonic cells, i.e., with the plasticity of becoming cells that normally would derive from endoderm, mesoderm, or ectoderm? Possible answers to questions regarding plasticity are that multiple tissue-specific stem cells are present in different organs; that this phenomenon is actually the result of fusion of the donor cell with resident cells in an organ; that cells undergo de-differentiation and re-differentiation; and, finally, that true multi- or pluripotent stem cells do persist in postnatal life. There is at least some scientific evidence supporting each of these theories. Thus, scientists need to continue carefully characterizing the mechanisms underlying apparent adult stem cell contributions to multiple or unexpected cell types in their studies, in order to truly understand the nature of plasticity.

Uses of Stem Cells–Basic Knowledge, Therapy, and Bioengineering

Dr Verfaillie stressed that one of the most important things that can be learned from stem cells is to understand the basic processes of self-renewal and differentiation of these cells in different tissues. This knowledge ultimately could lead to drug discovery, much in the same way that understanding stem cell biology and the hematopoietic system formed the basis for development of blood cell growth factors for therapeutic purposes. When stem cells in other tissues are better understood, pharmacologic agents–instead of cell replacement–may be used to therapeutically repair damage in the body.

For example, MAPCs that were experimentally induced to form cartilage and bone cells were studied using gene array technology. As expected, the lineage of these cells types was found to be very similar. However, there were a number of factors controlling gene expression (transcription factors) that were expressed differently by these two cell populations. These differences might help point to the gene switches that direct cells to become bone or cartilage. Thus, stem cells and their differentiated progeny may be used to define genetic programs that need to be activated or inactivated for cell differentiation to occur.

Clinically, ongoing studies are further characterizing MAPCs and developing them for use in therapy for a wide array of conditions–including genetic diseases, such as muscular dystrophy and lysosomal storage diseases, and degenerative diseases and disorders in which tissue repair is required, such as diabetes, Parkinson's disease, arthritis, heart muscle damage from a heart attack, and liver failure. Progress is already being made with regard to central nervous system abnormalities, diabetes, and liver disease. For instance, one of the best examples of what embryonic stem cells can do therapeutically has come out of research on brain disease at the NIH. Researchers created a rodent model with a Parkinson's disease-like deficit by destroying dopamine-producing (dopaminergic) neurons in rat brains. The researchers then injected mouse embryonic stem cells that had been induced to differentiate into dopaminergic-like neurons *in vitro* into the brains of these rats. They observed functional improvement of the rats, and showed by structural and electrophysiological analysis that the implanted ES-derived cells had repaired the brain defect.

In another series of experiments studying the potential of stem cells to correct central nervous system abnormalities, human MAPCs were used to repair brain damage in an animal model of stroke. In this case, the stem cells themselves were not directly responsible for the repair, but had acted by an as-yet unknown mechanism to minimize stroke damage, repair the blood vessel structures in the brain, or possibly recruit stem cells from the brain to go to the damage zone.

For type 1 diabetes, there is some evidence in animals to suggest that ES cells may be able to differentiate into insulin-producing beta cells. Other cells that might be sources for beta cells come from the pancreas itself, and from liver cells that have had transcription factors introduced that are important for beta cell development. In liver disease, a recent example of research on the potential of stem cells for correcting defects comes from studies in which hematopoietic stem cells have been shown to correct the liver disease, hereditary tyrosinemia, in animals. Notably, correction of this defect appears to have resulted from fusion of the healthy donor stem cells with diseased liver cells to produce functional hybrid cells–a phenomenon which requires further study to understand its implications for therapy development.

Finally, researchers foresee the use of stem cells to produce pieces of organs or whole organs–first relatively simple structures such as arteries or heart valves, then more complex organs such as the liver and kidney. This future is moving closer. At the University of Minnesota, muscle cells created from MAPCs have been used to engineer smooth muscle layers that have mechanical characteristics similarto newborn rat aortic smooth muscle cells. By co-culturing the engineered and aortic cells, a structure is produced that looks and functions like a normal artery.

Future Research Directions

Dr. Verfaillie emphasized that stem cell research has made significant progress over the past 6 years in expanding knowledge about developmental cell pathways and cell plasticity, and inspiring new concepts for treating disease. While stem cells hold the promise of new and improved therapies, she believes that clinical trials testing these approaches are at least 5 to 10 years away. Much additional research is still needed.

The NIDDK is continuing progress toward the twin goals of understanding the fundamental processes underlying stem cell development and applying that knowledge for clinical use and benefit. Large-scale research efforts include the Beta Cell Biology Consortium, which is supporting a number of stem cell research efforts with the ultimate goal of developing a cell-based therapy for insulin delivery to cure diabetes. The Institute is also supporting a number of Progenitor Cell Genome Anatomy Projects. These GAPs are conducting research on organ- and tissue-specific stem cells that includes their identification and isolation, characterization of their gene expression patterns during tissue development, and development of better tools for characterizing the functions of these genes. The NIDDK is also participating in the NIH-wide Stem Cell Task Force that is, in consultation with external scientists, developing strategies and initiatives to enhance scientific research and resources in this exciting and promising field.

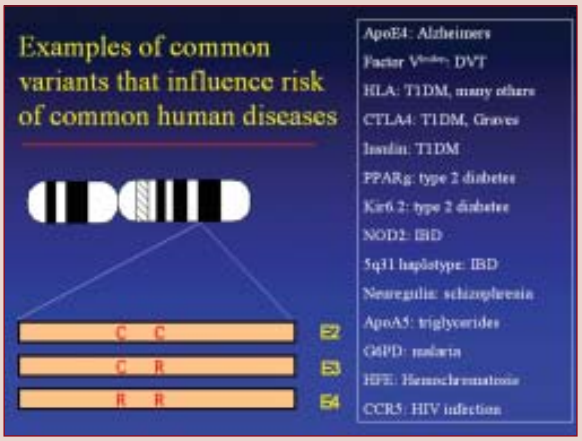
---

* Consistent with the policy announced by President George W. Bush on August 9, 2001, NIH funding of research involving human embryonic stem cells is in accordance with specific criteria established by the Administration.

Human Genome Variation and the Genetics of Common Human Disease

Dr. David Altshuler

David M. Altshuler, M.D., Ph.D., is Assistant Professor of Genetics and of Medicine at Harvard Medical School, and a member of the Department of Molecular Biology and Attending Physician in the Diabetes Unit at Massachusetts General Hospital (MGH). He is also Director of Medical and Population Genetics at the Whitehead Institute/MIT Center for Genome Research, and an Affiliate Member of the Whitehead Institute for Biomedical Research. He received his M.D. and Ph.D. from Harvard Medical School, and completed clinical training in Internal Medicine and Endocrinology at Massachusetts General Hospital. Dr. Altshuler's research focuses on the genetic basis of common diseases.



In the human population, genetic variation contributes to individual differences in susceptibility to common, complex diseases.

Identical twins excepted, everyone looks different. The scientific term for an organism's physical appearance is "phenotype," and there is a great deal of phenotypic variation among the human population. Some people are tall, some short, some have black hair, some red, and so on. These differences in phenotype are caused by subtle variations in people's genetic makeup–their "genotype." And, just as genetic differences can give rise to different appearances, they can also predispose some people to disease. From a scientific perspective, a central question is, to what extent do underlying genetic differences contribute to the common diseases?

Consider two people: next-door neighbors who live in the same environment. If one develops type 2 diabetes, what are the chances that the other will, too? If they are truly unrelated, that chance is around 5 to 10 percent. But, if they are siblings, the risk rises to about 30 percent. If they are identical twins–two people who share the same genotype–the risk rises to 80 to 90 percent. Clearly, then, genetic factors play an important role in the development of this disease. The challenge facing researchers is to identify which genetic elements are important.

For rare genetic diseases, especially those caused by a mutation in a single gene, researchers study families in which the disease regularly occurs. By correlating the appearance of the disease with the inheritance of specific chromosomal regions, scientists can narrow down the stretch of DNA that is likely to carry the responsible mutation. For common diseases that have a genetic component, this approach has not worked particularly well, because these diseases

result from the inheritance of multiple genetic variants, each of which may have only a subtle influence on the overall risk. It is much harder to find these subtle effects than the dramatic influences seen in diseases such as cystic fibrosis and Huntington's disease. For common conditions, scientists have to look at a large population of affected individuals and compare them with a large population of unaffected ones, and try to determine which genetic elements are associated in a more subtle way with development of the disease. Such studies are called "association studies," and are much more difficult to carry out and interpret than studies of diseases caused by a mutation in a single gene.

Genetic Variation, Environment, and Disease

Dr. Altshuler pointed out that it may seem counterintuitive to think that a widespread, common set of genetic variations could predispose a large number of people to a common disease. After all, one would think that mutations that cause people to get sick would be eliminated from the population by natural selection over many generations. Natural selection is the process by which minor genetic variations cause organisms to be more or less fit for their environments. Those who are more fit survive, and pass on their genes to their offspring. Those who are less fit do not pass on their genes, and these mutations are eliminated from the population. So, how could mutations that cause relatively common diseases today have persisted in the human genome for so long? The answer lies in the observation that mutations can be either beneficial or harmful in the context of the organism's environment. Thus, mutations that conferred a benefit in the past may no longer be beneficial in the modern environment.

One example of this sort of phenomenon is the "thrifty gene" hypothesis. For most of human history, people struggled to find enough to eat. Scientists have hypothesized that individuals who were able to accumulate fat might have had an advantage, because they were more likely to stockpile energy when food was plentiful and were therefore more likely to survive when food was scarce. However, the environment has changed dramatically in the last few hundred years, much too quickly for our genes to evolve to match it. The ability to accumulate fat is no longer beneficial in an environment in which calorie-rich food is plentiful and life is often sedentary. Obesity is emerging as one of the most important public health issues, as more and more people become overweight and develop diseases associated with obesity, such as type 2 diabetes and cardiovascular disease.

Human Genetic Variation

Dr. Altshuler noted that, to understand what exactly is meant by the term "genetic variation," it is helpful to think of it at the level of DNA. Chromosomes are made up of long stretches of DNA, which is composed of a double-stranded, linear sequence of nucleotides: adenosine (A), cytidine (C), guanosine (G), and thymidine (T). Geneticists have traditionally focused on mutations that alter the protein encoded by a gene. Scientists now appreciate that DNA sequences that do not code for proteins–which make up about 99 percent of our DNA–are also a potentially important source of genetic variation.

For long stretches, a given DNA sequence may be identical in two different people. However, every so often, one (or more) of the nucleotides differs. Such a site is called a "single nucleotide polymorphism"

or SNP. SNPs appear in the human genome roughly once every 1,000 nucleotides and a given SNP may be quite widespread. For example, in 60 percent of the population, a given chromosome may have an A at a certain position, while the other 40 percent of the population may have a G instead. These two forms–A and G–are called alleles, or variants, of that SNP. At some point in the distant past, there was a single form, but a mutation altered the base, and this mutation was passed down and spread throughout the population. "Haplotype" is the term used to describe a set of SNP alleles along a region of a chromosome. Theoretically, there could be many haplotypes for a chromosomal region, but studies typically find relatively few, common haplotypes in the entire human population.

One of the ways scientists used haplotypes is as a kind of molecular "signature" for a specific stretch of DNA. To examine large stretches of DNA comprehensively, scientists use a technique called "haplotype mapping." As its name implies, haplotype mapping uses a series of SNPs as markers to identify a stretch of DNA. Because all people today are descended from the same small population of humans that lived long ago, it is often the case that the same haplotype will be seen at a high frequency in an apparently unrelated set of individuals. When such a particular set of SNPs is seen more frequently in people with a disease than in those without it, those SNPs and their alleles are said to be associated with the disease. This finding suggests that there may be genes in that chromosomal region that contribute to developing the disease. Thus, haplotype mapping is a powerful and useful tool for narrowing down regions of DNA that might contain genes associated with common diseases. Scientists have used haplotype mapping to identify candidate genes that contribute to diseases such as Crohn's disease (an inflammatory bowel disease) and type 2 diabetes.

Common Genetic Variation, Gene Expression, and Disease

Dr. Altshuler described a complementary approach to looking for genes that contribute to disease, which is to examine genes, not one at a time, but in large sets of genes known collectively to contribute to a given biological system–for example, the mitrochondrion, the cell's energy "factory." Studies such as these focus, not on genetic variation at the DNA level, but on changes in which genes are turned on or off at a particular time or under certain conditions. By looking at changes in the expression levels of many genes at once, so-called "expression profiling," researchers can get a better view of the "big picture," and detect large-scale changes that might be missed in studies that focus on changes in a single gene.

Dr. Altshuler and his colleagues have recently used expression profiling in a small study of people with type 2 diabetes, pre-diabetes, and a control group with neither condition. They used DNA microarrays to measure gene expression of over 22,000 genes in these three groups. The researchers found that people with diabetes had, in general, lower expression of genes involved in metabolism in the mitochondria, the cell's energy centers. Out of 106 genes previously known to be involved in the chemical process known as oxidative phosphorylation, expression of 94 was decreased people with diabetes. Interestingly, these changes were modest on a gene-by-gene basis, but overall, the result was unmistakable: this important metabolic pathway was less active in people with diabetes. When the researchers looked closely at the genes affected, many of them were found to be influenced by the regulatory protein peroxisome proliferator activator protein-gamma co-activator-1 alpha. Thus, it might be that a subtle alteration of this single gene has a more dramatic effect, as it cascades down through the series of genes it controls.

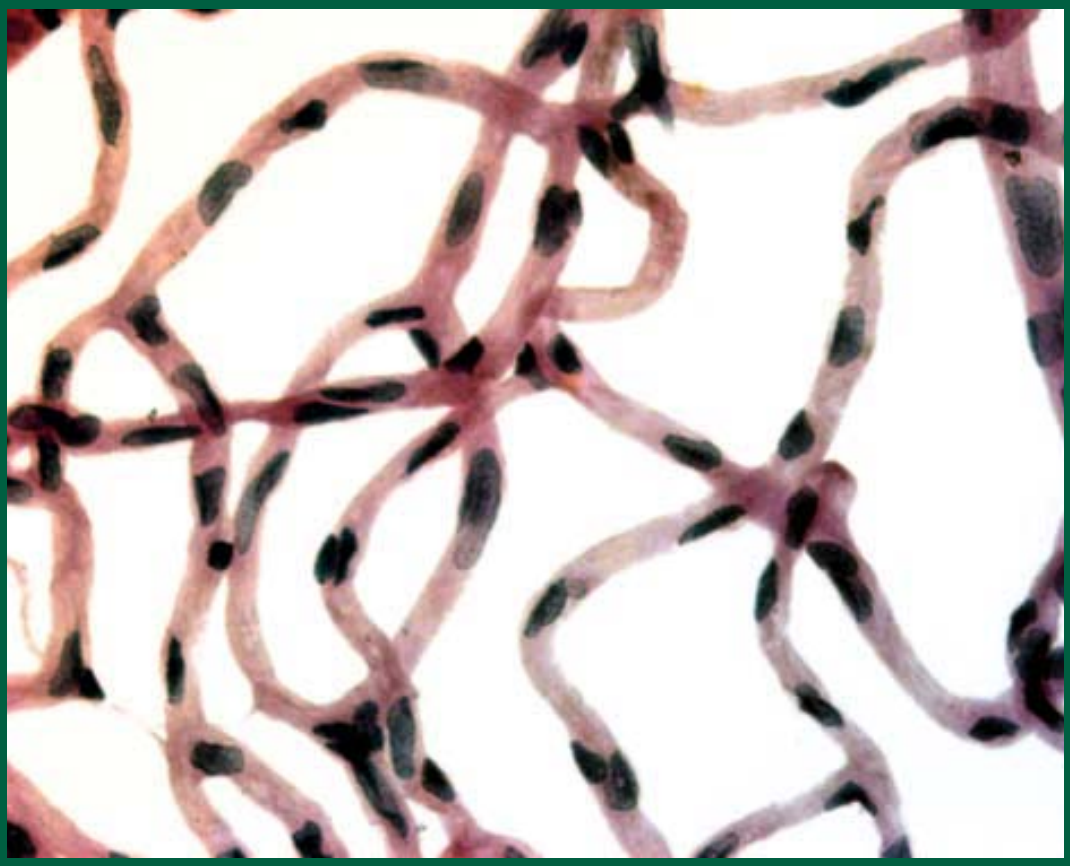
Although a small study, this investigation is significant because it demonstrates that it may be necessary to examine networks of genes in order to detect changes in activity. Studies of change in a single gene are practical when the change in that gene's expression is relatively large and overall expression patterns vary little from one person to another, but expression profiling permits researchers to examine subtle changes in gene expression patterns on a higher level. Such strategies ultimately may be necessary to detect small changes in pathways, which may ultimately turn out to be a defining feature of common, complex genetic disorders, such as type 2 diabetes.

The Future

One possible implication of the emerging appreciation for common genetic variation is how it could alter current approaches to disease treatment. For example, the knowledge that certain combinations of haplotypes might place a person a risk of developing a particular disease could influence treatment. Similarly, if people with one haplotype are less responsive to a particular treatment than others, it would be possible to give them alternative therapies from the outset. While such knowledge is years away, through studies of genetic variation and interaction with the environment, the possibility exists to "personalize" medicine through therapies tailored to a particular individual.

The NIDDK, along with multiple other NIH Institutes and Centers, is sponsoring an initiative entitled "Large-Scale Genotyping for the Haplotype Map of the Human Genome." This initiative will develop a map of the haplotype patterns and of the genetic variants that are most informative for detecting these patterns. The haplotype map is expected to be a key resource for finding genes affecting health, disease, and response to drugs and environmental factors, and for beginning to understand the pattern of human genetic variation.

Also, the NIDDK is sponsoring a Diabetes Genome Anatomy Project (DGAP). This overall goal of this project is to identify the genes and gene sets, as well as the proteins, involved in insulin action and the predisposition to type 2 diabetes. The DGAP is also examining secondary changes in gene expression that occur in response to the metabolic abnormalities present in diabetes. The DGAP will define the "normal" patterns of gene expression and response to insulin, the impact of diabetes on gene expression patterns and response to insulin, and the extent to which genetic variability might contribute to the alterations in expression or to the development of diabetes. This project, and the resultant database, will aid investigators as they strive to unravel the complexity of insulin action and its perturbation in diabetes, and ultimately will help develop more effective and specific modes for studying and treating the disease.



Healthy-looking blood vessels from the eyes of diabetic rats treated with benfotiamine, a B-vitamin related molecule. The therapeutic potential of this molecule represents one promising research advance toward the prevention of vascular complications of diabetes–including diabetic eye disease. Photo: Dr. Xuliang Du and Dr. Michael Brownlee, Diabetes Research Center, Albert Einstein College of Medicine, Bronx, NY. Reprinted with permission from Hammes HP *et al, Nat Med* 9: 294-9, 2003. © 2003 Nature Publishing Group (http://www.nature.com).

Diabetes, Endocrinology and Metabolic Diseases

NIDDK support of basic and clinical research in the area 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 18.2 million people in the U.S.–over 6 percent of the total population–and is the sixth leading cause of death. The number of people with diabetes continues to rise–only a year ago, estimates of the number of persons with diabetes stood at 17 million. Diabetes lowers average life expectancy by up to 15 years, increases cardiovascular disease risk two-to-four-fold, and is the leading cause of kidney failure, lower limb amputations, and adult-onset blindness. In addition to these human costs, the estimated total financial cost for diabetes in the U.S. in 2002–including costs of medical care, disability, and premature death–was $132 billion. Effective therapy can prevent or delay these complications, but one third of Americans with diabetes are undiagnosed. This has spurred the Department of Health and Human Services to launch the Secretary's "Diabetes Detection Initiative: Finding the Undiagnosed." The goal for this community-based effort is to help identify the several million Americans with undiagnosed diabetes, as well as those at high risk for the disease, and to refer them for follow-up testing as appropriate.

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 completely loses its ability to produce insulin; and type 2 diabetes, in which less insulin than needed is produced, and the body becomes resistant to its signals.

Type 1 diabetes affects approximately 5 to 10 percent of individuals with diagnosed diabetes. It most often occurs in children, but may appear at any age. Type 1 diabetes is an autoimmune disease, in which the immune system mistakenly attacks and destroys the beta cells of the pancreas. These beta cells, which are found within tiny cell clusters called islets, are the body's sole producers of 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 would 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 meant to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for up to 95 percent of diabetes cases in the U.S. 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 people with type 2 diabetes are overweight or obese. Type 2 diabetes occurs more frequently among minority groups, including African Americans, Hispanic Americans, Native Americans, and Native Hawaiians. 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 less insulin, and the timing of insulin secretion becomes abnormal. To control glucose levels, treatment approaches include diet, exercise, and medications; some patients also need to take insulin. There are also millions of individuals who have a condition called "pre-diabetes," in which blood sugar levels are higher than normal, but not as high as in diabetes. This population is at high risk of developing diabetes. Fortunately, the Diabetes Prevention Program (DPP) clinical trial has shown that patients 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, due to an increase in overweight in children, this form of diabetes is increasingly being diagnosed in children and adolescents, and it disproportionately affects minority youth. 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 sugar 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 vigorously pursuing research to understand the mechanisms that lead to the development of diabetes and its complications, as well as ways to prevent, treat, and cure the disease. This research is being propelled by emerging technological advances that enable scientists to gather a large amount of data in a short period of time. The impact of new technology is underscored by the recent use of high-throughput DNA sequencing to complete the Human Genome Project. Other novel experimental approaches–such as tools to assess changes in gene and protein expression in organs and tissues affected by diabetes and use of molecular libraries of thousands of compounds to identify potential therapeutic agents–are powerful tools to study disease rapidly. This will speed progress toward understanding, and ultimately curing, diabetes and many other metabolic and endocrine diseases within the NIDDK mission.

ADVANCES IN PREVENTING VASCULAR COMPLICATIONS OF DIABETES

Organs and tissues throughout the body are fed and cleansed by the blood *via* a complex network of capillaries, small blood vessels, and major veins and arteries. Compared with the rest of the population, patients with type 1 or type 2 diabetes have an increased risk for developing injury to these small and large blood vessels. This vascular damage can lead to serious health complications. Injury to the small blood vessels, or microvasculature, can cause blindness, kidney failure, and nerve damage, while injury to the large blood vessels, or macrovasculature, can lead to cardiovascular disease and stroke. Heart disease is the leading cause of death in persons with diabetes. Studies have shown that sustained high blood glucose (sugar) levels, or hyperglycemia, are a major factor in the development of many of

these complications. Researchers are building upon both fundamental investigations of how glucose damages blood vessels, and clinical investigations of its effects on health, to devise strategies to prevent or intervene in diabetes-induced vascular damage.

Reduction in Atherosclerosis from Intensive Blood Glucose Control: In one recent advance, investigators from the Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated an important link between blood glucose levels and macrovascular injury. The EDIC is a follow-up study to the landmark Diabetes Control and Complications Trial (DCCT), a large-scale multi-center clinical trial that demonstrated that tight control of blood glucose levels through intensive therapy can reduce the risk of developing small blood vessel complications causing kidney, eye and nerve damage in type 1 diabetes patients. The EDIC study continues to demonstrate enduring benefits of intensive therapy in these patients nearly a decade after the trial ended–most recently, its impact on the blood vessels that supply the heart and brain. (Please see the "Story of Discovery," "Preventing or Delaying Complications Of Diabetes: 20 Years of Study by the DCCT/EDIC Research Group.") While the DCCT proved that glucose control could prevent or delay small vessel damage, controversy remained about the effect of high glucose on the large vessels damaged in cardiovascular disease. Now, results from EDIC have shown that, in contrast to patients on standard therapy, patients who received intensive therapy in the DCCT developed less thickening of the wall of the carotid artery–the artery on which the brain depends for blood flow. Thickening of this artery is an important measurement of atherosclerosis. Reduced calcification, another marker of vascular damage, was also seen in the coronary arteries of the intensively treated group. These significant results demonstrate the importance of strict blood glucose control in preventing damage to large blood vessels, as well as small vessels.

Medicating the Microvasculature: Researchers are also gaining ground in pioneering potential treatments to combat vascular damage. In a recent study, scientists tested the ability of a vitamin-B related molecule, called "benfotiamine," to stop the adverse effects of high blood sugar levels on microvascular complications. Exploiting accrued knowledge about the biochemical pathways that are important in the development of glucose-induced vascular complications, the group hypothesized that benfotiamine could simultaneously block several of these pathways. They found that this was in fact the case, through experiments in both a cell culture model system and in retinas taken from diabetic rats. But was there an impact of this treatment on the development of disease? When the researchers directly tested the effect of benfotiamine in diabetic rats, they found that treated rats did not develop diabetic eye damage. In contrast, untreated rats developed the disease. These promising results in an animal model of diabetes provide an important first step in determining its therapeutic usefulness of benfotiamine for humans. If the results can be replicated in humans, this research could lead to a potential therapeutic agent that may enable diabetes patients to prevent development of diabetic eye disease and possibly other vascular complications.

The NIDDK is supporting numerous basic and clinical investigations like these to address the causes of, prevention of, and interventions for diabetes-associated vascular complications. The Institute is also currently collaborating with the National Heart, Lung, and Blood Institute to support the clinical study, Action to Control Cardiovascular Risk in Diabetes (ACCORD). The goal of ACCORD is to test the best approaches to lowering the risk of heart disease and stroke in adults with type 2 diabetes. ACCORD will compare the effect on cardiovascular outcomes of intensive or standard* blood sugar control, in combination with either aggressive control of blood pressure or blood fats. Moreover, to foster promising research on diabetes complications

* The current "standard" is based upon the more intensive recommendations from the DCCT and a clinical trial of blood sugar control in type 2 diabetes, the UKPDS.

more effectively, the NIDDK recently established a Working Group for Diabetes Complications (see also the "Kidney, Urologic, and Hematologic Diseases" chapter). The goals for this group are to provide seamless integration of NIDDK activities related to complications, including workshops, initiative planning and oversight of existing projects and trials; to establish liaisons with other Institutes and to develop activities that will increase interest in diabetes complications in other scientific communities; and to lead future strategic planning activities on diabetes complications. Through all of these efforts, the NIDDK seeks to ensure continued progress in research leading toward improved clinical management of diabetes complications.

Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I and Brownlee M: Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9: 294-9, 2003.

Nathan DM, Lachin J, Cleary P, Orchard T, Brillon DJ, Backlund JY, O'Leary DH, and Genuth S: Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N Engl J Med* 348: 2294-303, 2003.

CONTINUED INSIGHTS FROM THE DIABETES PREVENTION PROGRAM: SUSTAINED BENEFITS OF METFORMIN

As demonstrated by the EDIC follow-up to the DCCT clinical trial, investment in well-designed, large-scale clinical trials can yield important scientific fruits for many years. Similarly, a wealth of insight into the factors influencing onset of type 2 diabetes is continuing to arise from ancillary and follow-up studies to the Diabetes Prevention Program. The Diabetes Prevention Program (DPP) was a clinical trial that aimed to determine the relative effectiveness of drug or lifestyle modification in delaying or preventing the development of type 2 diabetes in an at-risk population. Over 3,000 people participated in the DPP, 45 percent of whom were from racial and ethnic minorities disproportionately affected by type 2 diabetes, and 68 percent of whom were women. The DPP reported that the incidence of diabetes in individuals with impaired glucose tolerance (or "pre-diabetes") could be reduced by 58 percent with intensive lifestyle modifications, and by 31 percent with metformin, an insulin-sensitizing drug, compared with standard medical advice and placebo.

Following the DPP, researchers examined participants who had received metformin or placebo and had not developed diabetes during the trial to learn more about how the drug worked to prevent diabetes. The goal was to determine whether the drug therapy had masked the development of diabetes, and its benefit would disappear if the medication was withdrawn, or if the effect were more lasting, and would persist after cessation of therapy. Patients were instructed to discontinue their medication–either placebo or metformin–for one to two weeks prior to further evaluation. After this "washout" period, the patients received an oral glucose tolerance test, a test that would reveal whether they had diabetes. Statistical analysis revealed that about one quarter of the 31 percent reduction in diabetes incidence seen with metformin therapy in the DPP was attributable to effects of the drug that do not persist when it is withdrawn. However, even after the washout, the incidence of diabetes was reduced by 25 percent in the metformin group. Thus, this study demonstrated that metformin therapy does provide some benefits that can persist after short term drug withdrawal. These observations are important for determining the role of metformin in the clinical management of pre-diabetes.

Extending the value of this clinical trial, several other components of the NIH and the Centers for Disease Control and Prevention (CDC) have joined the NIDDK in supporting a long-term follow-up study to the DPP, called the DPP Outcomes Study (DPPOS). The goal of the DPPOS is to determine the durability over time of the effect of the original

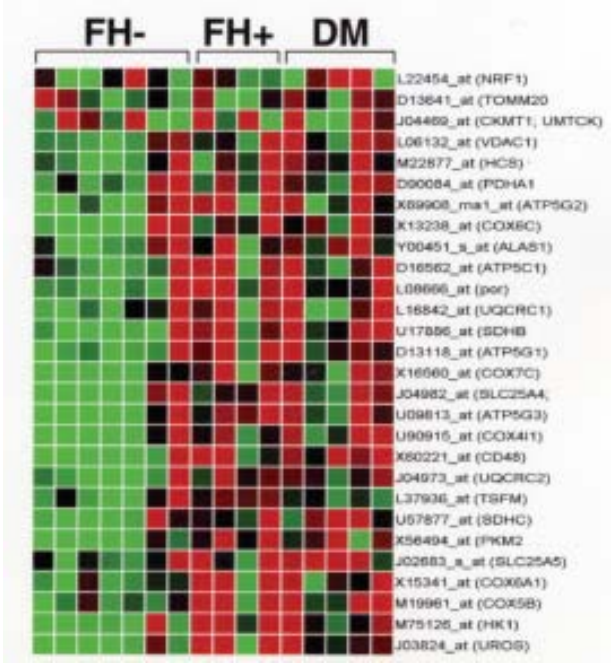
DPP interventions on onset of type 2 diabetes and their effects on diabetes-associated complications–particularly cardiovascular disease–in members of the large, diverse, and well-characterized cohort of DPP participants. It is hoped that results from the DPPOS will ultimately help improve clinical strategies to prevent the onset and/or progression of type 2 diabetes and diabetes complications in the millions of Americans already at-risk.

The Diabetes Prevention Program Research Group: Effects of withdrawal from metformin on the development of diabetes in the Diabetes Prevention Program. *Diabetes Care* 26: 977-80, 2003.

NOVEL USE OF MICROARRAY TECHNOLOGY FOR IDENTIFYING GENES INVOLVED IN TYPE 2 DIABETES

Both genetic and environmental factors contribute to the development of type 2 diabetes. The genetic contribution is likely due to multiple "susceptibility genes," each of which modestly increases risk. Because multiple genes are likely to be involved type 2 diabetes, scientists have used a powerful and efficient research tool, called "gene microarray technology (GMT)," or simply "microarrays," to identify genes that may contribute to disease development. GMT is a method for rapidly analyzing the expression of thousands of genes. It enables researchers to readily compare differences in gene expression in healthy and diseased tissues and cells.

However, although GMT is a powerful survey tool for finding individual genes whose expression changes in human disease, it is difficult to determine which of the modest changes in expression of multiple genes is important. Because there is already high variation in the expression of identical genes from person to person, it is statistically challenging–using the limited number of patient samples usually involved in these studies–to determine which small changes are potentially involved in a disease and which are due to normal population variation (see also the sidebar, "Human Genome Variation and the Genetics of Common Human Disease," in the



Gene microarray technology permits researchers to rapidly compare expression of multiple genes among different individuals. In this diagram, color indicates relative increases (green) or decreases (red) in gene expression. As shown, the expression of many genes involved in cellular energy production (listed at right) is relatively lower in muscle tissue from persons with a family history of diabetes (columns under FH+) or diabetes (DM) as compared to individuals with neither (FH-). Photo: Dr. Mary Elizabeth Patti, Research Division, Joslin Diabetes Center, Boston, MA. Reprinted with permission from Patti ME *et al, Proc Natl Acad Sci USA* 100:8466-71, 2003. © 2003 National Academy of Sciences, U.S.A.

"Cross-Cutting Science" chapter). Thus, researchers are developing novel approaches to capturing and analyzing microarray data that will help them discern when subtle changes are significant. These approaches are being developed by exploiting knowledge of the coordinated regulation of gene expression in discrete biological pathways and by enhancing statistical methods used to analyze the data from microarrays.

Two recent advances employed novel GMT approaches for finding genes that may be important in the development of type 2 diabetes. In one study, researchers investigated the expression of genes in skeletal muscle biopsies from healthy diabetic and non-diabetic individuals, both with and without a family history of diabetes. The expression of many genes was altered modestly in diabetic

patients and in those with a family history of diabetes. Using several special software programs, the researchers were able to uncover significant patterns of change in groups of genes that are related by function. Strikingly, when the scientists studied the resulting genes whose expression was decreased in diabetes, they found that many of them were part of a single pathway. This group of genes plays an integral role in a biological process involving the mitochondria–the power source of cell activity. The expression of this group of genes is regulated by a single master gene, *PGC-1*. Independently, another research team examined differences in skeletal muscle gene expression between diabetic and non-diabetic men using a new analytical strategy for GMT that they designed, called "Gene Set Enrichment Analysis." This team also found that genes regulated by PGC-1 were differentially expressed in the two groups.

These studies represent an ideal melding of basic knowledge of individual genes and gene pathways with high-throughput technologies to answer questions that neither could answer alone. They have not only described a novel approach that other researchers can use to identify additional type 2 diabetes susceptibility genes, but they have also provided insight into the pathogenesis of the disease. Obtaining new knowledge about genes and biological pathways that are important in disease development can be used to understand the underlying defects in diabetes as a basis for determining additional therapeutic targets for prevention or treatment. By demonstrating the importance of PGC-1, which may be a therapeutic target for type 2 diabetes, the researchers have paved the way to the discovery of other potential targets.

Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR and Mandarino LJ: Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of *PGC1* and *NRF1*. *Proc Natl Acad Sci USA* 100: 8466-71, 2003.

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D and Groop LC: PGC-1 alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267-73, 2003.

DUAL MECHANISM OF ACTION OF TYPE 2 DIABETES DRUG CANDIDATE

Insulin is critical for the body's use of blood glucose (sugar) as a cellular fuel. Problems in cellular processes involving insulin are apparent in type 2 diabetes patients, who are impaired in their ability both to produce and to respond to insulin. One result is that the liver produces too much glucose, sustained high levels of which lead to development of diabetic complications. Research has shown that an enzyme, called "glucokinase" (GK), is central to regulating glucose metabolism in the pancreas and the liver. Researchers implicated GK in diabetes when they showed that errors in the glucokinase gene were responsible for the development of a certain form of type 2 diabetes, called Maturity Onset of Diabetes in the Young (MODY). In addition, researchers have characterized the importance of GK in diabetes by genetically engineering mice to lack the GK gene in the liver and the pancreatic beta cells. Because of this central role of GK in glucose regulation and diabetes, researchers have considered GK as a possible therapeutic target for type 2 diabetes.

With this knowledge in hand, scientists hypothesized that a drug that would "activate," or increase the enzymatic activity of, normal GK would help to restore normal glucose levels in type 2 diabetes. Toward this goal, they screened 120,000 different drugs and identified a single one that activated GK. The drug dramatically and effectively restored normal glucose levels in diabetic mice. Importantly, the drug achieved this by a dual mechanism: it

stimulated insulin production by pancreatic beta cells and inhibited glucose production by the liver. This is the first identified therapeutic agent to have an effect on both insulin production and insulin action–two processes that are severely impaired in type 2 diabetes.

This study is a key example of how NIH-investment in basic research has directly enabled researchers to identify a therapeutic agent for type 2 diabetes. Only because of basic research on the importance of GK in regulating glucose levels in the pancreas and the liver, and also its role in MODY, could researchers hypothesize that therapeutically targeting this enzyme might be effective in treating diabetes. The novel dual mechanism of action of this drug, which attacks the defects in type 2 diabetes at both the level of the pancreas and the liver, makes this a promising potential treatment approach for type 2 diabetes in humans.

Grimsby J, Sarabu R, Corbett WL, Haynes NE, Bizzarro FT, Coffey JW, Guertin KR, Hilliard DW, Kester RF, Mahaney PE, Marcus L, Qi L, Spence CL, Tengi J, Magnuson MA, Chu CA, Dvorozniak MT, Matschinsky FM and Grippo JF: Allosteric activators of glucokinase: Potential role in diabetes therapy. *Science* 301: 370-3, 2003.

NEW INSIGHTS INTO ENDOCRINE PANCREAS GENE EXPRESSION

Historically, type 2 diabetes researchers have thought that the defect in the ability of beta cells to produce the proper amount of insulin, and the inability of muscle, fat, and liver to respond to the insulin–known as insulin resistance–are distinct problems. Now, however, there is evidence to suggest that the two defects may be related. A gene, called *Foxo1*, may be an important link.

Using genetically engineered mice, investigators showed that they can prevent diabetes in a mouse model of type 2 diabetes by reducing expression of the *Foxo1* gene product. Investigating how this might occur, they showed through several experiments that *Foxo1* was important in shutting off in beta cells the *Pdx1* gene–a gene that has been found to play an important role in promoting pancreatic cell development. The researchers also have evidence that *Foxo1* activity is regulated by insulin. Based upon these results and other research studies, they have proposed a scientific model in which, in the presence of functional *Foxo1*, there is no expression of the *Pdx1* gene and beta cells do not develop or proliferate; conversely, when insulin is present, insulin stops the *Foxo1* from working, and *Pdx1* is expressed–permitting development and/or proliferation of beta cells. Thus, if cells, including beta cells, become insulin resistant, then insulin can no longer inhibit *Foxo1* activity and enable beta cell development or proliferation. If this model is correct, *Foxo1* may link insulin action and beta cell development, making it a promising therapeutic target for promoting beta cell growth.

Understanding the underlying mechanisms of beta cell development is central to developing therapies for both type 1 and type 2 diabetes. For example, a major barrier to islet transplantation is a shortage of viable islets. If scientists uncover the mechanisms to turn progenitor cells into islet cells, then more cells for transplantation research can be made in the laboratory to help overcome the current shortage. This process will be enhanced by the PancChip 4.0, a microarray cDNA chip specific to the pancreas, which has been developed by researchers of the NIDDK-supported Endocrine Pancreas Consortium. This important research tool is widely accessible to diabetes researchers and will facilitate the identification of additional important genes in pancreatic cell development. Many of these genes may be promising therapeutic targets, just as *Foxo1* is a promising target for promoting beta cell growth. Greater knowledge of genes involved in endocrine pancreas development, coupled with improved tools to study them, will help researchers put together missing pieces of the puzzle of pancreatic cell growth and development.

Kaestner KH, Lee CS, Scearce LM, Brestelli JE, Arsenlis A, Le PP, Lantz KA, Crabtree J, Pizarro A, Mazzarelli J, Pinney D, Fischer S, Manduchi E, Stoeckert CJ, Jr., Gradwohl G, Clifton SW, Brown JR, Inoue H, Cras-Méneur C, and Permutt MA: Transcriptional program of the endocrine pancreas in mice and humans. *Diabetes* 52: 1604-1610, 2003.

Kitamura T, Nakae J, Kitamura Y, Kido Y, Biggs WH, III, Wright CV, White MF, Arden KC, and Accili D: The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic beta cell growth. *J Clin Invest* 110: 1839-47, 2002.

MODULATING AUTOIMMUNITY–IMPLICATIONS FOR TYPE 1 DIABETES

Type 1 diabetes is an autoimmune disease in which the patient's own immune system mistakenly attacks and destroys the beta cells of the pancreatic islets, the sole producers of insulin. To prevent autoimmune destruction of body cells, it is very important that the immune system distinguishes between "self," or one's own cells, and "non-self," or foreign matter. The body has a way to ensure this recognition happens, which involves destroying any immune cells that will react with "self" cells. A recent study showed that a protein, called AIRE, plays a role in this process. Researchers genetically engineered mice to lack AIRE, and found that those mice developed autoimmune disease. Furthermore, they found that AIRE turned on genes that had a role in the process of destroying immune cells that will react with "self" cells. Researchers can use these results as a basis to elucidate the processes that are important in development of human autoimmune diseases–including type 1 diabetes.

Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C and Mathis D: Projection of an immunological self shadow within the thymus by the Aire protein. *Science* 298: 1395-401, 2002.

REMOVING BARRIERS TO ISLET AND ORGAN TRANSPLANTATION

For patients with type 1 diabetes, replacing the destroyed beta cells through transplantation of fresh, undamaged islets that can restore normal insulin production offers the hope of a real cure. However, there are still significant barriers in pursuing this research avenue that could limit its widespread use in clinical application: (1) inadequate supplies of islets, and (2) limitations of current methods to prevent transplant rejection and recurrent autoimmunity. Barriers in the area of transplant rejection are present not only with islet transplantation, but also with organ transplantation in general. Transplant recipients are put on a strict regimen of immunosuppressive drugs–drugs that stop the immune system from working to its full potential–in order to prevent them from rejecting the transplant. However, these drugs can have severe side effects, and can lead to an increase in morbidity and mortality. Therefore, researchers are actively pursuing novel transplantation technologies and improved immunosuppressive therapeutic strategies both to increase organ acceptance and to decrease the adverse side effects of drug treatment.

Encapsulation–A Biological Cloak of Invisibility: Researchers investigating ways to prevent islet transplant rejection are making strides in their work with a technique known as "encapsulation." Encapsulation is used to coat cells with a material that makes them resistant to attack by the body's immune system, thereby obviating the need for immunosuppressive drugs. Encapsulation is challenging because the cell coating must permit exchange of signals, such as glucose and insulin, but block access of the immune system to the transplanted cells. Scientists have tried for years to improve the composition of the coating to achieve these goals. Recently, scientists used a modified encapsulation method to coat immature pig islets. They then transplanted the islets into diabetic mice to determine whether they would mature, grow and start to produce insulin. The encapsulated pig islets were not destroyed by the mouse's immune

system, for up to 20 weeks after transplantation (when the experiment was ended). The islets were able to function properly and produce insulin.

Modeling Mice into Men: New insights are emerging from the observation that, although methods to prevent transplant rejection may work very well in rodent models, the same methods do not work well in non-human primates or in humans. Researchers have found that the ability of the immune system to accept or reject a transplanted organ depended on immune cells that developed because of prior exposure to viruses. They tested mice that had either been virus-free or infected with viruses before transplantation. The mice treated with viruses better mimic a human, because humans have been exposed to many viruses and bacteria during their lifetimes. Both sets of mice were then given immunosuppressive drugs. The mice infected with the viruses were more likely to reject their transplants. The researchers identified the cells in the immune system that were causing this transplant rejection in the virally-infected mice, and they used a drug, called DSG, to stop the immune cells from working. Treatment with DSG increased transplant acceptance.

New Strategies for Immunosuppression: While new approaches to transplantation are going forward, improvements are also being sought for handling immune system rejection of islets and organs. Researchers recently tested a new immunosuppressive regimen in patients receiving kidney, liver, pancreas, or intestinal transplants. They were able to increase the interval between doses of immunosuppressive drugs given to the patients after transplantation (for example, from daily to once a week). This advance, in combination with pre-treating the patients with immunosuppressive drugs before their transplant surgery, has decreased organ rejection and improved the quality-of-life of the patients. Importantly, these results were not dependent on the type of organ transplanted, which suggests that these new approaches can yield wide-reaching benefits for organ transplantation recipients.

Collectively, these studies have demonstrated that novel strategies can improve transplant acceptance over current protocols. In one study, the new methods were tested on human transplant recipients, so the success can be directly translated into improved treatment strategies for patients. The other studies in animal models show much promise, and will have to be extended to non-human primates and humans. These new methods have the potential to increase organ acceptance and decrease the severe side effects of immunosuppressive therapy in order to improve quality-of-life.

The NIDDK will continue to foster progress in this area through its support of the Immune Tolerance Network (ITN), an international consortium led by the National Institute of Allergy and Infectious Diseases (NIAID) that is dedicated to the clinical evaluation of novel, tolerance-inducing therapies for autoimmune diseases, asthma and allergic diseases, and also to research to prevent rejection of transplanted kidneys and pancreatic islets. The NIDDK has also recently launched a new initiative through which it intends to stimulate research focusing specifically on the biology of human beta cells and human pancreatic islets. These large-scale initiatives complement ongoing efforts by individual basic and clinical research scientists committed to improving health outcomes for people with diseases, including type 1 diabetes, that may be cured through the gift of an organ and/or tissue transplant.

Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, Kaech SM, Wherry EJ, Onami T, Lanier JG, Kokko KE, Pearson TC, Ahmed R, and Larsen CP: Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest* 111: 1887-95, 2003.

Omer A, Duvivier-Kali VF, Trivedi N, Wilmot K, Bonner-Weir S, and Weir GC: Survival and maturation of microencapsulated porcine neonatal pancreatic cell clusters transplanted into immunocompetent diabetic mice. *Diabetes* 52: 69-75, 2003.

Starzl TE, Murase N, Abu-Elmagd K, Gray EA, Shapiro R, Eghtesad B, Corry RJ, Jordan ML, Fontes P, Gayowski T, Bond G, Scantlebury VP, Potdar S, Randhawa P, Wu T, Zeevi A, Nalesnik MA, Woodward J, Marcos A, Trucco M, Demetris AJ, and Fung JJ: Tolerogenic immunosuppression for organ transplantation. *Lancet* 361: 1502-10, 2003.

CLINICAL EFFORTS TO FIGHT DIABETES IN YOUTH

The onset of diabetes in youth–whether type 1 or type 2 diabetes–has long-term consequences for the health and development of the child. The NIDDK is supporting new and continuing clinical initiatives to reduce the burden of diabetes on youth in the U.S. To combat the alarming rise in diagnosis of type 2 diabetes in children and adolescents, the NIDDK plans new trials to identify the best interventions available treat and to prevent type 2 diabetes in youth. A multi-center trial, TODAY (Treatment Options for Type 2 Diabetes in Adolescents and Youth), will compare three approaches to therapy of type 2 diabetes, and STOPP-T2D (Studies to Treat or Prevent Pediatric Type 2 Diabetes) is conducting pilot studies for a school-based trial directed at preventing the risk factors for type 2 diabetes in middle school children. The NIDDK is also collaborating with the National Institute of Child Health and Human Development (NICHD) to stimulate research on the precursors of the "metabolic syndrome" in children and adolescents. Greater knowledge of this clustering of metabolic disorders, which in adults predicts the development of type 2 diabetes and/or coronary heart disease, may lead to new therapies to prevent its development in youth, or to mitigate its consequences later in life.

The NIDDK will also continue vigorous support of research on type 1 diabetes. Support will continue for TrialNet–a nationwide network of clinical trial centers that supports the development and implementation of clinical trials of agents to prevent or slow the progression of type 1 diabetes. Another study, The Environmental Determinants of Diabetes in the Young (TEDDY), will analyze the infectious agents, dietary factors, and other environmental conditions that might trigger type 1 diabetes in genetically susceptible individuals. A major new clinical trials network will expand support for clinical trials on islet transplantation in type 1 diabetes patients. With the continued Congressional support for research on type 1 diabetes through the Special Statutory Funding Program for Type 1 Diabetes Research, the NIDDK is spearheading a series of initiatives to understand, treat, prevent and cure type 1 diabetes, together with other NIH Institutes and other agencies within the Department of Health and Human Services. More information on efforts made possible through this program is available on the worldwideweb at http://www.niddk.nih.gov/fund/diabetesspecialfunds/.

USING SMALL MOLECULES TO CORRECT CYSTIC FIBROSIS

Cystic fibrosis (CF) is a disease caused by mutations in the gene encoding the CFTR protein. The most common mutation, called ΔF508 (delta F508), causes cells to produce a misfolded protein that is unable to move to its proper location at the cell membrane, or function properly as a chloride ion channel. These abnormalities cause patients to have impaired lung function, caused by thick mucous secretions and bacterial infection, as well as digestive problems.

While the CFTR protein is expressed in high levels in the kidney, mutations in CFTR have little or no effect on kidney function. To help understand this paradox, researchers studied mouse kidney cells expressing the mutant CFTR grown under a special condition. This condition, called "hyperosmotic stress," mimics the environment for cells in the fluid filtering parts of the kidney. This hyperosmotic stress had the effect of correcting the mutant CFTR protein folding defect. They further showed that a small molecule, GSNO, which is a substrate for an abundant enzyme in the kidney, also promoted mutant CFTR protein maturation and function. These results may explain why CF patients do not have impaired kidney function, and demonstrate the feasibility of using a small molecule approach to promote proper folding and cellular trafficking of mutant CFTR protein as a therapeutic treatment. Another research group also used a small molecule

approach, and screened 100,000 small molecules. They identified six classes of molecules that were able to restore the ion channel function to ΔF508, using a cell culture model system. Both of these studies successfully used small molecules to correct defects in the ΔF508 mutant protein in cell culture, which suggests that small molecule intervention may be a useful approach for treating CF.

Howard M, Fischer H, Roux J, Santos BC, Gullans SR, Yancey PH and Welch WJ: Mammalian osmolytes and S-nitrosoglutathione promote Delta F508 cystic fibrosis transmembrane conductance regulator (CFTR) protein maturation and function. *J Biol Chem* 278: 35159-67, 2003.

Yang H, Shelat AA, Guy RK, Gopinath VS, Ma T, Du K, Lukacs GL, Taddei A, Folli C, Pedemonte N, Galietta LJ and Verkman AS: Nanomolar affinity small molecule correctors of defective Delta F508-CFTR chloride channel gating. *J Biol Chem* 278: 35079-85, 2003.

BONE HEALTH AND OSTEOPOROSIS

Although seemingly static, bone is constantly being broken down and reformed in a process called "remodeling." A person's skeleton is completely remodeled every 10 years. The protein-mineral complex of bone is created by one set of cells called osteoblasts, and broken down by another set of cells called osteoclasts. Many factors influence the synthesis and demolition of bone by these cells, including nutrition, hormones, the immune system, medications, and exercise. Building and maintaining good bone health are important for proper development of body architecture and strength in youth, and for preventing potentially debilitating bone fractures in older age. The NIDDK supports basic and clinical research on the hormonal regulation of bone and mineral metabolism in health and disease, and the exploitation of this knowledge to develop approaches to maintaining bone mass and preventing bone loss.

Novel Assay for Measuring Protein Involved in Phosphate-Wasting Disorders: Phosphate is a compound obtained from food that is necessary for maintaining bone health. When the body cannot properly use the phosphate, phosphate-wasting disease can develop, such as autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemia, and oncogenic (cancer-related) osteomalacia. Patients who suffer from these three diseases have similar clinical symptoms, such as defective bone growth. Research has shown that ADHR is caused by errors in a protein called fibroblast growth factor 23 (FGF-23). The role of FGF-23 in regulating phosphate levels in healthy people is currently unknown. In addition, since X-linked hypophosphatemia and oncogenic osteomalacia are clinically similar to ADHR, it is possible that FGF-23 also plays a role in those diseases. However, a current limitation in understanding the function of FGF-23 is that there is no easy way to measure a person's FGF-23 protein levels precisely.

Researchers recently developed a novel assay, or test, in order to measure the levels of FGF-23 in the blood. They used this assay to measure FGF-23 levels in healthy people, and also in patients with X-linked hypophosphatemia and oncogenic osteomalacia. They found that most patients with either X-linked hypophosphatemia or oncogenic osteomalacia have much higher levels of FGF-23 in their blood than people without bone disease, suggesting that FGF-23 plays a role in regulation of phosphate levels in healthy people. Interestingly, after surgery to remove tumors causing oncogenic osteomalacia, the FGF-23 levels return to normal.

This new assay may be a very useful tool to diagnose patients with phosphate-wasting disorders. For example, patients with oncogenic osteomalacia often have tumors that are very small and difficult to locate. This assay could be used to measure FGF-23 levels in blood sampled at different locations in the body in order to help find the tumors. The assay could also be used to monitor the health of these patients after

tumor removal–if the FGF-23 levels start to increase, then the tumor may have returned. In addition to these promising clinical applications, researchers can now use the assay to study FGF-23 in the laboratory to understand more fully the underlying mechanisms by which the protein normally regulates phosphate levels.

Hormone Treatment for Osteoporosis: Osteoporosis is a disease characterized by low bone mass and bone deterioration. According to the National Osteoporosis Foundation, 10 million people in the U.S. have osteoporosis and an additional 34 million people have low bone mass, which increases their risk for developing the disease. Although the disease strikes both men and women, approximately 80 percent of patients with osteoporosis are women. Osteoporosis occurs when new bone is not formed as quickly as old bone is broken down. NIDDK-supported researchers previously identified parathyroid hormone (PTH) as a key hormone responsible for regulating the osteoblast cells involved in bone formation. PTH both stimulates new bone formation and promotes bone breakdown; careful studies demonstrated that with intermittent administration, the net effect is an increase in bone formation. Because of this beneficial effect, PTH was recently approved as an effective treatment for osteoporosis. Another hormone, PTH-related protein (PTHrP), is similar in many respects to PTH, but has not been tested for its ability to treat osteoporosis.

Researchers recently conducted a 3-month clinical trial of 16 post-menopausal women with osteoporosis to determine if PTHrP treatment had any effect on bone formation. All of the women were on menopausal hormone replacement therapy (MHT) and given vitamin D and calcium supplements during the study. A subset of the women was treated with PTHrP while others were given placebo. The researchers found that the women treated with PTHrP had a 4.7 percent increase in their spine bone mineral density (BMD), a measurement of bone mass. The women treated with the placebo had only a 1.4 percent increase in BMD. This study showed that, over a short period of time, PTHrP could significantly increase bone formation in post-menopausal women who have osteoporosis. Because this was a short-term study with few patients, these results must be confirmed in a clinical study with more patients over a longer time frame to determine if PTHrP can produce beneficial effects long-term. Further studies may compare PTHrP and PTH to define their relative risks and benefits, and establish whether PTHrP represents an alternative bone forming agent that could be used to to stimulate new bone formation in patients with osteoporosis.

Jonsson KB, Zahradnik R, Larsson T, White KE, Sugimoto T, Imanishi Y, Yamamoto T, Hampson G, Koshiyama H, Ljunggren O, Oba K, Yang IM, Miyauchi A, Econs MJ, Lavigne J and Jüppner H: Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. *N Engl J Med* 348: 1656-63, 2003.

Horwitz MJ, Tedesco MB, Gundberg C, Garcia-Ocana A and Stewart AF: Short-term, high-dose parathyroid hormone-related protein as a skeletal anabolic agent for the treatment of postmenopausal osteoporosis. *J Clin Endocrinol Metab* 88: 569-75, 2003.

SUSTAINED BENEFITS OF INSULIN-SENSITIZING DRUG THERAPY IN HIV-POSITIVE INDIVIDUALS WITH METABOLIC COMPLICATIONS AND FAT REDISTRIBUTION

Before the advent of highly active anti-retroviral therapy (HAART), individuals with Acquired Immunodeficiency Syndrome caused by the human immunodeficiency virus (HIV-AIDS) often developed a wasting syndrome characterized by catastrophic weight loss. The widespread adoption of HAART has markedly improved survival in HIV-infected individuals and the incidence of AIDS wasting syndrome has declined dramatically. Unfortunately, in many cases, HAART is associated with a different set of metabolic complications that can also be life-threatening, including elevated

blood lipid (fat) and cholesterol levels, insulin resistance, and abnormal distribution of body fat (lipodystrophy). These metabolic abnormalities are major risk factors for the development of serious diseases, such as diabetes and cardiovascular disease. Researchers have previously found that short-term (three month) treatment with metformin (a drug approved to improve glucose metabolism and insulin sensitivity in patients with diabetes) decreases insulin resistance and improves several cardiovascular risk factors in HIV-positive patients with fat redistribution. However, the risks and benefits of prolonged metformin therapy for these patients have been largely unknown.

The metabolic and cardiovascular benefits of continued metformin therapy for HIV-infected patients with lipodystrophy have now been examined in a follow-on study to the earlier clinical trial. Researchers found additional benefits of metformin in participants in the original three-month trial treated with metformin for an additional six months. Significant reductions were found in levels of tissue plasminogen activator (tPA) antigen levels in the blood–a marker for cardiovascular disease risk–as well as in waist circumference and body mass index, and insulin levels. Although metformin moderately improves lipid levels in type 2 diabetes, lipids were not affected by continued metformin therapy in patients with the metabolic complications of HIV.

Future studies will help to determine if higher doses of metformin are more effective, as well as to assess whether metformin therapy reduces the likelihood of the development of full-blown cardiovascular disease or type 2 diabetes in HIV-positive patients with metabolic complications and fat redistribution.

Hadigan C, Rabe J and Grinspoon S: Sustained benefits of metformin therapy on markers of cardiovascular risk in human immunodeficiency virus-infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab* 87: 4611-5, 2002.

STORY OF DISCOVERY

Preventing or Delaying Complications of Diabetes: 20 Years of Study by the DCCT/EDIC Research Group

In type 1 diabetes, the patient's own immune system mistakenly attacks and destroys the beta cells of the pancreatic islets, the sole producers of insulin. Without insulin, the tissues of the body cannot absorb or use glucose (sugar), the major cellular fuel. Type 1 diabetes patients require insulin administration for survival. Insulin, however, is a treatment for the disease and not a cure. Diabetes slowly damages major organs in the body, such as the eyes, kidneys, and cardiovascular system. Thus, it is imperative to better understand and intervene in the development of diabetes complications to improve longevity and quality-of-life of type 1 diabetes patients.

Impressive research progress toward combating diabetes complications was achieved through a large clinical trial which the NIDDK launched in 1983. The Diabetes Control and Complications Trial (DCCT) was a multi-center clinical trial of over 1,400 people with type 1 diabetes. Completed in 1993, the trial compared the relationship between intensive versus conventional treatment of blood glucose levels and the development of disease. Patients on intensive treatment kept their blood glucose levels and hemoglobin A1c (HbA1c) levels (which reflect average blood glucose levels over a 2- to 3-month period) as close to normal as safely possible with frequent monitoring of blood glucose, and at least three insulin injections a day or use of an insulin pump. Conventional treatment consisted of one or two insulin injections a day, with once-a-day urine or blood glucose testing. The result was a large difference in the mean HbA1c levels in the two groups and a striking difference in their development of microvascular complications. The DCCT proved conclusively that intensive therapy reduces the risk of microvascular complications, such as diabetic eye, kidney, and nerve disease, by 35 to 76 percent compared with conventional treatment. This dramatic, positive result has had a profound impact on clinical practice for the management of type 1 diabetes: it led to the development of clinical guidelines by the American Diabetes Association and other groups; it spurred the creation of the National Diabetes Education Program to disseminate the findings to the public; and it stimulated multifaceted research efforts to develop tools and therapies that enable patients to achieve tight control of blood glucose levels.

Upon completion of the DCCT, participants who had received conventional treatment were taught intensive treatment, and all patients were encouraged to use intensive treatment. Nearly all patients who participated in the DCCT volunteered for the Epidemiology of Diabetes Interventions and Complications (EDIC) study, which began in 1994. EDIC was established to determine the long-term outcome of reducing exposure of the body's tissues and organs to high blood glucose levels.

Now, 10 years after the end of the DCCT, further seminal insights are emerging regarding long-term benefits of intensive blood glucose control. In May 2002, EDIC investigators reported that the 6.5 year

period of intensive treatment during the DCCT continued to reduce the risk of eye disease as long as 7 years after the study ended. Building on this exciting finding, a study in October 2003 showed that the former intensive treatment group had a decreased incidence of kidney damage and high blood pressure compared to the former conventional treatment group eight years after the end of the DCCT. These long-term benefits were observed despite nearly identical blood glucose control in the patients after completion of the DCCT. Analysis shows these long lasting differences in development of complications can be explained by the difference in control of glucose levels between the two treatment groups during the DCCT.

While DCCT proved that glucose control could prevent small vessel damage that causes kidney, eye and nerve problems, controversy remained about the effect of glucose on cardiovascular disease (CVD). Studies had already shown that high glucose levels correlated with CVD, but the effectiveness of intensive glucose control in preventing or delaying CVD had not been proven. In June 2003, the DCCT/EDIC research group showed that patients in the former intensive therapy group had a decreased progression toward atherosclerosis compared to the patients in the former conventional therapy group. This was demonstrated using both ultrasound to measure thickening of the wall of the carotid artery and also electron beam computed tomography (EBCT) to measure coronary calcification. Twenty years after the beginning of the DCCT, there is now evidence that intensive glucose control prevents damage to large blood vessels. This is a significant finding because CVD causes death in two-thirds of patients with diabetes.

These findings of the DCCT/EDIC research team raise interesting questions about the "metabolic memory" that enables the beneficial effect of intensified blood glucose control to persist long after the period of intensive therapy has ended. The biologic basis of metabolic memory–how a difference in glucose control for a finite period can have striking effects long after the conclusion of the study–has been explored in a symposium that marked the 20th anniversary of the initiation of the DCCT. Held at the NIH in April 2003, the symposium, "Metabolic Imprinting and the Long-Term Complications of Diabetes Mellitus: Bench to Bedside and Back," included an overview of the DCCT/EDIC trials, as well as presentations from leading investigators studying diabetic complications. These investigators are vigorously pursuing possible explanations for the enduring effects of intensive therapy that outlast the period of improved glucose control. One possibility is suggested by the demonstration of longstanding tissue changes associated with high blood sugar, particularly the attachment of end products of sugar metabolism to collagen, a component of the matrix that surrounds most cells. Continued efforts by scientists will unravel the underlying molecular mechanisms by which elevated glucose levels damage small and large blood vessels, and the tissues and organs that are affected. The symposium underscored that, even though the results of the DCCT/EDIC studies show that intensive therapy is beneficial for long-term prevention of complications, a severe limitation to the practice of intensive therapy is the potential for acute episodes of hypoglycemia, or low blood sugar. Thus, it is imperative that researchers seek new methods to improve blood glucose monitoring and insulin delivery, or develop new beta cell replacement therapy to cure type 1 diabetes.

STORY OF DISCOVERY

The DCCT and the EDIC studies have directly and positively affected the manner in which patients and physicians manage diabetes. They have provided conclusive evidence that patients should begin intensive therapy as early as safely possible. By maintaining intensive therapy, patients have significantly reduced development of diabetic complications, which directly translates into an improved quality-of-life. Researchers will continue to investigate mechanisms by which glucose exerts its devastating effects, in the development of complications, with a goal of discovering therapeutic targets to treat or prevent complications.

PATIENT PROFILE

Dan Lamb

For Dan Lamb and Many Others, Participating in the DCCT/EDIC Has Been a Life-Altering Experience

"For those of us with diabetes, it was amazing to see the results of 20 years of research," Dan Lamb recalls. Dan and several other participants in the Diabetes Complications and Control Trial, mostly from Iowa and neighboring states, had gotten together in the summer of 2003 for a 20-year reunion. Many had not seen each other in years. One fellow participant came up to Dan the night of the reunion dinner and introduced himself. "I had been this guy's camp counselor many years ago," says Dan, "and when I found out then that he had diabetes, I strongly advised him to get involved with the study. His coming up to me all these years later to tell me the important role the study played in his life, and how appreciative he was that I had pointed him in that direction was extremely heart-felt. It made me feel as if I had made a real difference in someone's life."

Dan Lamb

The Diabetes Control and Complications Trial (DCCT), conducted from 1983 to 1993, included 1,441 volunteers with type 1 diabetes and was conducted in 29 medical centers in the United States and Canada. The goal of the trial was to determine whether or not tight control of blood sugar levels could prevent or delay microvascular complications of the disease. As a result of the DCCT, researchers determined that intensive control of blood sugar dramatically reduces the incidence of eye, nerve, and kidney disease in people with type 1 diabetes. (Please see the accompanying "Story of Discovery," "Preventing or Delaying Complications of Diabetes–20 Years of Study by the DCCT/EDIC Research Group.") Referring to those who had attended the summer reunion, Dan says that most had good stories to tell. "Even those who had a rougher time with their diabetes had good things to say about their experience in the study," he adds.

Today, nearly everyone who participated in the DCCT is now part of an NIDDK-sponsored follow-up study called the Epidemiology of Diabetes Interventions and Complications (EDIC). The EDIC study has followed several diabetes-related health outcomes in DCCT participants since the end of the trial. It is reaffirming the importance of beginning, as early as possible, intensive treatment to control blood sugar.

PATIENT PROFILE

Taking Part in the DCCT

Dan Lamb has been an athlete all his life. As a youngster, he played soccer and football, and also swam. Today, as a 34-year-old, Dan still plays soccer on an adult league and likes to go skiing with his family. With athletics seemingly in his blood, he wasn't about to let being diagnosed with type 1 diabetes at age 10 stop him from playing sports. Much to the chagrin of his mother and his physician, shortly after his diagnosis, Dan signed up for peewee football. Although regular exercise is now considered important for all people with diabetes, "Back then, physical activity wasn't recommended for people with [type 1] diabetes," says Dan.

At the same time that Dan was determined to play sports despite his type 1 diabetes, Dan's mother was just as determined to take care of her son's new-found health care needs. Wanting to be as informed as possible about diabetes, she got involved with the American Diabetes Association. Five years later, after coming upon an NIDDK announcement of a clinical trial that was recruiting volunteers, Dan's mother enrolled him in the DCCT at age 15.

Results of the Diabetes Control and Complications Trial (DCCT) demonstrate that, by strictly monitoring and controlling their blood sugar, people with type 1 diabetes can significantly reduce their risks of developing eye, kidney, and nerve complications.

The DCCT trial was testing the hypothesis that, in persons with type 1 diabetes, more intensive control of blood sugar would significantly prevent or delay the onset or progression of eye, nerve, and kidney disease that are common complications of the disease. The DCCT trial participants were randomly assigned to one of two treatment groups. Dan was randomized into the "intensive" therapy group, which controlled blood sugar levels using a variety of treatment approaches, including different insulin preparations, "jet injectors," and glucose memory meters. As part of the study protocol, and in an effort to simulate the activity of a normal pancreas, Dan's insulin shots went from one or two a day to four shots a day. "I took insulin with my meals and before bed, and recorded my blood sugar results daily," says Dan. For the 10-year duration of the study, Dan was seen once a month at the DCCT clinic to which he was assigned in Iowa, where, he says jokingly, he was "poked and prodded" and given instructions on how to aggressively control his glucose levels. In contrast to this intensive therapy, DCCT participants in the parallel "control" group followed what was then a conventional monitoring and treatment regimen. To determine how well the DCCT participants controlled their blood glucose levels throughout the trial, DCCT clinicians regularly administered a blood test called the HbA1c test. This test measures blood levels of a molecule called HbA1c. HbA1c measurements reflect average blood glucose levels for a 2-to-3 month period, and are given as percent values. The goal for DCCT participants in the intensive

Loren Kirkpatrick
(Intensive treatment group)
"I saw a patient recruitment announcement for the DCCT in the newspaper. I'd never participated in anything like this before. I greatly appreciated the DCCT's approach to teaching us volunteers as much as possible about how to manage our diabetes."

Becky Murphy
(Intensive treatment group)
"After participating in the DCCT for nine years and the ongoing EDIC follow-up study for 11, I feel that I'm being studied by the best medical teams there are…I also feel that these studies will help many, many other people who currently have diabetes, as well as those yet to be diagnosed with the disease."

treatment group was to achieve and maintain an HbA1c measurement as close to 6.0 percent as possible, which would indicate sustained blood glucose levels within a normal, healthy range. At the end of the DCCT, there was a dramatic difference in the HbA1c values between the two treatment groups: participants in the intensive treatment group had an average HbA1c of 7.2 percent, while participants in the conventional treatment group had an average HbA1c value of 9.0 percent. Most importantly, the lower HbA1c values correlated directly with reduced risk for microvascular complications. As soon as the trial ended, those in the control group were instructed in intensive therapy, and both groups were strongly encouraged to use the intensive therapy for life. Since then, glucose control has drawn closer and been similar between the two groups, with better control in the former conventional treatment group, and looser control in the former intensive treatment group.

Dan's experience has been even better than the average for the intensive treatment group, and he has maintained excellent HbA1c values since the end of the trial: Dan started the DCCT in February 1984 with an HbA1c measurement of 10.4 percent. Over the last 10 years, Dan's HbA1c has averaged 6.9 percent. Currently it is 6.5 percent. "As a result of the DCCT, I lowered my numbers relatively quickly and have been able to maintain that level over a long period of time," Dan says. "Had I not been part of the DCCT, I probably would not have paid attention to my diabetes as closely as I have, nor possess the same understanding of the disease and its complications that I have now. The study has been a huge part of my life, and has contributed greatly to my success as a person with diabetes." As a result of the DCCT and a separate study of persons with type 2 diabetes, it is now recommended that all persons with diabetes try to maintain HbA1c values as close to normal as safely possible, at 7.0 percent or less.

Findings of the DCCT Study

The DCCT found that lowering average blood glucose levels for several years **reduces the risk** of:

- *Eye disease by 76 percent*
- *Kidney disease by 50 percent*
- *Nerve disease by 60 percent*

The EDIC

Dan says that the EDIC follow-up study is quite different from the DCCT, but just as valuable. "Instead of once a month, I come in once a year. The physicians review what I've been doing and where I'm at. They [measure my HbA1c values], take a look at my eyes, kidneys and neurological functions and see if I'm staying within a good range of glucose levels," he says. According to Dan, who, as a result of both the DCCT and EDIC, has been followed through his high school, college, marriage, and now his fatherhood years, he still wants that information. "I never

Photo: Melikian Studio

Ruth Thomasian
(Intensive treatment group)
"Before entering the DCCT, I had difficulty managing my diabetes. I'd practically given up being able to deal with erratic blood sugars. The DCCT experimental protocol provided me the ability to take charge of my health and control my blood sugars. I'm thrilled to participate in the EDIC follow-up study to learn more about the benefits of taking charge of my diabetes."

Ralph Dinneen
(Intensive treatment group)
"The DCCT has helped me to reduce the health complications of diabetes, which has improved my life. I could not have asked for a better opportunity than to have participated in the DCCT and now the EDIC."

miss my annual appointment, and I plan on staying with the EDIC for as long as it continues." Currently, Dan's father, mother and brother are also taking part in an ancillary study to the EDIC follow-up in order to help researchers gain a clearer understanding of the genetic aspects of diabetes.

After living with type 1 diabetes for nearly 25 years, Dan has had no complications as a result of his disease. But he also realizes his good fortune. "I'm lucky that my sugar levels stay very much on an even keel," he says. "I have relatives, including an aunt, who did everything according to the book, but had difficulty maintaining good control."

The Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study is revealing the durability of the benefits of stricter blood sugar control and the importance of beginning intensive treatment to control blood sugar as early as possible. So far, the continuing benefits of DCCT intensive therapy observed in EDIC participants include:

- *Reduction in progression of diabetic eye disease by 66 to 77 percent*
- *Reduction in the risk for development of or progression to diabetic kidney disease by 84 percent*
- *Reduced progression of diabetes-related atherosclerosis*

Benefits in Balance

Strict control of glucose levels comes with its own risks, including dangerously low blood sugar, or hypoglycemic episodes, which in severe cases can result in life-threatening comas. In fact, in the DCCT, the intensive treatment group had three times the rate of episodes of serious low blood sugar reactions compared to the control group. Thus, balance is key: While it is recommended to keep blood sugar as close to normal as safely possible to prevent long-term complications, physicians and patients need to be aware of the risks of hypoglycemia that accompany intensive glucose management, especially for those who may be at increased risk of problems due to hypoglycemia. Some individuals–young children, individuals with severe heart disease, or those with hypoglycemia unawareness–may be at increased risk of problems due to hypoglycemia, and thus, for them, it may not be appropriate to manage glucose as intensively. Even though Dan periodically has hypoglycemic episodes, he says that he's always been able to recognize them coming on early and treats them with glucose tablets, or sometimes with orange juice or a sugar-based soda. As far as Dan is concerned, the benefits that come from maintaining tight control over blood sugar far outweigh the potential risks of hypoglycemia. "There's just no substitute for a healthy heart, eyes that are able to focus and work correctly, and kidneys that function well," says

Steve Cook
(Conventional treatment group)
"There were times it seemed like an inconvenience to be part of the DCCT study, but in retrospect the inconvenience was minor in comparison to what I learned about my health and the impact that lifestyle has on diabetes. I'm convinced I'm still not totally aware of all the benefits I received by taking part in both the DCCT and EDIC."

Coretha Rozendaal
(Intensive treatment group)
"I didn't know it before entering the DCCT, but I had something called 'dawn syndrome,' a condition in which my blood sugars would automatically rise, starting at 3 in the morning. The insulin pump offered to me in the trial corrected the condition. The pump certainly has made my life a lot easier, and both the DCCT and EDIC have helped me learn about diabetes, its complications, and what could happen if I don't take care of myself."

Dan. “I know people who have lost their eyesight or have had pancreas transplants because of their diabetes. Living with diabetes is tough, and sometimes people are unable or unwilling to control their blood sugars.”

The message Dan would like to convey to others with diabetes is: “Make sure you’re educated about the disease; follow your doctor’s advice; and take good care of yourself.” As an adult athlete, Dan continues to stay in shape and eat right. “My kids and my wife mean the world to me,” says Dan. “I can’t imagine losing my eyesight and not seeing them grow up, and that’s a distinct possibility for people with diabetes.” He advises everyone he knows with diabetes to “exercise, watch your diet and maintain your blood sugar levels so that you can experience those important moments of your life.” And for Dan, that includes attending his DCCT group’s 40 year reunion–20 years from now.

If doctors and patients adopt stricter standards regarding the monitoring and control of blood sugar, it is likely that we can prevent or delay the development of long-term complications in the estimated 18.2 million Americans with diabetes, both type 1 and type 2, and minimize the risk of hypoglycemia.

Jake Pokita
(Conventional treatment group)
“Because of the study, I’m more in tune with my body. I can tell when my blood sugar is low and when it is high. I have access to the best doctors and latest technologies and treatments. It’s really made a difference.”

Diabetes Education at NIDDK: The National Diabetes Education Program

Although making new discoveries about diseases and how to prevent them is a critically important aspect of the NIH mission, the process of discovery cannot benefit Americans if research findings are not put into practice to improve health. Thus, disseminating new knowledge through education programs directed at healthcare providers and the public is also an important part of the NIH mission. The National Diabetes Education Program (NDEP) is a health information and education service that was launched in 1997 to improve diabetes management and thus reduce the morbidity and mortality from diabetes and its complications. The NDEP is sponsored by the NIDDK and by the Division of Diabetes Translation of the Centers for Disease Control and Prevention (CDC). The program's goals and objectives support major federal government public health initiatives, such as "Steps to a Healthier U.S." and the President's "Healthier U.S." programs.

On November 13, 2003, Health and Human Services Secretary Tommy G. Thompson announced that the number of Americans with diabetes had reached an all-time high. It is estimated that 18.2 million Americans have diabetes and that 90 to 95 percent of these individuals have type 2 diabetes. Of the 18.2 million persons with diabetes, approximately 5.2 million have undiagnosed or unrecognized diabetes.

In response to the startling 2003 numbers, Secretary Thompson announced a new community-based program, "The Diabetes Detection Initiative: Finding the Undiagnosed (DDI)". With the support of the NDEP, the DDI will utilize health education/communication and community health interventions to increase the number of at-risk individuals that undergo risk assessment and, if appropriate, receive blood testing to determine if they have diabetes and need the necessary follow-up. Appropriate information about risk reduction will also be given to individuals who are identified with pre-diabetes or considered to be high-risk. The DDI is supported by several federal agencies, including the CDC and the NIH.

The new DDI program is built on previous clinical studies and ongoing NDEP initiatives. Scientific and clinical studies have demonstrated that if diabetes is well managed, the potentially devastating complications of this disease can be prevented or delayed. However, diabetes must first be diagnosed to be effectively treated and managed. Thus, it is critically important to identify the millions of people with unrecognized diabetes early in the course of the disease, so that they can benefit from earlier interventions to reduce both the microvascular and macrovascular diseases that can occur from diabetes. This early identification has the potential to reduce morbidity and mortality, improve quality of life, and lower the financial costs to individuals and society that result from diabetes complications.

A national diabetes prevention campaign, launched on November 20, 2002, by Secretary Thompson, is being coordinated by the NDEP. The program, entitled "Small Steps, Big Rewards," represents the first major NDEP effort to translate the Diabetes Prevention Program (DPP) results on a national level. The DPP found that modest weight loss and regular physical activity, such as brisk walking for 30 minutes a day five times per week, could cut the risk of developing type 2 diabetes by more than half in people at high-risk for diabetes. These lifestyle changes worked for people of every ethnic or racial group who participated in the study, and they were especially successful for people over age 65. The program emphasizes the practical application of the DPP findings and includes lifestyle-change tools for those at risk, patient education materials for healthcare providers,

web-based resources for both healthcare providers and consumers, and TV, radio and print public service announcements. The NDEP will be tapping its partners at local, state and national levels for help in disseminating the new program's message, and will also recruit businesses and consumer-based programs as partners in this effort.

While working to increase awareness about diabetes and effective means for prevention, the NDEP continues to promote a core campaign, "Be Smart About Your Heart: Control the ABCs of Diabetes." This campaign is designed to make people with diabetes aware of their high risk for heart disease and stroke–the leading causes of death in these patients–and the steps they can take to dramatically lower that risk. The campaign emphasizes that good diabetes management is more than lowering blood glucose (best measured by the HbA1c test). Control of blood pressure and cholesterol is crucial to help prevent heart disease and stroke in people with diabetes.

Because diabetes disproportionately affects minority groups and older adults, the NDEP has developed tailored campaigns for these special population groups. Educational materials, public service announcements (PSAs), and other products have been developed for African Americans, American Indians and Alaskan Natives, Hispanic/Latinos, Asian Americans and Pacific Islanders and senior citizens. NDEP partner organizations representing these audiences help develop and deliver these health messages.

Working with its many partners and community contacts, the NDEP hopes to close the gap between what is known about the best diabetes treatments and what is actually practiced at doctors' offices and health clinics throughout the U.S. A new online comprehensive resource, *www.betterdiabetescare.nih.gov*, is designed to help health care providers, educators, policy makers and purchasers make changes in systems of care. The website provides tools and materials to help improve patient outcomes. Another online resource, *www.diabetesatwork.org*, helps businesses and managed care companies to assess the impact of diabetes in the workplace. It also provides easy-to-understand information for employers to help their employees manage their diabetes and take steps toward reducing the risk for diabetes-related complications. Ultimately, the NDEP aims to help reduce the illness and deaths associated with diabetes and its complications.

PATIENT PROFILE

Krystle Kelly

Living with Type 2 Diabetes as a Teen

Going through adolescence is tough enough. Being a 19-year-old girl with type 2 diabetes makes the going that much tougher. Just ask Krystle Kelly. Diagnosed with the disease at age 13, Krystle's high school classmates tease her about what she eats and are aghast when she has to prick her finger to check her blood-sugar, or glucose, levels. As for those fast-food franchises where teens like to hang out, these establishments present a real risk for someone with Krystle's disease. In addition, her diabetes increases her appetite, which makes it doubly difficult for her to control her weight. "The kids in school call me the peanut butter girl because I eat a lot of peanut butter, and go 'ooh, that's sick' when they see me eat cottage cheese for lunch or prick my finger to check my blood. I try to tell them what diabetes is," says Krystle, "but they don't understand."

At age 19, it may be of little consolation to Krystle, but the fact is she's not alone in her adolescent fight against type 2 diabetes. Once a disease diagnosed in adults, type 2 diabetes is rising dramatically among children, especially minority adolescents, including African Americans, Hispanic Americans, and Native Americans. Currently, there are no national population-based data. However, studies conducted in several cities across the United States indicate that the percentage of children with newly diagnosed diabetes who are classified as having type 2 diabetes has risen from less than five percent before 1994 to 30-50 percent in subsequent years.

Krystle Kelly

Why this dramatic surge?

Type 2 diabetes in children, as in adults, is closely linked to a sedentary lifestyle, a family history of the disease, and obesity–and the prevalence of obesity in adolescents has nearly tripled in the past 20 years. According to recent estimates, 15.3 percent of children six to 11 years old, and 15.5 percent of adolescents 12 to 19 years old were overweight in 2000 in the United States.

Overweight children are at increased risk of developing type 2 diabetes during childhood and later in life. Genetic susceptibility, as well as lack of physical activity and unhealthy eating patterns, all play important roles in determining a child's weight. They also contribute to a child's risk for type 2 diabetes and other complications of being overweight.

Living with Type 2 Diabetes as a Teenager

Krystle was never obese as a child, but she was overweight by 10 or 15 pounds–enough to be considered a risk factor for diabetes. She also had another risk factor that could not be ignored; her father has type 2 diabetes. He was diagnosed in his early 30s. So at around age 13, when Krystle began feeling tired and lethargic, never wanting to do anything, her family had her tested for the disease. Her first blood test turned out negative. However, the second time she was tested, the test proved positive for type 2 diabetes. "I was upset and angry when I found out I had diabetes," says Krystle. As a result of her father's having the disease, she knew what it was like to live with type 2 diabetes "and I just didn't want to go through that." Despite her anger and disappointment, Krystle has made a noble effort to control her weight and blood-sugar levels through exercise, diet and medications in order to stay healthy. But it has not been easy.

"Having this disease is extra hard when you're a teenager," says Krystle's mom, Sharan Kelly. "It's more difficult for kids like Krystle to be accepted by their peers," she says. "The dietary choices teens are constantly confronted with are certainly not good choices for teens with type 2 diabetes, and the fact that kids with diabetes want to avoid being embarrassed by their classmates whenever they need to prick their fingers means they're not taking as good care of themselves as they should." It also has a lot to do with the day and age we live in, adds Mrs. Kelly. "I must confess that when you're a family with two working parents, some nights it's hard to put a balanced meal on the table."

Being the parent of a teen with type 2 diabetes also presents a constant concern. "I'm always worried about the potential long-range complications of this disease as Krystle gets older," says Mrs. Kelly. "I feel like I'm always hounding her, but that's because I understand the complications better than she does." And Mrs. Kelly has every reason to be concerned. Complications of diabetes can result in heart disease, stroke, high blood pressure, blindness, kidney disease, nervous system disease, amputations, and dental disease, as well as other health difficulties. To date, there is no cure for diabetes.

Because there is no known cure for type 2 diabetes, researchers agree that the best one can do is to bring his or her blood-sugar levels into a healthier target range through diet, weight loss, physical activity, stress reduction, and diabetes medication. Therapies that reduce blood pressure and cholesterol are also critical for decreasing the risk of developing complications.

Treating the Disease

So far, Krystle manifests no complications as a result of her diabetes. One factor may be that she tries to exercise at least three or four times a week. "I'm a member of the YMCA, I walk, and ride my bike or roller blade as often as I can," says Krystle. Exercise has been shown to improve insulin sensitivity in people with type 2 diabetes.

If exercise is a good thing for Krystle, the worst thing about having diabetes for her is "the eating part." Her favorite foods are bread, pasta and desserts. At 5-feet 4-inches tall and 146 pounds, Krystle is 10 to 15 pounds overweight, and has remained in that range for several years. Although she stays in fairly good control of her diet, "We're

constantly counting carbohydrates," says Mrs. Kelly. "Some days she eats the wrong foods and her blood sugar rises. Other days she'll eat the right foods, but too much of it." Portion control is a problem for most people with diabetes. The disease increases appetite, which makes the person feel hungry. This can often lead to bad eating habits.

Fortunately, the advent of new medications and technologies is helping people with type 2 diabetes control their blood-sugar levels. Up until recently, for example, Krystle took two types of insulin: one a quick acting insulin before meals or for corrections in between meals to help bring down her sugar levels; the other, a longer-acting insulin to help regulate her blood glucose levels throughout the day and night. She also continues to take metformin, an oral diabetes medication, at breakfast and dinner. Today, almost one-third of the people with type 2 diabetes take some form of insulin to effectively control their sugar levels.

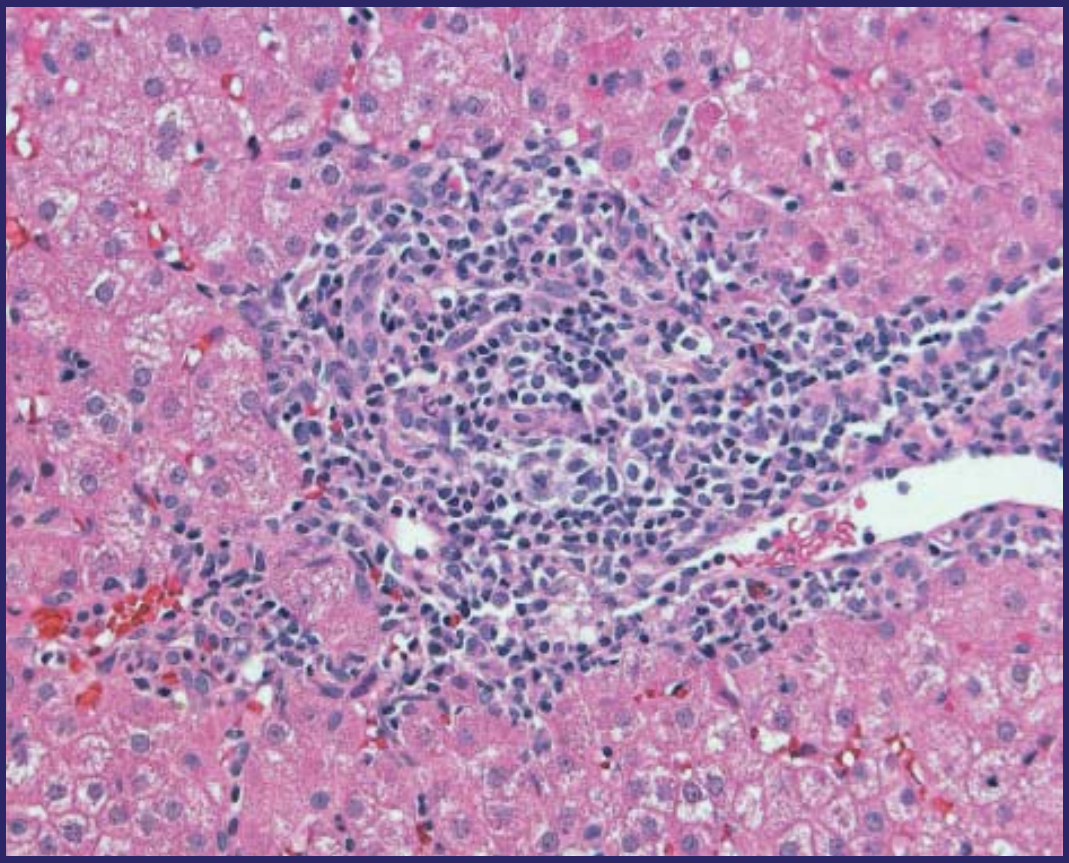
However, many people with type 2 diabetes, including Krystle, who require insulin are now opting to use an insulin pump. "Krystle just loves the pump," says Mrs. Kelly. "It's freed her from all the paraphernalia needed for insulin injections, as well as given her a lot more independence because she's no longer held to the rigid schedule of these injections. It's made a world of difference."

The NIH is funding clinical trials to prevent and treat type 2 diabetes in children. The trials will focus on developing cost-effective interventions to prevent diabetes that can be widely applied in schools and communities across the country, and on determining how best to use diabetes medications to treat children with type 2 diabetes.

Despite these pharmaceutical and technological advances, much more still needs to be done. The NIH is funding clinical trials to prevent and treat type 2 diabetes in children. These studies will try to develop ways to stem the rising tide of type 2 diabetes in children and to treat the disease safely and effectively in those who do develop it. The prevention trials will focus on developing cost-effective interventions that can be widely applied in schools and communities across the country.

"For children like Krystle, who already have type 2 diabetes, it's critical to give the safest, most effective therapy as early as possible," says the NIH study chair Francine Kaufman, MD. "Yet we can't assume that the therapies used in adults have the same safety and efficacy profiles for children," adds Dr. Kaufman, who also is past-president of the American Diabetes Association and director of the Comprehensive Diabetes Center at the Childrens' Hospital of Los Angeles.

The overriding concern is that the longer a person has diabetes–meaning, the earlier the onset–the greater the chances of developing the disabling, life-threatening complications that go along with diabetes. "We are seeing young people in their late teens who are already developing the complications of type 2 diabetes," says Dr. Kaufman. As far as 19-year-old Krystle is concerned, "I just hope they find a way to get rid of this disease."



In a patient with active hepatitis C infection, inflammatory cells of the immune system (stained dark blue) congregate in infected liver tissue (stained pink) in reaction to presence of the hepatitis C virus. Hepatitis C infection is a leading cause of liver disease and liver transplantation. The NIDDK supports basic and clinical research aimed at improving treatments for this disease and preventing its occurrence. Photo: Dr. David Kleiner, National Cancer Institute, National Institutes of Health.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. They include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. NIDDK-supported scientists are vigorously pursuing research to understand how common these diseases are across the U.S. population, to identify the causes of these diseases and how they progress, and to test pharmacological, surgical, and behavioral interventions for treatment and prevention.

A functional liver is essential for life. Several types of liver disease have serious adverse impacts on health, and some can lead to complete liver failure and the need for a liver transplant for survival. Scientists are intensifying research on a variety of liver diseases, from those primarily affecting children, such as biliary atresia, to those commonly affecting adults, such as non-alcoholic steatohepatitis. Some, such as hepatitis C, are caused by infection, while others result from such diverse factors as autoimmune reactions, genetic mutations, drug toxicity, and as-yet-unknown triggers. With livers from deceased donors in short supply, and the death rate among patients on the waiting list for a liver transplantation having risen by a factor greater than 10 during the past decade, identifying ways to facilitate safe and effective transplantations from living donors is critical. Efforts are under way to coordinate and bolster each of these areas of liver disease research within the NIDDK, across the NIH, and with other organizations to respond to this health problem.

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. While multiple factors contribute to obesity, dietary intake clearly plays a key role in weight gain. 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. Investigators are also continuing behavioral research to help people achieve healthy lifestyles that include increased physical activity and improved diet. (Additional information on research endeavors focusing on obesity supported by the NIDDK is provided in the next chapter.)

Intestinal disorders include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. One such disorder is irritable bowel syndrome (IBS), which causes pain and constipation or diarrhea. IBS more frequently affects women, who, in comparison to men, display a different range of symptoms and respond differently to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Symptoms may be influenced by abnormal functioning of the intestinal nervous system and altered perception of intestinal stimuli by the brain. Scientists are accelerating research to better understand these intestinal disorders.

Another serious intestinal disorder is inflammatory bowel disease (IBD). IBD, which encompasses Crohn's disease and ulcerative colitis, is marked by destructive inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. Surgical treatment is often required. Scientists are dissecting the complex interactions among the genetic, environmental, and cellular factors that contribute to

the development of IBD. The continued identification of predisposing genetic variations and their interactions, as well as other factors, such as potential autoimmune and microbial influences, will help spur the design of novel therapeutic strategies.

Small but powerful players in tipping the balance towards digestive health or disease are the microorganisms that inhabit the gastrointestinal tract. These microbes can affect intestinal health in some surprising ways, depending on whether they work with, or against, the cells of their host. Scientists are gaining insights into how these microorganisms that normally reside in the gut influence the development and function of the digestive tract.

Among other diseases of the digestive tract are those of the pancreas, including various forms of pancreatitis. An inflammation of the pancreas, pancreatitis, results in abdominal pain, weight loss, poor digestion, and, in more serious cases, tissue damage and infection. Chronic pancreatitis, serious in and of itself, increases susceptibility to pancreatic cancer, one of the deadliest malignancies. Scientists are identifying both genetic and environmental factors associated with pancreatic disease and pancreatic cancer.

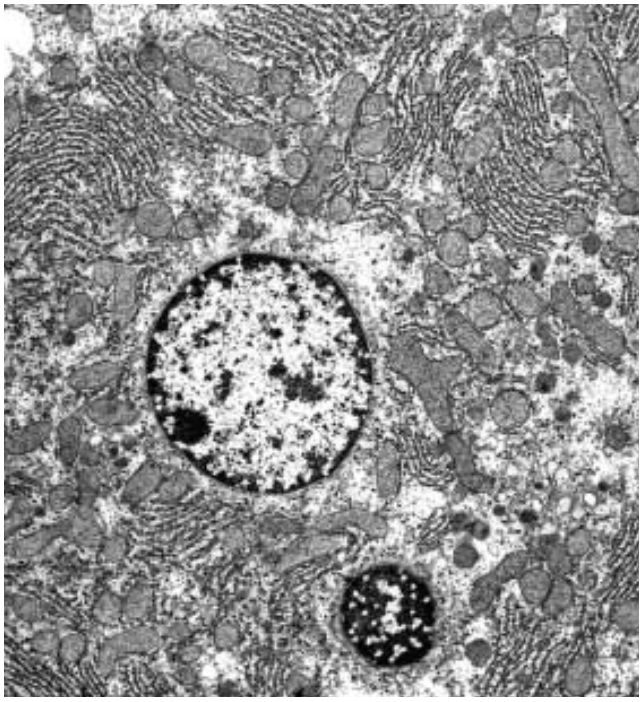
Finally, digestive disease can also be triggered by foods. In individuals with celiac disease, the immune system reacts to a protein called gluten, which is a component of wheat, barley, and rye. This reaction leads to damage to the small intestine, consequently interfering with its ability to absorb nutrients from foods and resulting in chronic diarrhea, bloating, anemia, and, in children, growth failure. Celiac disease is also associated with other serious conditions, such as osteoporosis, and, rarely, increased risk of certain cancers. Following a gluten-free diet is difficult, but is the only effective treatment. The greater challenge now facing patients and their healthcare 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 through genetic-based screening in the future.

STRENGTHENING EFFORTS IN LIVER DISEASE

The liver, the largest organ in the body, is essential for keeping the body functioning properly. It removes or neutralizes poisons from the blood, processes drugs, produces immune agents to control infection, and removes germs and bacteria from the blood. The liver also makes proteins that regulate blood clotting, produces bile to help absorb fats and fat-soluble vitamins, and stores nutrients used for energy. Liver diseases that interfere with these essential functions can therefore severely threaten health. Unlike degenerative diseases, which typically manifest in old age, liver diseases in the United States frequently strike individuals in some of the most productive years of life, between the ages of 40 and 60 years. Even some of the very young are, in rare cases, afflicted with neonatal liver diseases, such as biliary atresia. Liver disease in the U.S. also disproportionately affects minorities and the economically disadvantaged. For those whose condition reaches "end stage," or liver failure, liver transplantation offers hope for survival, but demand for donor organs far exceeds supply. New planning and research activities within the NIDDK are currently strengthening efforts in liver disease research to advance knowledge in this area, with the ultimate goal of reducing the burden on patients suffering from liver disease.

Liver Disease Research Branch: In June 2003, the NIDDK Director established the Liver Disease Research Branch within the Institute's Division of Digestive Diseases and Nutrition. This Branch brings together experts in liver disease research to accelerate progress and coordinate liver-related research activities across the NIH and with other federal agencies. Among the Branch's long-term responsibilities are the planning and management of research and training. However, one of its first and most important tasks is to coordinate the

preparation of an Action Plan for Liver Disease Research, with a targeted release date of Spring 2004. Already, the Branch has assembled representatives from across NIH as part of the Liver Disease Subcommittee of the statutory Digestive Diseases Interagency Coordinating Committee. This Subcommittee will oversee and guide the development of the Research Action Plan, which will draw on the insights of these NIH representatives, as well as those of experts from other federal agencies and from the external liver disease research and advocacy communities. This Research Action Plan will provide an overview of current research funding in liver disease; challenges to advancing liver disease research; opportunities for future research; and a tactical plan for addressing these challenges and opportunities. The Research Action Plan will strengthen the collaborations necessary to advance NIH-wide planning for liver disease research to achieve optimal scientific and clinical benefits.



Transmission electron micrograph of a rat liver cell (hepatocyte), showing the nucleus (center) surrounded by a high density of cellular organelles important for carrying out the many functions of these highly active cells–including protein synthesis and energy production and storage. Photo: Prof. M.V. Parthasarathy, Cornell Integrated Microscopy Center, Cornell University, Ithaca, NY. © CIMC.

Adult Liver Transplantation–New Hope from Living Donors: For patients with end stage liver disease, liver transplantation represents the only available cure. Yet, more than 17,000 Americans are currently awaiting transplantation due to the shortage of livers available for transplant from deceased donors. The practice of transplanting portions of livers from living donors began over a decade ago with transplants from adult donors to children, due to the shortage of livers from deceased donors. Because of the liver's amazing ability to regenerate, the donor's liver eventually regrows to its previous size, and the portion transplanted also grows in the recipient.

Adult-to-adult living donor liver transplantation, first accomplished in the late 1990s and introduced into the U.S. in 1997, is a promising procedure that enables adult patients as well to receive part of a liver from a living adult donor, rather than from a deceased donor. Since its introduction, the number of adult-to-adult living donor transplants performed has grown considerably, now accounting for approximately 5 percent of all liver transplants in the U.S. However, liver transplantation between two adults is a more extensive and life-threatening operation for the donor than the adult-to-child operation, because it requires more of the donor's liver to be removed in order for the tissue to function successfully in the adult recipient. Indeed, between 1998 and 2003, there were two widely known deaths of healthy, adult donors after adult-to-adult living donor liver transplantations. The potentially serious, but as yet ill-defined, health risks posed to living donors, coupled with the enormous potential benefit for the vast number of patients on transplant waiting lists, make adult-to-adult living donor liver transplantation an important and timely area of study.

In order to understand both how this increasingly popular procedure is being performed on a national scale and the complications that can result from it, NIDDK-supported researchers recently collaborated on a survey of all liver-transplantation programs in the U.S. Reviewing data from 69 percent of the programs, they found that the number of liver transplants from living donors rose rapidly in just a few

years, increasing from one transplant in 1997, when this procedure began, to 266 transplants in 2000. Importantly, they found that overall mortality among donors is low, approximately 0.2 percent. However, serious complications occur in approximately 14 percent of donors. This study highlighted the critical importance of continuing research on the effects of the procedure on both the donors and recipients in order to improve strategies to combat adverse effects.

Given that adult-to-adult living donor liver transplantation procedure in the U.S. is expected to become even more widespread in years to come, it is important to comprehensively assess and monitor the risks to potential donors and to develop uniform criteria for matching donors with recipients. An ongoing effort to address these issues is the Adult-to-Adult Living Donor Liver Transplant Cohort Study (A2ALL). This study is supported by the NIDDK in collaboration with the Federal Health Resources and Service Administration (HRSA) and the American Society of Transplant Surgeons. The initiative was launched in 2001 to carefully evaluate the risks and outcomes for donors and patients. This multi-center clinical cohort study currently consists of nine liver transplant centers experienced in performing the procedure, and a data coordinating center responsible for maintaining a clinical database of patients. The study will follow both donors and recipients before and after the operation to assess their clinical outcomes and quality-of-life. The primary goal of the study is to provide valuable information on the outcomes of living donor liver transplantation that can be used to aid decisions made by patients, potential donors, and physicians in considering this potentially life-saving procedure.

Facilitating Research on Pediatric Liver Disease–The Biliary Atresia Research Consortium: The need for liver transplantation is not limited to adult patients with liver disease. The neonatal liver disease known as biliary atresia is the single most common reason for liver transplantation in children, and is a major challenge for early detection, diagnosis, and management. Biliary atresia is characterized by a progressive inflammatory process in the liver beginning soon after birth. The inflammation causes obstruction of the ducts that drain bile from the liver, damage to the liver cells, and scarring of liver tissue, leading to jaundice and weight loss. The cause(s) of the disease, however, remains elusive and its optimal management is still unsettled. Because biliary atresia and other forms of neonatal liver disease are relatively rare, no single referral center in North America treats a sufficient number of new patients each year to permit an intensive analysis of etiology and risk factors, or to assess critically novel means of diagnosis or treatment.

In 2002, the NIDDK created the Biliary Atresia Research Consortium to facilitate and perform clinical, epidemiological, and therapeutic research in children with biliary atresia and other neonatal liver diseases. At present, the Consortium consists of nine pediatric liver disease Clinical Centers and a Data Coordinating Center. The Consortium has recently developed a clinical trial to optimize the success of the Kasai procedure. This surgical procedure removes the biliary ducts outside the liver and attaches the small intestine to the liver, at the site where bile is formed. If this procedure is successful, it can reverse the effects of biliary atresia on the liver, removing the need for liver transplantation. It is also hoped that the establishment of this Consortium and the serum and tissue bank will stimulate other scientists to develop an interest in investigating the etiology and pathogenesis of neonatal liver diseases.

In addition to these initiatives, many new and ongoing clinical efforts in liver disease research are being supported by the NIDDK. These include two sets of multi-center clinical trials of treatments for hepatitis C, known as the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) Trial and the Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C). Also, the Acute Liver Failure Study Group combines a prospective database with a study of acute liver failure to test a treatment for cases of the disease that result from

factors other than damage due to acetaminophen. With respect to one of the most common causes of liver disease in the U.S., nonalcoholic fatty liver disease, the NIDDK supports an effort on one form of the disease, the Nonalcoholic Steatohepatitis (NASH) Clinical Research Network. This network includes a database of patients and provides additional resources and support for clinical studies of new therapies. Finally, to address the problem of liver injury due to medications, which is one of the most common causes of acute liver disease, the NIDDK has established a Hepatotoxicity Clinical Research Network. This multi-center network will characterize drug-induced liver injury and provide samples to collaborating researchers. Collectively, these clinical research efforts should spur research advances in various types of liver disease.

Brown RS, Jr., Russo MW, Lai M, Shiffman ML, Richardson MC, Everhart JE and Hoofnagle JH: A survey of liver transplantation from living adult donors in the United States. *N Engl J Med* 348: 818-25, 2003.

COMPREHENDING THE COMPLEXITY OF INFLAMMATORY BOWEL DISEASE

Within the human gastrointestional tract exists an ecosystem teeming with life. Microbes coexist alongside nutrients, molecules, ions, and debris. Cells forming the wall of the gastrointestinal tract serve as a permeable barrier that permits beneficial molecules to pass through, while preventing entry of harmful microbes and molecules. Under healthy conditions, the ecosystem is beneficial to the host. Pathogens are kept at bay, commensal bacteria reside peacefully, and probiotic bacteria break down molecules so that they can be absorbed through the intestinal wall as nutrients for the host. Under certain disease conditions, however, the ecosystem is perturbed when the host's immune system spins out of control and attacks friendly as well as pathogenic bacteria, which causes inflammation and eventual breakdown of the intestinal wall.

One million Americans suffer from inflammatory bowel disease (IBD), the general name for diseases causing inflammation in the small and/or large intestine. The two primary IBDs are Crohn's disease and ulcerative colitis (UC). While the cause(s) of IBD is not entirely known, it appears that IBD arises from a complex interplay of factors involving the environment, heredity, and the immune system. IBD is characterized by a breakdown in the regulation of immune responses to microbes residing in the gastrointestinal tract. The mechanisms underlying these aberrant responses are very complex and the target of intense research investigation. Another important aspect of IBD research is the genetics of this complex disease. The intricacy of mechanisms of this disease is reflected in the diverse research being conducted, as illustrated by the following recent advances.

"Trading Spaces"–Modulating Gut Bacteria to Reduce Inflammation: Researchers are finding that the composition of gut microbes is one factor influencing IBD and intestinal irritation. In a rat model of colitis (inflammation of the large intestine), called SPF B27 TG, administration of antibiotics that kill gut bacteria can prevent or treat this condition. Symptoms recur when treatment is stopped, however. In a recent study, researchers demonstrated for the first time in this model that administering both antibiotic drugs and a "probiotic" commensal bacterium synergistically confers partial protection against the relapse of colitis. When the probiotic bacterium, called *LGG*, was introduced to SPF B27 TG rats with established colitis following antibiotic treatment, the rats were less likely to have a relapse. Other species of the bacteria were tested to see if they conferred partial protection against relapse, but when the rats were treated with other related bacteria, they were not protected. Thus, the partial protection demonstrated with *LGG* bacteria is species-specific. These results are particularly significant because regimens combining probiotic bacteria with antibiotics have shown promise as

treatments for intestinal maladies in humans–including as a therapy to prevent post-operative recurrence of Crohn's disease. Interestingly, at the end of the experiments, the total amount of colon bacteria was the same between rats which did or did not receive the *LGG* bacteria, but the treated rats had 10-fold more of this type of bacteria in their colons. While further investigation is necessary to confirm these results and to elucidate the mechanisms involved, this work in an animal model suggests that specific modulation of the bacterial species present in the intestine, through a combination antibiotic/probiotic approach, may help reduce the inflammation in diseases such as IBD.

Insights Into Inflammatory Mechanisms: Amazingly, despite constant exposure to up to 1,000 species of commensal bacteria and their pro-inflammatory molecules, the gut maintains a state of controlled inflammation. In this state, immune system cells capable of attacking "good" bacteria are present, but their activity, and hence their potential to do harm, is being held in check. It is thought that intestinal inflammation in diseases such as IBD may be due in part to faulty control mechanisms. Scientists recently examined the contribution of certain immune system cells, called $CD4^+$ T cells, to the delicate balance of immune tolerance and sensitivity observed in the intestine. Previously, using a mouse strain that spontaneously develops colitis, the researchers had identified a subset of CD4+ T cells that react to bacteria that are normally tolerated in the colon, leading to inflammation. The mice recover from their colitis; however, the pathogenic $CD4^+$T cells can still be found in these mice. Moreover, if transferred to another mouse strain, these cells can still cause colitis. In the new study, the researchers have found evidence for why this is so. They were able to isolate and culture a specific set of regulatory T cells from the special mouse model that can communicate with the pathogenic $CD4^+$ T cells to rein in their growth and killing activity *in vitro*. These regulatory cells were also able to prevent colitis when transferred with the pathogenic cells into new mice. Importantly, when the researchers examined intestinal tissue from normal mice, they found evidence for the same regulatory activity in the $CD4^+$T cells in that tissue. These experiments provide further evidence of regulatory mechanisms that may contribute to the inflammatory process in colitis.

Researchers are also uncovering the contribution of other body components to inflammation that may be highly relevant to IBD. Platelets are particles that circulate in the blood where they are available to stop bleeding by forming blood clots, or scabs. Platelets normally circulate in an inactive state until injury occurs. At that time, they are activated through a series of molecular events initiated by the injury. Recently, scientists found that activated platelets express a protein, CD40 ligand (CD40L), on their surface. This discovery meant that platelets are capable of interacting with cognate CD40-positive cells lining the intestine and of initiating a cascade of events leading to intestinal inflammation. Scientists have also discovered that the platelets of IBD patients circulate in an activated state. Based on this new knowledge, researchers wanted to determine if platelets from IBD patients also express enhanced levels of CD40L. They found that, indeed, the platelets of IBD patients express increased amounts of CD40L compared to healthy controls, and that the activated platelets are located in the inflamed intestine, as well as in the circulation. Through additional experiments, they determined that the binding of platelet CD40L to human intestinal microvasculature cells *in vitro* results in increased expression of soluble and cell adhesion molecules that attract and retain inflammatory cells in the vasculature. The platelets from IBD patients also produce higher amounts of a molecule called RANTES, which may contribute to the chronic nature of IBD. This knowledge of the properties of the highly activated platelets presents a potential target for therapeutic intervention.

The IBD5 Gene Confers Susceptibility to Inflammatory Bowel Disease: Major progress is being made in understanding the genetic underpinnings of both forms of IBD–Crohn's disease and ulcerative colitis. In 2001, scientists made a landmark achievement when they demonstrated that the *NOD2* gene (also called *CARD15*) confers susceptibility to Crohn's disease. In a recent NIH-supported controlled study of Canadian Crohn's disease patients, scientists subsequently identified *IBD5*, located in part of chromosome 5 (specifically, the chromosomal region 5q31), as a Crohn's disease susceptibility region that contains a number of candidate susceptibility genes. Now, these scientists have replicated the findings of that study in a German population of Crohn's disease patients. In addition, *IBD5* was found to contain one or more likely susceptibility genes for ulcerative colitis. Further analysis revealed that *IBD5* and *CARD15* act independently to confer either risk for (*IBD5*) or clinical manifestations of (*CARD15*) Crohn's disease. The data also suggest that *IBD5* and *CARD15* may act synergistically to promote the development of ulcerative colitis. These findings provide the basis of a model for IBD diseases in which *IBD5* is a general risk factor for IBD, and genes such as *NOD2/CARD15* determine the clinical expression of the disease. Molecular classification of patients with IBD, combined with clinical data, may lead to the identification of patient subgroups and to greater precision in tailoring treatments for IBD patients.

IBD research has experienced tremendous progress in the face of enormous challenges, but much remains to be understood about this set of diseases. The NIDDK is continuing to advance IBD research through its strong support and scientific leadership. The recently established, NIDDK-supported IBD Genetics Consortium is attempting to identify additional susceptibility genes for IBD. To that end, the Consortium is creating a repository of patient data, immortalized cells lines, and DNA samples, and will ultimately be forming an extensive database for analysis. Other recent efforts include an initiative to study in great detail non-endocrine progenitor cells of the digestive tract, including the intestine, which will provide investigators with tools and knowledge to better understand both normal and diseased cells more fully. Furthermore, NIDDK-supported Digestive Diseases Centers with a focus on IBD continue to enhance interdisciplinary research efforts.

Finally, the NIDDK is actively engaged in strategic efforts to enhance IBD research. The statutory Digestive Diseases Interagency Coordinating Committee, which is led by the NIDDK, meets four times yearly to discuss current issues and to coordinate research activities among agencies within the Department of Health and Human Services and extramural organizations. In its April 2003 meeting, the DDICC focused on research advances and needs in IBD. The group discussed the latest advances in research on the intestinal epithelium, the microbiota, and the immune system, and their interactions in disease, as well as predisposing genetic factors and IBD epidemiology. The NIDDK also partners with the Crohn's and Colitis Foundation of America (CCFA) to enhance research in IBD. The Institute will work in partnership with the CCFA to attempt to address the scientific opportunities and hurdles outlined in the CCFA Strategic Plan entitled, "Challenges in IBD Research." These steps toward building a comprehensive understanding of the mechanisms underlying disease pathology, as well as the normal intestinal ecosystem (see "'Good' Bacteria–How Do They Help?"), will provide the springboard for more effective prevention and treatment strategies for IBD.

Cong Y, Weaver CT, Lazenby A and Elson CO: Bacterial-reactive T regulatory cells inhibit pathogenic immune responses to the enteric flora. *J Immunol* 169: 6112-9, 2002.

Danese S, de la Motte C, Sturm A, Vogel JD, West GA, Strong SA, Katz JA and Fiocchi C: Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. *Gastroenterology* 124: 1249-64, 2003.

Dieleman LA, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, Grenther WB and Sartor RB: *Lactobacillus GG* prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut* 52: 370-6, 2003.

Giallourakis C, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S and Rioux JD: IBD5 is a general risk factor for inflammatory bowel disease: Replication of association with Crohn's disease and identification of a novel association with ulcerative colitis. *Am J Hum Genet* 73: 205-11, 2003.

"GOOD" BACTERIA—HOW DO THEY HELP?

Many strains of bacteria cause illness. They are unwanted invaders that the immune system must eliminate. However, not all bacteria are unwanted; "good" bacteria live throughout the body with benefit to both host and microbe. Recent studies have shed light on how the "good" bacteria found in the gut are beneficial.

A type of cell found in the gut, the "Paneth" cell, appears to have a significant role in fighting disease. Researchers showed that the Paneth cells in mice produced a molecule, called Ang4, which is important in preferentially attacking harmful, invading microbes. Interestingly, the resident "good" bacteria in the gut were responsible for directing the Paneth cells to make Ang4. Another research team showed that the "good" bacteria, again working through Paneth cells, had an important role in developing the capillary networks found in the small intestine. In the absence of bacteria, these networks–which are important for absorption of nutrients–do not form properly.

In addition to providing insights into intestinal infections, this research is paving the way toward improved understanding of how bacteria and cells interact to guide the formation of new blood vessels–knowledge that could provide the basis for future experimental therapies for intestinal injury and cancer. The significance of appropriate interactions between intestinal cells and bacteria in creating a healthy intestinal immunity was also demonstrated by this research. With this knowledge, researchers have a better perspective on what goes awry in some intestinal diseases.

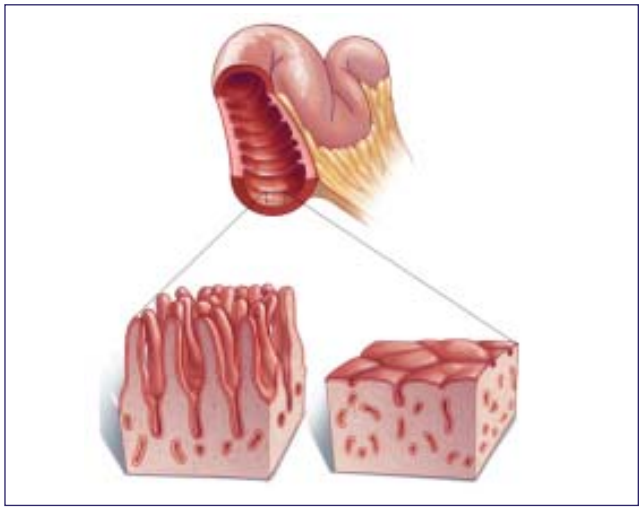
Hooper LV, Stappenbeck TS, Hong CV and Gordon JI: Angiogenins: A new class of microbicidal proteins involved in innate immunity. *Nat Immunol* 4: 269-73, 2003.

Stappenbeck TS, Hooper LV and Gordon JI: Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl Acad Sci USA* 99: 15451-5, 2002.

A DIFFERENT KIND OF FOOD FIGHT—MAKING PROGRESS IN CELIAC DISEASE

Celiac disease is an autoimmune, gastrointestinal disorder characterized by intolerance to a protein found in many foods–gluten. Children with the disease have symptoms that can include chronic diarrhea, bloating, anemia, and failure to grow at a normal rate. Early intervention is key to preventing damaging complications of this disease, especially in childhood cases. There is a genetic predisposition to developing celiac disease, and a large majority of patients have at least one copy of a gene, called HLA-DR3. (See also the "Patient Profile," "Celiac Disease: A Family Affair.")

In order to estimate incidence of the disease in the general population, researchers conducted a large, genetic screen of over 22,000 newborns in Denver, Colorado. A subset of the infants were followed for five years to compare disease development in those having zero, one, or two copies of the susceptibility gene. Overall, roughly one percent of all children at age 5 were estimated to have the disease. The children who had either one or two copies of the



This diagram illustrates how the structure of the tissue in the small intestine (top) is altered in celiac disease. Normal tissue from the surface of the small intestine (bottom left) has finger-like protrusions called villi, which are crucial for nutrient absorption. In patients with celiac disease, the villi become smoothed out and no longer function properly (bottom right)–leading to malnourishment and consequent complications. Illustration: Stephen Graepel. Copyrighted by and used with permission from the Mayo Foundation.

susceptibility gene were at an increased risk compared to children without the gene. In addition, the researchers found that females had a higher risk for disease development than males.

These results show that celiac disease is common in a population representative of the general population, and new screening strategies based on the study may help identify children at increased risk. Interestingly, other new studies have been revealing that onset or clinical symptoms of celiac disease is not restricted to children. New knowledge about prevalence of celiac disease in all ages and other pressing scientific and diagnostic issues will be the subject of a scientific conference at the NIH in June 2004.

Hoffenberg EJ, Mackenzie T, Barriga KJ, Eisenbarth GS, Bao F, Haas JE, Erlich H, Bugawan Tl T, Sokol RJ, Taki I, Norris JM and Rewers M: A prospective study of the incidence of childhood celiac disease. *J Pediatr* 143: 308-14, 2003.

TREATING FUNCTIONAL BOWEL DISORDERS

Functional bowel disorders, also called motility disorders, result from poor nerve and muscle function. Symptoms such as gas, pain, constipation, and diarrhea come back again and again, but–unlike intestinal diseases such as IBD, which can have similar symptoms–there are no signs of disease or damage in intestinal tissues. However, the symptoms themselves can be quite debilitating, and limiting to normal life activities. Irritable bowel syndrome (IBS), which specifically affects the colon, is a commonly encountered functional bowel disorder. The cause of IBS is unknown, but diet, emotions, and stress contribute to IBS symptoms. It affects an estimated one in five Americans, but is more common in women. New hope for understanding and treating IBS and other functional bowel disorders is arising from recent research advances.

Comparing Psychological Treatment Strategies for Women with Bowel Disorders: Patients with functional bowel disorders (FBD) have symptoms, such as abdominal pain and altered bowel habits, that can vary from person to person. Patients with moderate to severe symptoms often suffer from greater depression and psychological distress than those with less severe symptoms. A randomized, multi-center clinical trial of 431 women compared the value and utility of different approaches for treating psychological disabilities associated with FBD. In one comparative study, researchers compared two different types of sessions with trained psychologists. One group of women received educational training on their disorder, while the other group underwent cognitive-behavioral therapy (CBT). The latter is a type of therapy that emphasizes the role of using conscious thoughts to develop more effective coping strategies. In a second comparative study, separate groups of patients were treated with either an antidepressant or a placebo. Researchers found that CBT was far more beneficial than education therapy. Antidepressant therapy was equally effective as CBT, but the drug had side effects that prevented some

patients from staying on the medication. The researchers compared subpopulations of patients, such as those with differing severity of illness or depression. Subgroup analysis demonstrated that both treatments were more effective in patients with moderate symptoms than in those with severe symptoms. However, FBD symptoms of patients who also had depression were not improved with CBT or antidepressant therapy. This study suggests that patients with FBD can benefit from certain types of treatment strategies which can, in turn, improve their quality-of-life.

Sex Differences in Neurological Responses to Bowel Distention in IBS Patients: Women are more likely than men to develop IBS, to experience certain IBS symptoms, and to respond differently to pharmacological treatments for the disease. Though the reasons for these sex/gender-related differences are unknown, previous research has suggested that differences may exist between men and women in the brain's response to pain and stress experienced with IBS. Previous knowledge of which areas of the brain process such signals as pain emanating from the pelvic area (where the large intestine is located) or emotions of fear or stress, provided researchers with a number of places to look for differences in brain region activity of female *versus* male IBS patients.

To test for gender differences in the activity of brain areas associated with IBS symptoms, researchers measured blood flow to specific parts of the brain, as a reflection of activity level. Men and women were tested under three conditions: at rest, during inflation of a balloon inserted into the colon to mimic painful bowel distention during IBS, and while anticipating the balloon inflation. They found that, though there were some similarities, clear differences were seen in the activity of certain brain regions during colon distention or even anticipation of distention between women and men with IBS. In response to both distention and expectation of distention, women showed greater activation of emotion-processing areas, while men showed more activity in brain areas involved in pelvic pain.

This study's findings improve our understanding of the unique neurological mechanisms underlying IBS symptoms in female *versus* male patients. Because the cause of IBS is unknown and better treatments are needed, a more sophisticated understanding of the neurological basis of IBS symptoms will help spur the development of more effective treatments for these patients. These findings will be particularly useful as a basis for designing gender-specific therapies that relieve symptoms by targeting specific neural pathways at work in female and male patients.

Drossman DA, Toner BB, Whitehead WE, Diamant NE, Dalton CB, Duncan S, Emmott S, Proffitt V, Akman D, Frusciante K, Le T, Meyer K, Bradshaw B, Mikula K, Morris CB, Blackman CJ, Hu Y, Jia H, Li JZ, Koch GG and Bangdiwala SI: Cognitive-behavioral therapy *versus* education and desipramine *versus* placebo for moderate to severe functional bowel disorders. *Gastroenterology* 125: 19-31, 2003.

Naliboff BD, Berman S, Chang L, Derbyshire SW, Suyenobu B, Vogt BA, Mandelkern M and Mayer EA: Sex-related differences in IBS patients: Central processing of visceral stimuli. *Gastroenterology* 124: 1738-47, 2003.

STORY OF DISCOVERY

The Art of Liver Transplantation

It was the 2002 Winter Olympic Games at Park City, Utah and the world looked on in awe as 29-year-old American snow boarder Chris Klug competed in the giant slalom race wearing a broken boot held together with duct tape. The crowd cheered as he crossed the finish line. Chris had won the bronze medal with tape securing his boot, but even more amazing, 18 months earlier he had undergone a liver transplant. Chris had spent years training for this event, encountering hurdles, large and small, and now he had medaled. Chris continues in his pursuit for excellence and in the 2006 Winter Olympic Games, he will be aiming for the gold.

Success in clinical research is similar to striving for the gold. It does not come with one major victory, but instead it requires persistent small but steady progress, punctuated with major achievements along the way. One scientist has shown the courage and commitment to perfect liver transplantation therapy in just this manner. Dr. Thomas Starzl, with continued NIDDK support, performed the first human liver transplant in 1963 and has spent the last 40 years perfecting this procedure. The achievements of Dr. Starzl and many other dedicated scientists save the lives of thousands of patients annually who have end-stage liver disease (ESLD).

Liver transplantation in humans was preceded by careful animal studies that enabled researchers to develop and test surgical procedures, and the result is that the procedure is safer and more successful. For example, NIDDK investigators developed a venous-venous bypass technique that reduces excessive blood loss and renal failure that sometimes seriously compromised the health of previous liver transplant recipients.

Preserving donor organs to be used as transplants was another research hurdle. In 1984, an NIDDK grantee developed a preservation solution-called the "UW solution"– that effectively doubled the time a donor liver remains usable, to an average of 12 hours. Donor livers could then be procured from much greater distances and still arrive in a viable state for transplant.

In the late 1950s, animal research provided the foundation for determining that the drug combination of azathioprine and prednisone was the most effective immunosuppressive therapy at that time. Immunosuppressive therapy was greatly enhanced in the early 1980s with the introduction of cyclosporine, first as a single agent and then in combination with steroids. Largely as a result of cyclosporine's arrival, the frequency and success of liver transplant therapy began to grow. Indeed, a 1983 National Institutes of Health Consensus Development Conference concluded that liver transplantation was a therapeutic modality for ESLD.

Now, tacrolimus is the drug of choice. Discovered in 1984, it is effective and has fewer side effects than cyclosporine. Drugs like tacrolimus prevent graft rejection by inhibiting the immune system, but this does not come without a price. An inhibited immune system leaves the body susceptible to opportunistic infections and puts patients at higher risk for cancer. Thus, the drugs that prevent graft rejection can cause serious illnesses themselves.

STORY OF DISCOVERY

Dr. Starzl and his colleagues are now taking a new approach to the concept of graft rejection. Rather than striving to suppress the immune response that threatens graft survival, they are seeking to minimize the patient's dependence on anti-rejection drugs following transplant surgery. Patients are pretreated with a broadly reacting drug before their transplants, and then are treated by tacrolimus monotherapy beginning the day after transplantation. This regimen diminishes, but does not destroy, the immune system's ability to attack the new organ. It also enables the immune cells from the host and the "passenger" immune cells that are transplanted with the donor organ to interact with each other. Eventually, a type of peaceful co-existence develops, imparting a degree of host tolerance to the engrafted organ. At that point, medication is gradually reduced. Weaning from tacrolimus is continued until the patient is receiving a very low dose. Mild graft rejection is allowed; however, if serious symptoms begin to develop, the patient's medication is increased to a higher dose until he or she is ready to begin the weaning process again. This new regimen has been highly successful for patients who have received the treatment. Most are able to reduce their medication, and some take as little as one dose of tacrolimus a week. The timing and dosage of this regimen is based on principles of organ engraftment and acquired tolerance and, therefore, is effective for the transplantation of other organs, as well as the liver.

Although great strides have been made in facilitating liver transplantation, a chronic problem remains. There are far fewer livers available than are needed, and many lives are being lost because of the lack of available donor livers. Therefore, novel approaches to transplantation are being developed to fill this void. In December 2000, the NIDDK held a workshop on Living Donor Liver Transplantion. With this protocol, a donor gives part of his or her liver to an individual with ESLD. Ideally, the partial livers regenerate quickly into complete livers. However, this protocol presents a serious potential risk to the donor. The NIDDK is supporting research to improve the safety and outcomes of living donor transplantation therapy. Another therapeutic approach is the transplantation of liver cells (hepatocytes). This avenue of treatment is particularly suitable for patients with metabolic disorders. With this regimen, liver cells from a donor are infused into to the portal vein of a recipient, take up residence in the patient's liver, and become fully functional. In 1998, a child with Crigler-Najjar syndrome, which causes a build up of serum bilirubin levels, received a hepatocyte transplant. The engraftment improved her condition and lasted 11 months until she was able to receive a liver transplant. Recently, an infant received a hepatocyte transplant for a urea cycle disorder, a condition that results from a missing enzyme normally produced by liver cells. Her condition was also improved temporarily as a result of the transplanted cells.

Dr. Starzl and his colleagues are also exploring genetically-altered pigs as potential organ donors. Pigs normally express the antigen 1,3-galactose (1,3-Gal) on their cells surface. Because this protein is not synthesized in humans, it is the major cause for rejection of pig-to-human liver grafts. Starzl's group developed genetically engineered pigs that no longer make the 1,3-Gal protein by "knocking out" both copies of the pig's 1,3-Gal gene. Without this antigen on their cells surface, the pig organs are much safer to use as donors for human transplants.

Improved surgical procedures, preservation solutions, immunosuppressive drugs, and protocols designed to use the body's natural mechanisms for tolerance are major successes that were achieved because of smaller research accomplishments along the way. These advances, as well as the novel approaches to liver transplantation that are in various stages of development today, are being pursued by dedicated scientists "aiming for the gold," a cure for liver disease.

PATIENT PROFILE

The Traffs

Celiac Disease–A Family Affair

Elizabeth and R.J. Traff say that when Emily was 4 years old they felt they were watching their daughter starve to death in front of their very eyes. "Emily had stick arms and a bloated belly," says Mrs. Traff, "and other than her having had a bad case of the flu, we couldn't figure out what was wrong." A procedure called endoscopy, whereby a thin, flexible optical fiber is inserted down the esophagus, the interior of which can be viewed through or seen on a TV monitor, finally revealed that Emily has celiac disease. Celiac disease is a digestive disease that damages the small intestine and interferes with absorption of nutrients from food. At the time of Emily's diagnosis, the Traffs had never heard of celiac disease or that people who have it cannot tolerate a protein contained in many foods, called gluten. What they also didn't know is that the disease runs in families and, unlike Emily, some people may not have symptoms–yet, they are still at risk for the complications of the disease. These complications range from cancers, such as lymphoma and adenocarcinoma, to osteoporosis, to short stature and seizures. It wasn't until nine years after Emily's diagnosis, and completely by chance, that the Traffs learned that several other members of their immediate family either have the disease or are at risk of contracting it.

Research supported by the NIDDK is discovering that celiac disease may be far more prevalent in the U.S. than previously believed. Once thought to be a pediatric, or children's, disease, epidemiological studies are finding that celiac disease can actually develop in adulthood or remain undiagnosed well into adulthood. People at greater risk of developing celiac disease include those with type 1 diabetes, and researchers continue to uncover other risk factors.

The Traff family. Clockwise from the top: R.J., Elizabeth, Joseph, David, Laura, and Emily Traff.

Facts About Celiac Disease

- People with celiac disease cannot tolerate gluten, a protein in wheat, rye, or barley.
- The disease damages the small intestine and interferes with nutrient absorption.
- Treatment is important because people with celiac disease could develop complications, such as anemia, nutritional deficiencies, short stature (in children) or, rarely, cancer.
- A person with celiac disease may or may not have symptoms.
- Methods of diagnosis include blood tests and biopsy of tissue from the small intestine.

- Because celiac disease is hereditary, family members of a person with celiac disease may need to be tested.
- Celiac disease is treated by eliminating all gluten from the diet. The gluten-free diet is a lifetime requirement.

About Celiac Disease

Because the body's immune system causes the damage in celiac disease, celiac is considered an autoimmune disorder. When a person with celiac disease eats foods that contain gluten, which many foods do, his or her immune system responds by damaging the small intestine. Specifically, tiny fingerlike protrusions, called villi, on the lining of the small intestine become smoothed out and no longer function properly. Nutrients from food are absorbed into the bloodstream through these villi. Without protruding villi to absorb nutrients, a person becomes malnourished, regardless of the quantity of food eaten. This explains why Emily looked emaciated before her diagnosis.

"Knowing what we know now, if someone in a family is diagnosed with celiac disease, it makes good sense to test everyone else in the family for the disease," says Mrs. Traff.

Celiac disease also is a genetic disease, meaning it runs in families. About 10 percent of an affected person's first-degree relatives (i.e., parents, siblings, or children) will have the disease. Unfortunately for the Traffs, their percentage turned out to be much higher. About 9 years after Emily was diagnosed, the family voluntarily decided to take part in an NIDDK-funded study on the familial incidence of celiac disease–and it was fortunate that they did. It was during the course of the study that the family learned that Mr. Traff, as well as then 10-year-old daughter, Laura, both totally symptom-free, actually have the disease, and that the Traffs' younger son, David, has the genetic marker but currently shows no disease.

The Traff's older son, Joseph, is the only one in the family aside from Mrs. Traff who shows no genetic marker. In Laura's case, the blood tests used to detect celiac disease showed particularly troublesome values. At the same time, her villi were in the process of being severely damaged, despite the fact she manifested no symptoms. "Knowing what we know now, if someone in a family is diagnosed with celiac disease, it makes good sense to test everyone else in the family for the disease," says Mrs. Traff.

"The disease appears to be on my side of the family," says Mr. Traff. "My mother has had stomach problems all her life, but she was never diagnosed as having celiac," he adds. "She's now had a blood test and an endoscopy and is waiting for the results of a biopsy for a firm diagnosis. My brother is in the same situation."

Living with Celiac Disease

Emily, now 15, would be the first to tell you that living with celiac disease isn't exactly fun, but after 10 years she's gotten used to her gluten-free diet. "There are lots of foods I can eat, like hamburgers without the bun and French fries," she says. "It's really not that bad. There are a lot worse things than not eating anything with gluten in it." Mrs. Traff offers a slightly different perspective, however. "Emily plays by the rules," says Mrs. Traff, "but try finding something gluten-free to eat!"

After Emily was diagnosed, Mrs. Traff devoted a lot of her time to researching the disease and the impact of gluten, and quickly learned that many of the foods we eat contain the protein. Obvious sources are foods containing wheat, rye, and barley, including most breads, pastas, cereals and processed foods. But there are hidden sources as well, including food additives, preservatives and stabilizers not always clearly marked on processed-food labels. Some medicines and mouthwashes also contain gluten. Mrs. Traff's rule of thumb is "When in doubt, don't eat it."

She also strongly recommends connecting with a celiac support group, especially when the diagnosis is new. "Dealing with all the details of diet can become overwhelming," she says. "To be connected with people who have 'been there, done that' can be a great source of information and comfort."

Mrs. Traff was heartened recently when she picked up a processed-food product in her grocery store that said "gluten-free" on the packaging. She also said that some national restaurant chains now have menus that give information about whether their offerings are gluten-free. "It's good seeing nationally known companies taking these kinds of steps," she says, but adds that much more still needs to be done. Consequently, the local celiac disease support group Mrs. Traff belongs to has gone to several restaurants in her area to educate them about the disease. Mrs. Traff also has visited the cooks in the school cafeteria where her daughters eat to explain what her children can and cannot eat, "and they have cooperated beautifully," she says.

Cost is yet another issue. "It can be expensive to eat gluten-free," says Mrs. Traff. In her experience, a pound of gluten-free pasta, for example, can cost as much as $6.65. But considering the long-range health implications to her family if they do not eat gluten-free foods, she feels it is well worth it.

Recent Findings

Joseph Murray, MD, is an NIDDK-supported researcher at the Mayo Clinic in Rochester, Minnesota, who is conducting a study on the epidemiology and familial incidence of celiac disease. It was through Dr. Murray's study that the Traff family learned that several of their members, previously undiagnosed, have celiac disease. Dr. Murray says that many celiac disease cases go undiagnosed because many physicians are not familiar with the disease or the way it presents itself. "Many people with celiac do not manifest severe symptoms," he says.

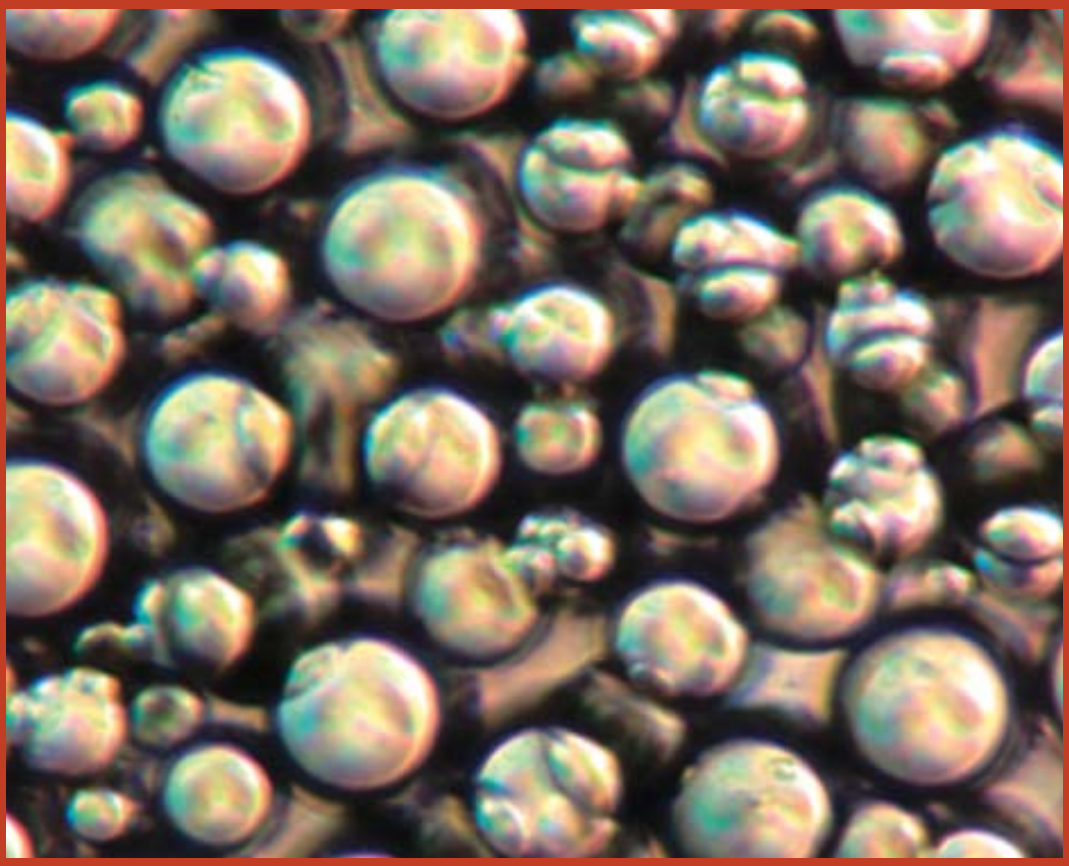
Other research appears to support Dr. Murray's findings. It is estimated, for example, that 1 in 4,700 Americans have been diagnosed with celiac disease. However, a study in which random blood samples from the Red Cross were tested for celiac disease markers suggests that as many as 1 in every 250 Americans may have it; more recent studies indicate prevalence may be as high as 1 in 133 across the nation, and even as high as 1 in 100 in children in a major metropolitan area. "We're learning that celiac disease appears to be primarily an urban/suburban phenomenon and more common in Caucasians than in any other ethnic group," says Dr. Murray. More than 90 percent of the diagnoses made in Dr. Murray's study are in Caucasians. "Celiac disease is thought to be rare in Asian and sub-Saharan African ethnic groups though no one knows for sure as those populations have not been subject to detailed study."

According to Dr. Murray, over the past 10 years, because of increased awareness and new and improved blood tests, many more people are being diagnosed. His study also has found patients can be overweight or even obese and have celiac disease, "which flies in the face of it being a malabsorption disease." Celiac disease is also an explanation for anemia and osteoporosis and is linked to people with type 1 diabetes. "We're finding that 30 percent of those being diagnosed with celiac disease are either overweight or obese, that weight loss is only a symptom in half the cases we see, and that the disease affects women more than men two-to-one." Also, Dr. Murray's findings indicate that most recent diagnoses are being made in people 45 years and older.

PATIENT PROFILE

As for the Traffs, they are very grateful that everyone in their family has been tested, and that by eating gluten-free, they can control the disease. “As a result of Emily’s diagnosis 10 years ago, we know how to eat gluten-free,” says Mr. Traff, who, since being diagnosed, has cut back on going out to lunch at work because he feels uncomfortable asking people in restaurants if their food contains gluten or not. “It’s more an annoyance than anything else.” And this is a healthy way to look at it.

The NIDDK fosters research on celiac disease. Research studies solicited by the Institute have yielded insights into the pathogenesis, genetics, and prevalence and diagnosis of celiac disease. Furthermore, many recent findings in celiac disease–including the greater than expected prevalence in the U.S., insights into the underlying causes of disease symptoms, and further characterization of the links between celiac and other autoimmune and digestive diseases–were the topic of a recent meeting of the statutory Digestive Diseases Interagency Coordinating Committee, a federal group led by the NIDDK, that promotes information exchange and agency collaborations geared toward combating digestive diseases. As a result of discussions at this meeting, the NIDDK is planning an “NIH Consensus Development Conference” on celiac disease. The NIH convenes these conferences to address complex issues of medical importance to health care providers, patients and the general public. The goal for the June 2004 conference is to rigorously assess the state of the science and medical practice for celiac disease and to identify the most pressing clinical research questions for pursuit in the near future. Through these efforts, the Institute hopes to reach a better understanding of celiac disease, and to facilitate improved rates of diagnosis and treatment, and the development of prevention strategies for this serious condition.



These fat cells were isolated using a method developed by Nobel laureate Dr. Martin Rodbell, whose prize-winning work was supported by NIDDK. Understanding fat cell biology is a key element in learning how to prevent and treat obesity. Photo: Dr. Joseph Brzostowski and Ms. Mary-Jane Zarnowski, NIDDK.

Obesity

Obesity is one of our nation's most pressing health problems, and it disproportionately affects racial and ethnic minorities–especially minority women. A strong risk factor for type 2 diabetes, obesity is also associated with other health conditions within the mission of the NIDDK, including, for example, gallbladder disease, urinary incontinence, and the fatty liver disease non-alcoholic steatohepatitis (NASH). Nearly 31 percent of adults in the U.S. are considered obese based on body mass index (BMI), a measure of weight relative to height.[1] Furthermore, 15 percent of children and teens in the U.S. are overweight, and are thus at risk for serious health problems early in life and as adults.[2]

The increase in prevalence of obesity in the U.S. in the past two decades is thought to result from the interaction of genetic susceptibility with behavior and factors in our environment that promote increased caloric intake and physical inactivity. The NIDDK has thus been supporting a multidimensional research portfolio on obesity ranging from basic studies to large clinical trials. This research includes, for example, investigations to elucidate the hormones and signaling pathways that influence appetite and energy expenditure; exploration of genetic factors that predispose individuals to obesity; studies of nutrition, including diet composition; research encompassing physical activity; and studies aimed toward obesity prevention through the development and testing of modifications of environmental factors in schools, the home, and other settings. A large clinical trial, Look AHEAD (Action for Health in Diabetes), will be examining the health effects of an intervention designed to achieve and maintain weight loss over the long term, primarily through exercise and decreased caloric intake, in obese individuals with type 2 diabetes. In another area of clinical research, a Bariatric Surgery Clinical Research Consortium has been established to facilitate and accelerate research on these surgical procedures, which are used to treat severe obesity. The NIDDK additionally supports research on eating disorders that are associated with obesity in some people. Highlights of recent advances from NIDDK-supported research on obesity are provided later in this chapter.

The NIDDK also sponsors education and information programs to bring the results of research to the public and health care providers. Through its Weight-Control Information Network (WIN), the NIDDK produces and distributes science-based information on obesity, weight control, nutrition, and physical activity to health professionals and consumers. The information includes fact sheets, educational brochures, and other publications. Another education effort is "Small Steps. Big Rewards. Prevent Type 2 Diabetes." This educational campaign is promoting the dramatic effects of modest weight loss and moderate changes in diet and physical activity on reducing the risk for type 2 diabetes. The "Small Steps" campaign is based on the results of the Diabetes Prevention Program clinical trial, which was supported by the NIDDK and others. The campaign is sponsored by the National Diabetes Education Program (NDEP), a partnership of the NIDDK, the Centers for Disease Control and Prevention, and over 200 public and private organizations.

---

[1] This information is from data published at the time this document went to press; see, for example: Statistics Related to Overweight and Obesity. NIH Publication No. 03-4158, July 2003. http://www.niddk.nih.gov/health/nutrit/pubs/statobes.htm. New statistical information is expected to become available in 2004.

[2] Because there is no generally accepted definition for obesity, as distinct from overweight, in children and adolescents, this document uses the terms overweight and obesity interchangeably for this age group.

ORGANIZATIONAL ENHANCEMENTS

New efforts are under way to accelerate progress in research to address the increasingly severe obesity epidemic and its serious implications for public health. Within the NIDDK, the Office of Obesity Research was created by the Institute Director in early FY 2003 to encourage multidisciplinary approaches to obesity and to coordinate obesity-related research within the Institute. The Office is located organizationally under the auspices of the Office of the Director, NIDDK, and its co-directors represent the two NIDDK Extramural Divisions with primary responsibility for obesity-related grants: the Division of Digestive Diseases and Nutrition and the Division of Diabetes, Endocrinology, and Metabolic Diseases. The NIDDK Director has also established an Obesity Research Working Group in the Institute. The responsibilities of this Working Group are to provide a forum for sharing and coordination of trans-NIDDK and trans-NIH obesity research activities; to assist the Director, NIDDK, in identifying research opportunities, initiatives, and advances; to identify and plan appropriate workshops and conferences; and to assist in preparation of obesity-related reports and responses to inquiries. An integral and essential component of the ongoing obesity research planning process is the solicitation of advice from external scientific and lay experts.

New Research Initiatives: Since its inception, the NIDDK Office of Obesity Research, with input from external experts, has developed new obesity-related initiatives and has planned several conferences on a variety of obesity-related topics. For example, one initiative will encourage studies on diet composition and energy balance to understand more fully how different attributes of foods (such as the amount and types of fats, carbohydrates, and proteins they contain) may affect appetite, weight loss, and other biological processes relevant to obesity. Another effort is soliciting grant applications for ancillary studies to existing obesity-related clinical trials and networks; such studies would help maximize the value of resource investments and contributions of volunteers. This effort is also one of several planned to foster collaborations between basic scientists and clinical investigators. In another area, to enhance genetic research, the NIDDK is encouraging new studies in animals or other "model organisms" in which techniques for gene discovery are currently much more powerful than in humans. Identification of obesity-related genes in other organisms can lead scientists to discovery of similar obesity-related genes in humans. New multidisciplinary research collaborations will be promoted to bridge the gap between our understanding of behavioral influences on human obesity and our understanding, at the molecular and genetic levels, of biological pathways in the brain that are involved in food intake. Because of the critical importance–and extraordinary difficulty–of maintaining weight loss over the long term, the NIDDK is planning to solicit new studies to elucidate factors associated with weight maintenance and weight regain after intentional weight loss. The NIDDK is planning to solicit new research to help clarify the effects on overweight and obesity of environmental factors relevant to development, such as, for example, nutritional conditions present during fetal development. Another planned effort will promote research to identify biological markers associated with obesity-related health conditions using proteomics technology. Such studies seek to gain a comprehensive understanding of proteins in cells, tissues, and body fluids and how they function together.

Scientific Meetings–Setting the Stage for the Future: Examples of recent and upcoming meetings sponsored by the NIDDK to glean information and advice from external experts include the following. A workshop in the fall of 2003 fostered discussion of a variety of obesity-associated phenotypes (traits), aside from measurements of BMI, that might be useful to implement in a future large-scale human genetics study. The role of biological factors secreted by fat cells in the development of obesity-associated health conditions was the topic of another recent workshop. In early 2004, the NIDDK convened a conference on issues relating to "translating" the results of clinical trials on diabetes and obesity. One form of translational research aims to determine what can improve outcomes in diverse,

real-world populations and how to achieve these goals in a practical way that positively affects public health. An upcoming workshop will explore the role of lipids (such as different types of fatty acids, cholesterol, and other molecules) in the development of obesity and its associated diseases. All of these meetings will help inform the planning of future research efforts in obesity.

NIH OBESITY RESEARCH TASK FORCE

At the agency level, the NIDDK and the National Heart, Lung, and Blood Institute (NHLBI) are co-leading the NIH Obesity Research Task Force, which was established by the NIH Director in the Spring of 2003 to facilitate progress in obesity research across the agency. The Task Force is co-chaired by the NIDDK Director and the Acting Director of NHLBI, and its membership includes representatives from these Institutes and numerous other NIH components. Among the activities of the Task Force is the ongoing development of trans-NIH research initiatives for FY 2005 that would address pediatric obesity, neurobiologic research relating to obesity, and other areas. In response to a key element of the NIH Director's charge to the Task Force, an NIH strategic plan for obesity research is under development and is expected to be available in early 2004. To complement the strategic plan document, the Task Force is also preparing a website on NIH obesity research, also to be launched in early 2004. The primary purposes of this new website will be to help inform investigators of NIH funding opportunities for obesity research, to provide information on NIH-sponsored scientific meetings relevant to obesity, and to provide other information relevant to obesity research. In providing this information, the website will reflect the dynamic and ongoing planning process for obesity research at the NIH. Additionally, while the focus of this website will be research, the site will also include links to other NIH websites that provide information to the public and health professionals on weight loss, nutrition, physical activity, health problems associated with obesity, and other topics relevant to obesity.

Examples of Recent NIDDK-Supported Obesity Research Advances

BASIC RESEARCH

Basic research is providing insights into the molecular mechanisms underlying the development of obesity and its associated health conditions. The research advances highlighted below are examples of studies in animal models that are elucidating the roles of hormones and other molecules important in regulating body processes relevant to obesity.

Hunger Pangs in the Brain? A Potential New Brain Circuit for Appetite Regulation: Scientists have recently defined what may be a novel circuit in the brain for regulating appetite and energy balance. This circuit involves the appetite-stimulating hormone ghrelin. Levels of this hormone not only rise just before a meal to stimulate eating, but also rise after diet-induced weight loss–with a resulting increase in appetite. These effects provide one possible explanation for why dieters find it difficult to keep pounds off. Because ghrelin is made primarily in the stomach, and because there are "receptors" (docking sites) for ghrelin in the brain, this hormone was thought to be a signal from the gut to the brain to indicate when it was time to start a meal. While this gut-brain signaling may occur, ghrelin is also known to be manufactured within the brain itself. Taking this information as a potential clue that there may be another pathway for ghrelin's actions, a team of scientists has now further explored the brain's production of ghrelin. Many cells in the brain have been shown to be involved in energy balance. However, by studying the brains of rodents, the scientists discovered that ghrelin is made by a group of brain cells not previously known to influence energy balance. Intriguingly, these ghrelin-producing cells are located adjacent to brain cells that produce a protein called NPY, which functions to increase appetite–and which has also been known to help mediate the effects of ghrelin. The scientists also found that ghrelin can stimulate the activity of NPY-producing cells. Based on these results and several other experiments, the scientists

proposed that the brain's own indigenous source of ghrelin may activate the production of NPY by neighboring brain cells, thus leading to increased appetite. By uncovering what may be a previously unknown brain regulatory circuit, these studies provide further insights into the body's complex regulation of energy balance.

Animal Model To Study the Metabolic Syndrome: Animal models of specific diseases or syndromes are critical tools to advance research. The metabolic syndrome is a cluster of medical problems, including obesity, insulin resistance, high blood pressure, and hyperlipidemia (high lipid levels), that appear in varying combinations, and that put people at increased risk for cardiovascular disease and for type 2 diabetes and its complications. Researchers have recently generated a mouse model that mimics the human metabolic syndrome. These mice were genetically-engineered to have fat cells that contained extra amounts of a cortisol-producing enzyme. The researchers had previously shown that the mice developed abdominal obesity and insulin resistance. They have now determined that the mice also have high blood pressure. This mouse model will be a useful tool to understand biological processes that are important in the metabolic syndrome. In addition, the cortisol-producing enzyme appears to play a role in regulating many aspects of the metabolic syndrome, including high blood pressure. Identifying agents that target this enzyme may be a useful approach for treating the metabolic syndrome.

Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, and Horvath TL: The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37: 649-61, 2003.

Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, Shinyama H, Sharp MG, Fleming S, Mullins JJ, Seckl JR, and Flier JS: Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *J Clin Invest* 112: 83-90, 2003.

OBESITY, DIET AND ACTIVITY–EXAMPLES OF STUDIES WITH ADULT VOLUNTEERS

Obesity results from an energy imbalance–that is, when the number of calories eaten exceeds the number of calories burned for energy in order to maintain essential body functioning and to power physical activities such as walking. Thus, investigators are conducting research on behavioral changes in diet and activity that may help people to lose excess weight or prevent weight gain. A number of studies are exploring various types of dietary modifications and how they may affect health and body weight. With respect to physical activity, researchers are addressing both the problems associated with too much sedentary behavior and the potential benefits of increasing activity.

Low Carbs *versus* Low Fat–Is Either Diet Better?: Investigators recently compared the effects of a specific low-carbohydrate diet with a more conventional diet in a small-scale study of obese men and women. The test diet was low in carbohydrates and high in fat and protein; the "conventional" diet was high in carbohydrates and low in fat and calories. Participants in the study were assigned to follow one of these two diets for a year. While the participants on the low-carbohydrate diet lost more weight early on than those on the conventional diet, by the end of the year there was no significant difference in weight loss between the two groups. With respect to several health conditions associated with obesity, participants on the low-carbohydrate diet had a greater improvement in some risk factors for heart disease, including a greater increase in high-density lipoprotein cholesterol ("good" cholesterol) and a greater decrease in triglyceride concentration. As is often the case with diets, however, many participants from each group did not adhere to their assigned diet and dropped out of the study before the end of the year. Overall, the researchers concluded that longer and larger studies would be necessary to adequately assess whether low-carbohydrate, high-protein, high-fat diets are safe and effective.

Increased Risk for Obesity and Type 2 Diabetes From TV Watching and Other Sedentary Behaviors: In a large study of thousands of women, researchers found that sedentary behaviors–and especially sitting while watching television–are predictive of significantly greater risk of obesity and type 2 diabetes. Those who, on average, watched more TV per day were at higher risk. In fact, each 2 hour per day increase in TV watching during the course of the study was associated with a 23 percent increase in risk for obesity, and a 14 percent increase in risk for developing type 2 diabetes. This research adds to earlier findings that related TV watching and obesity in children.

Exercise and Weight Management: In a new study, investigators observed beneficial effects of moderate intensity exercise on weight management in young men and women who previously had sedentary lifestyles and were overweight or moderately obese. Because the participants did not change their diets during the study, the results highlight specific effects of exercise on weight. Participants were assigned to either to a supervised 16-month exercise program or to a control group instructed to maintain their usual activity levels. The exercise program consisted mainly of walking on treadmills, and the participants started with an initial 20 minutes and built up to 45 minutes per session, five sessions a week. Their goal was to burn a minimum of 400 calories per session. The men in the study lost an average of approximately 6 percent of their body weight, and most of this weight loss came from body fat. Weight losses of similar amounts have been shown previously to have positive effects on health. Visceral fat–that is, fat surrounding the internal organs in the abdomen–declined significantly in the men who exercised; this result is encouraging because visceral fat is thought to be particularly associated with health risks. The results of the exercise program were different in women, although still beneficial. The female participants did not lose weight, but they also did not gain weight. By contrast, the women in the sedentary control group gained extra weight by the end of the study period. The women who exercised also had slightly less total body fat and visceral fat as compared to the women in the control group. The reasons for the differences seen between the men and women are as yet unclear, and it may be that diet modification to reduce energy intake, in addition to this level of exercise, would be needed to achieve weight loss in women. In considering the positive effects of the study, the investigators pointed out that the challenge now is to develop effective ways to help overweight and moderately obese people maintain an exercise program over the long-term.

Donnelly JE, Hill JO, Jacobsen DJ, Potteiger J, Sullivan DK, Johnson SL, Heelan K, Hise M, Fennessey PV, Sonko B, Sharp T, Jakicic JM, Blair SN, Tran ZV, Mayo M, Gibson C, and Washburn RA: Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: The Midwest Exercise Trial. *Arch Intern Med* 163: 1343-50, 2003.

Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, Rader DJ, Edman JS, and Klein S: A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 348: 2082-90, 2003.

Hu FB, Li TY, Colditz GA, Willett WC, and Manson JE: Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 289: 1785-91, 2003.

OBESITY IN CHILDREN AND ADOLESCENTS

The dramatic increase in overweight and obesity in children and adolescents has ominous implications for our Nation's future health. There is an ominous link between obesity and such serious diseases as type 2 diabetes–once viewed as a disease of older adults but now increasingly seen in children. Thus, the rise in childhood obesity may portend a lifetime of devastating health problems for many of our country's children and adolescents, as well as an escalation in the demands on our health care system. Following is one example of recent NIDDK-supported research on adolescent obesity.

Effect of Diet Macronutrient Content on Weight Loss in Obese Adolescents: As noted, type 2 diabetes is increasingly being diagnosed in young people. Most youth with this form of diabetes are obese, and it is therefore imperative to design interventions to prevent obesity, or to promote weight loss in adolescents who are already obese. In order to understand the role of diet macronutrient content in weight loss in obese adolescents, researchers have compared a conventional, low-fat diet with an experimental diet that has a reduced glycemic-load (GL). GL is based on another parameter, called the "glycemic index," which is a measurement of how a food changes blood glucose levels in a short period of time. The researchers found that obese adolescents who ate a low-GL diet lost more weight compared to those on a conventional diet. This was an especially interesting result, because the adolescents on the low-GL diet were allowed to eat until they reached satiety, whereas those in the low-fat diet group maintained a certain number of calories. Another benefit from the low-GL diet was that it reduced the progressive rise in insulin resistance seen during the study more than the low-fat diet. Because this was a small study of 14 adolescents, further studies will have to be performed to determine if a low-GL diet has the same benefit in a larger group. If so, this may be a potential intervention strategy to promote weight loss and improve insulin sensitivity in obese adolescents.

Ebbeling CB, Leidig MM, Sinclair KB, Hangen JP, and Ludwig DS: A reduced-glycemic load diet in the treatment of adolescent obesity. *Arch Pediatr Adolesc Med* 157: 773-9, 2003.

OBESITY–ENVIRONMENTAL INFLUENCES AND LONG-TERM HEALTH EFFECTS

Years of Life Lost Due to Obesity: As one way to address the seriousness of the obesity epidemic, a variety of public health messages can be developed to convey the deleterious health effects of obesity. Thus, to build upon existing information available for health campaigns, a group of scientists recently sought to express the impact of obesity on individual health in terms of "years of life lost." This approach assesses the difference between the expected life span of an individual who is not obese (or overweight) and the number of years the person might live if he or she were obese. To determine the "years of life lost" due to obesity, the scientists evaluated data on Caucasian and African American men and women from the third National Health and Nutrition Examination Survey (NHANES III), which collects information on the health and diet of people in the U.S. The scientists found that the "years of life" lost associated with obesity was most dramatic at younger ages. Caucasian men 20 to 30 years old who are severely obese can expect lifespans up to 13 years shorter than someone who is not overweight; severely obese Caucasian women may lose up to 8 years of life. Among young African-American women, those who are severely obese may lose up to 5 years from their expected remaining life span. Alarmingly, severely obese young African-American men may lose up to 20 years of life. While the reasons for the race and gender differences are not yet clear, the study presents a striking picture of the effects of obesity. The scientists further placed their findings in the context of yet another harmful aspect of obesity: not only does obesity shorten life, but previous research suggests that it also impairs the quality of life.

A Few Less Bites and a Few Extra Steps–Dealing with Obesity in Our Current Environment: Given the dramatic increases in levels of obesity in the population, a team of researchers recently proposed a short-term strategy that may help individuals prevent further weight gain in our current "obesogenic" environment. Their strategy consists of small behavior changes that would hopefully fit to into most people's lifestyles: eating "a few less bites" at each meal, even without changing the types of foods eaten, or walking an extra mile–amounting to 15 to 20 minutes per day–even if the extra walking is not done all at once. Essentially, these changes would be designed to shift energy balance by 100 kilocalories per day, through reducing calorie intake and/or increasing calorie burning through physical activity. How did the researchers end up

with this number of calories? By reviewing data from large-scale national studies, they first estimated the average weight gain per year of adults in the U.S. Then, estimating the number of calories needed to form each extra pound of body weight, the researchers calculated an "energy gap" of 100 kilocalories–the amount of energy that people who are gaining weight may be taking in and storing as excess pounds, rather than burning off. The strategy of small behavior changes to close this energy gap has yet to be tested with volunteers, and it is not clear how it may need to be adjusted to prevent excess weight gain in children. Nevertheless, the researchers suggest that their strategy, with a specific target of preventing even further weight gain–rather than attempting to reduce current levels of obesity–may be something feasible that can be pursued now to halt the rising obesity epidemic.

In addition to their short-term strategy, these researchers also advocate longer-term and more challenging goals. These include: (1) promoting broad social changes to build an environment more conducive to healthy lifestyles, and (2) developing improved ways to help people change behaviors in an environment that may never revert to a time when a lesser food supply and a greater need for physical labor might have made worries about body weight unnecessary.

Fontaine KR, Redden DT, Wang C, Westfall AO, and Allison DB: Years of life lost due to obesity. *JAMA* 289: 187-93, 2003.

Hill JO, Wyatt HR, Reed GW, and Peters JC: Obesity and the environment: Where do we go from here? *Science* 299: 853-5, 2003.

WIN: The Weight-Control Information Network

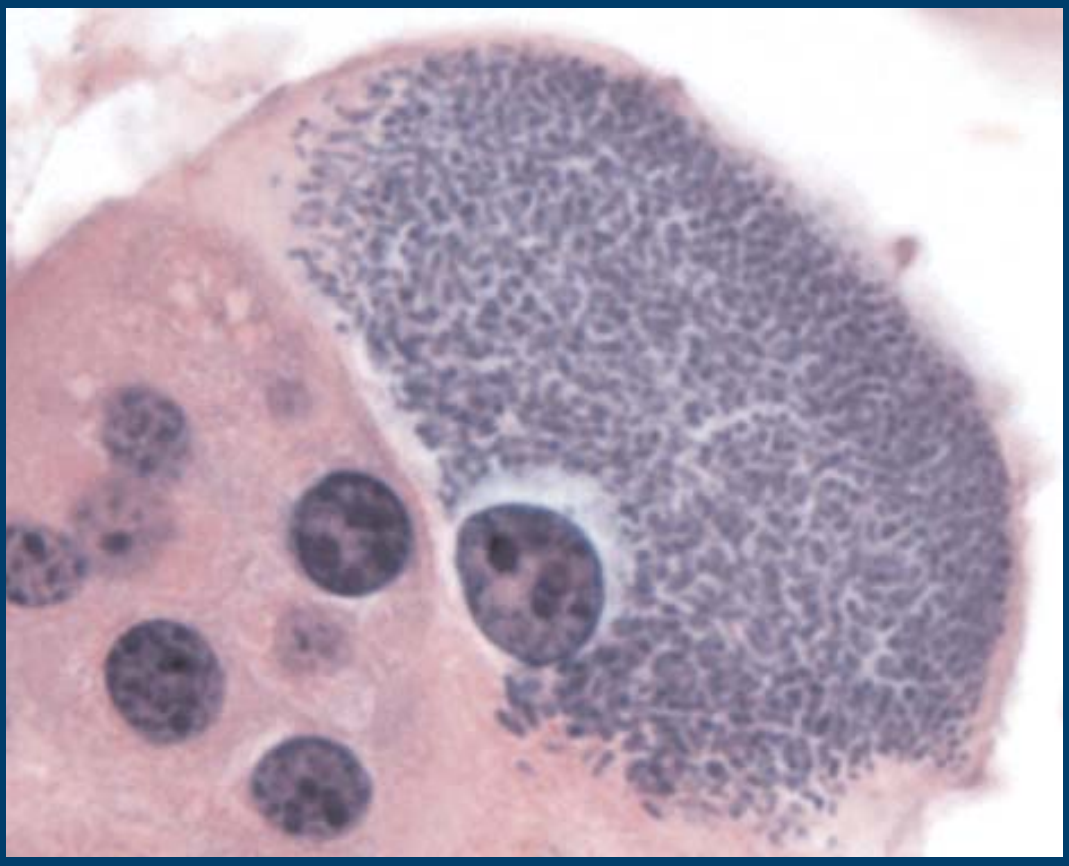
When the Department of Health and Human Services (HHS) released its report entitled, "The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity," Secretary Tommy G. Thompson warned that, "Overweight and obesity are among the most pressing new health challenges we face today." It has been estimated that 300,000 deaths each year in the U.S. are associated with obesity and overweight, and the numbers are increasing. Obesity is an epidemic that must be brought under control. The WIN is an NIDDK health information service directed at helping to reach this goal.

Established in 1994, the NIDDK's Weight-control Information Network (WIN) is a national information service that produces and provides science-based information on obesity, physical activity, weight control, and nutrition to health professionals, people who are overweight or obese, and other information consumers. The WIN has reached out to all age groups and diverse ethnic and racial groups with its materials.

Recently, the WIN published a series of booklets, "Healthy Eating and Physical Activity Across Your Lifespan," to encourage better eating and physical activity habits. The series contains four booklets entitled, "Tips for Parents," "Tips for Adults," "Tips for Older Adults," and "Tips for Pregnant Women." These booklets are published in both English and Spanish language versions. The latest publication produced by the WIN is "Just Enough for You." This publication defines the difference between food portions and food servings, and provides information on ways to control the size of food portions and to improve nutrition with minimal changes in eating habits.

Non-Hispanic African-American women have been hardest hit by the nation-wide rise in overweight and obesity. Based on body mass index (BMI)–a measure of weight relative to height–77.3 percent of adults in this group are overweight or obese. The WIN's "Sisters Together: Move More, Eat Better" initiative was developed in the 1990s to encourage African-American women 18 years of age or older to maintain a healthy weight by increasing physical activity and eating more healthful food. A planning guide and kit based on the pilot phase of this initiative are available to provide step-by-step instructions for planning, promoting, implementing, and evaluating community health awareness programs to prevent African-American women from becoming overweight. "Sisters Together" has also produced other informational brochures.

The WIN is also coordinating with the Institute's Look AHEAD (Action for Health in Diabetes) clinical trial. The Look AHEAD trial is a large-scale, multi-center trial that is examining whether a lifestyle intervention designed to achieve voluntary long-term weight loss will improve cardiovascular and other outcomes over the long term in obese individuals with type 2 diabetes. The WIN is providing a portion of the information on physical activity and healthy eating that is given to trial participants.



In mouse bladder tissue, a dense "biofilm" of bacteria (at right side, stained dark blue) completely fills a cell. Forming biofilm-like pods within cells may be a way for infection-causing bacteria in the urinary tract to survive antibiotic treatments and cause recurrent infections. Unaffected bladder cells (stained pink, with dark purple nuclei) are visible at left. Photo: Dr. Joseph Palermo and Dr. Scott Hultgren, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO. Reprinted with permission from Anderson GG *et al, Science* 301:105-7, 2003. © 2003 AAAS.

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 is committed to enhancing research to understand, treat, and prevent these diseases.

Normal, healthy kidneys filter toxins from the blood so that they may be excreted. In people with chronic kidney disease, these organs are less able to perform this life-sustaining function. Persons whose disease progresses to irreversible kidney failure, also known as end-stage renal disease (ESRD), require dialysis or kidney transplantation to live. Conservative estimates find that 4.5 percent of American adults 20 years of age and older–about 7.4 million adults–have substantially impaired kidney function. The leading cause of kidney disease is diabetes, with hypertension (high blood pressure) the second-leading cause. The recent increases in obesity and type 2 diabetes in the U.S., if left unchecked, will have grave implications in several years, as more people begin to develop renal complications of diabetes.

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

The U.S. has seen an enormous increase in people with ESRD. The NIDDK-supported United States Renal Data System, a nationwide database covering kidney disease, reports that nearly 100,000 people developed ESRD in 2001 and a total of nearly 400,000 patients were living with the disease at the end of that year. These numbers have doubled since 1990 and are expected to nearly double again by 2010. The cost of ESRD is high–nearly $23 billion in public and private spending for healthcare alone in 2001.

The NIDDK devotes significant resources to understanding the basic mechanisms underlying chronic kidney disease. The 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. Research areas 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 the hemolytic uremic syndrome. A major new, pilot educational outreach effort is the National Kidney Disease Education Program.

Urologic diseases affect persons of all ages, result in significant health care expenditures, and, if mis-diagnosed or improperly treated, may lead to irreversible kidney and/or bladder damage and possibly death. The NIDDK's urology research portfolio includes basic and clinical research on the normal and abnormal development, structure, and function of the genitourinary tract. Nonmalignant

urologic diseases include benign prostatic hyperplasia, prostatitis, urinary tract infections, urinary tract stone disease, interstitial cystitis, urinary incontinence, and congenital anomalies of the genitourinary tract.

Benign prostatic hyperplasia, or BPH, is a condition that affects an estimated 9 percent of men 30 years of age and older, with men 55 and older accounting for most cases. Prostatitis is inflammation of the prostate gland that accounts for a significant percentage of all office visits by young and middle-aged men for complaints involving the genital and urinary systems. The NIDDK is committed to enhancing research to understand, treat, and prevent these diseases.

Infections of the urinary tract are extremely common in women, and many women suffer repeated urinary tract infections (UTIs); according to one recent national health survey, over half of women 20 years of age or older report they have had at least one UTI or related bladder infection (cystitis). In 2000, UTIs and cystitis accounted for over 9 million physician visits. Interstitial cystitis (IC) is a debilitating, chronic, painful bladder disease that has been estimated to affect as many as 847,000 Americans adults, over 90 percent of whom are women. Millions of Americans, most of them women, suffer from urinary incontinence. For both men and women, kidney stones, formally known as urinary tract stone disease, accounted for over 2.2 million physician visits in 2000. In children, one of the most common causes of kidney failure, vesicoureteral reflux, occurs in an estimated 1-to-2 percent of newborns. In fact, abnormalities of the genitourinary tract are the most common birth defects.

To address these and other urologic problems, the NIDDK's urology research efforts support basic, applied, and clinical research in prostate and prostate diseases; diseases and disorders of the bladder; male sexual dysfunction; urinary tract infections; urinary tract stone disease; and pediatric urology, including developmental biology of the urinary tract. A research emphasis of the urology program is the study of chronic inflammatory disorders of the lower urinary tract.

The NIDDK's hematology research program uses a broad approach to understanding the normal and abnormal function of blood cells and the blood-forming system. Research areas include a number of blood diseases, such as 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 have broader application in gene therapy research. An additional priority of the Institute's hematology research programs 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 such as Cooley's anemia (thalassemia major).

BUILDING INSIGHTS IN PKD AND OTHER CYSTIC KIDNEY DISEASES

Polycystic kidney disease (PKD) affects people of all ages and is the fourth leading cause of kidney failure in the U.S. PKD causes massive enlargement of the kidneys due to the progressive development of fluid-filled cysts. Mutations in the PKD1 and PKD2 genes are responsible for the most common forms of the disease. The proteins encoded by *PKD1* and *PKD2*, polycystin-1 (PC1) and -2 (PC2), respectively, form a functional complex implicated in cell signaling in kidney epithelial cells, but the precise mechanism by which PKD mutations interfere with kidney tubule development is still unclear. Fascinating new studies in model organisms have led PKD researchers to focus on a single hair-like structure on kidney epithelial cells called the primary cilium. An emerging scientific model suggests that structural or functional defects in this single cilium interfere with normal kidney tissue development, and may be at the root of PKD and other cystic diseases of the kidney. Scientists are rapidly building this picture of PKD mechanisms.

Cilia and Cystic Kidney Disease: A recent study strengthens the link between ciliary defects and PKD, and reinforces the importance of the cilium to normal kidney development. Previously, researchers had shown that PC1 and PC2 co-localize to the cilium, and that loss of functional PC1 and PC2 disables a cell signaling mechanism normally triggered by mechanical stress on the cilium. Loss of this pathway may abrogate the cells' ability to sense and respond to environmental cues regulating kidney tissue development and function. In related work, researchers have now found that proteins implicated in another cystic kidney disease, nephronopthisis (NPHP), also co-localize to the cilium. NPHP is the most frequent inherited cause of ESRD in children and young adults. Researchers recently identified the causative gene for a particular form of the disease that attacks in infancy. This gene encodes a protein called inversin. Inversin is important for determining left-right body axis symmetry, or "patterning," during development, but appears to have other developmental tasks as well. In experiments using a common, simple model of animal development, the zebrafish, the investigators found that if they reduced expression of this animal's inversin gene, it not only disrupted normal development, but caused PKD-like renal cyst formation. In other experiments, they found that inversin interacts with another NPHP disease protein (nephrocystin)–findings which culminated in the discovery that inversin and nephrocystin can be observed together in the cilium of cultured kidney epithelial cells. These results suggest that inversin is important in animal kidney development and that NPHP proteins, like PKD proteins, may fulfill some of their functions through their presence in the cilium.

The roles of the cilium in normal development or maintenance of kidney epithelial cells–and hence, its possible role in cyst formation–are still under investigation. In particular, because PC1, PC2, and other PKD and cystic kidney disease-related proteins are found in other parts of the cell in addition to the cilium, it is unknown whether ciliary dysfunction alone is sufficient to cause disease. However, the results of this recent research bolster the significance of cilia and ciliary proteins in cystic kidney diseases. Continued investigation of both cilia themselves and the PKD-related proteins is required to determine exactly how defects in the kidney epithelial cell cilium may contribute to the pathogenesis of PKD and other cystic kidney diseases.

Potential Therapeutic Target for PKD and NPHP: Another team of researchers has uncovered a promising therapeutic approach for PKD and other cystic kidney diseases. Basic research studies have shown that levels of an important intracellular molecule, cAMP, are increased in kidney cells from PKD animals, and the expression of genes that are responsive to cAMP levels is also altered. This "upregulation" of cAMP levels thus has a multitude of downstream effects on kidney cell growth and function that may influence PKD pathogenesis. Building upon these studies, the researchers found that, in two rodent models of childhood cystic kidney disease, levels of VPV2R are increased. VPV2R is a cell receptor for vasopressin, an anti-diuretic hormone secreted by the pituitary gland in the brain. When vasopressin interacts with VPV2R, it increases levels of cAMP in kidney cells. Using their two animal models, the researchers tested whether a drug that could block this vasopressin receptor could specifically reduce renal accumulation of cAMP and influence the course of PKD. They found that, indeed, early administration of the drug prevented the accumulation of cAMP and inhibited cystic kidney disease development in both models, with an estimated protective effect of up to 75 percent in a rat model of autosomal recessive polycystic kidney disease (ARPKD). Excitingly, they also observed disease regression in the other rodent model, a model of nephronopthisis (NPHP3), when the drug was administered later in life, to adolescent animals–the first interventional study in an animal model of cystic kidney disease to show such an effect. Vasopressin receptor-blocking drugs that have been or are being tested in pre-clinical and clinical studies

are apparently safe, with minimal adverse effects. Importantly, although the researchers worked with models of ARPKD and NPHP3, not ADPKD (the most common form of PKD), the tissues affected by kidney cysts are similar in all three diseases. Thus, such VPV2R blocking drugs may be well-worth studying in future prevention and treatment trials for cystic kidney disease.

In addition to these important fundamental investigations of PKD pathology and treatments, the NIDDK is supporting several clinical initiatives in PKD. The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, or CRISP, is an NIDDK-sponsored study designed to find ways through imaging techniques to monitor changes in the kidneys and kidney cyst size in patients with PKD. The goal is to improve clinicians' ability to monitor the progression of kidney disease in these patients, in order to assess possible strategies for clinical intervention. Furthermore, the Polycystic Kidney Disease Clinical Trials Network has been established to design and implement clinical trials of treatments that will slow the progressive loss of renal function in PKD. The first large interventional clinical trial conducted by this network, the HALT-PKD trial, will be a randomized, controlled trial of the efficacy of blocking the renin-angiotensin system on slowing the rate of decline of kidney function in patients with PKD. The commonly used "ACE inhibitors" target this system, and an ACE inhibitor will be one of the drugs tested in this trial. It is expected that patient recruitment will be initiated in the Spring of 2004. Finally, investigation of PKD and other kidney diseases will benefit from the NIDDK-supported Kidney Disease Clinical Studies Initiative (KDCSI), a new paradigm for kidney disease clinical research. This initiative aims to improve the quality and quantity of clinical studies by maximizing outcomes and reducing costs through sharing of resources obtained in previous studies, such as samples, specimens, and data, and through innovative funding mechanisms.

Gattone VH, II, Wang X, Harris PC and Torres VE: Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med* 9: 1323-6, 2003.

Nauli SM, Alenghat FJ, Luo Y, Williams E, Vassilev P, Li X, Elia AEH, Lu W, Brown EM, Quinn SJ, Ingber DE and Zhou J: Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet* 33: 129-37, 2003.

Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, Ruf RG, Hoefele J, Beekmann F, Landau D, Foreman JW, Goodship JA, Strachan T, Kispert A, Wolf MT, Gagnadoux MF, Nivet H, Antignac C, Walz G, Drummond IA, Benzing T and Hildebrandt F: Mutations in *INVS* encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. *Nat Genet* 34: 413-20, 2003.

FUNDAMENTAL INSIGHTS INTO KIDNEY DISEASE

Each day, the kidneys cleanse the blood by passing it through millions of tiny filtering units called glomeruli. The glomeruli permit waste molecules to move out of the blood to form urine, while holding back other molecules, such as albumin and other critical blood proteins. Diseases that injure the glomeruli–including systemic diseases such as diabetes, and kidney-specific diseases such as focal segmental glomerulosclerosis (FSGS)–lead to excess protein in the urine (proteinuria), edema, hypertension, reduced waste clearance, and other symptoms of kidney dysfunction. Recent research on both systemic molecules and kidney cell proteins is providing new insights into glomerular disease susceptibility and injury.

sFlt1 Antagonism of Angiogenic Factors May Underlie Symptoms of Preeclampsia: Preeclampsia affects approximately 3 to 4 percent of pregnancies and is the leading cause of maternal and fetal death in the U.S. Mothers with preeclampsia develop hypertension, proteinuria, and edema–all resulting from widespread endothelial dysfunction. Abnormal endothelial growth in the glomeruli is the typical kidney lesion of preeclampsia. Although the symp-

toms are known, the cause of preeclampsia has remained elusive. One hypothesis is that a soluble factor(s) secreted by the placenta triggers maternal endothelial dysfunction and subsequent clinical disease. Identifying that factor(s) could be key to developing a treatment or preventive therapy for preeclampsia.

In an important new advance, researchers have found that a soluble protein, sFlt1, is strongly associated with the symptoms and lesions of preeclampsia. Following a screen to identify candidate factors, the researchers found that serum levels of this protein–which are normally elevated during pregnancy–are nearly five-fold higher in patients with severe preeclampsia than in pregnant women with normal blood pressure. This is significant because the protein is a known antagonist of two promoters of new blood vessel development (angiogenesis)–VEGF (vascular endothelium growth factor) and PlGF (placental growth factor). Loss of VEGF activity has also been implicated in the development of hypertension and excess levels of protein in the urine, which are indicative of kidney damage. Consistent with their initial finding, the research team observed that serum levels of free VEGF and free PlGF were significantly decreased in preeclampsic mothers. Using an *in vitro* model system, they also found that that both serum from preeclampsic mothers and control serum supplemented with sFlt1 could disrupt angiogenesis–an effect that could be overcome by adding back VEGF and PlGF. Furthermore, sFlt1 blocked the ability of VEGF and PlGF to relax blood vessels in another experimental system, suggesting that excess levels could induce hypertension. Most significantly, the team was able to reproduce human preeclampsia symptoms and lesions in a rat model by the addition of the sFlt1 protein. Notably, pregnancy was not required for the induction of these symptoms in rats, suggesting that this protein is interfering directly with the maternal endothelium, rather than through a second pregnancy-related factor. The effects were seen in rats with both a high dose of the protein and with lower dosages more closely resembling levels in human preeclampsia patients, differing only in severity of symptoms.

Currently, there is no specific treatment for preeclampsia, and severe cases often require premature delivery of the infant. These results from work done with humans, animal models, and *in vitro* suggest that overproduction of the sFlt1 protein, possibly by the placenta, tips the balance between proangiogenic and antiangiogenic factors in the body, thereby causing generalized endothelial dysfunction. This new knowledge is encouraging, for if abnormalities in levels of this protein are the primary cause for some or all of preeclampsia symptoms in pregnant women, then treatments to overcome its effects may ameliorate symptoms. Furthermore, if this protein is overexpressed early in pregnancy, it may serve as a diagnostic marker for patients at high risk of developing the condition.

Deficiency in Podocyte Protein May Increase Susceptibility to Kidney Disease: How well a person's glomeruli function normally may determine how well they can resist certain types of injury. In each glomerulus, specialized epithelial cells (podocytes) and their associated thin cell junctions (slit diaphragms) are the ultimate filtration barrier preventing loss of crucial blood components to the urine. Several proteins necessary for podocyte function have been identified, including CD2 adaptor protein (CD2AP). Scientists have now uncovered a possible link between CD2AP deficiency and susceptibility to glomerular kidney disease. The CD2AP protein is a component of podocyte slit diaphragms, where it is thought to play an important structural role. In mice, lack of this protein leads to massive proteinuria and early death from kidney failure. Investigators recently examined the kidneys of mice which had some, but much less than normal, CD2AP, and compared them with normal siblings. They found that while none of the mutant mice developed proteinuria during a 1 year study, older CD2AP-deficient mice developed lesions in the glomeruli–including protein deposits similar to those observed in human glomerular disease. Furthermore, the mutant mice were more susceptible than normal mice to experimentally-induced glomerular damage. Finally, electron microscopy studies revealed that the podocytes of the mutant mice had certain defects in an important cellular

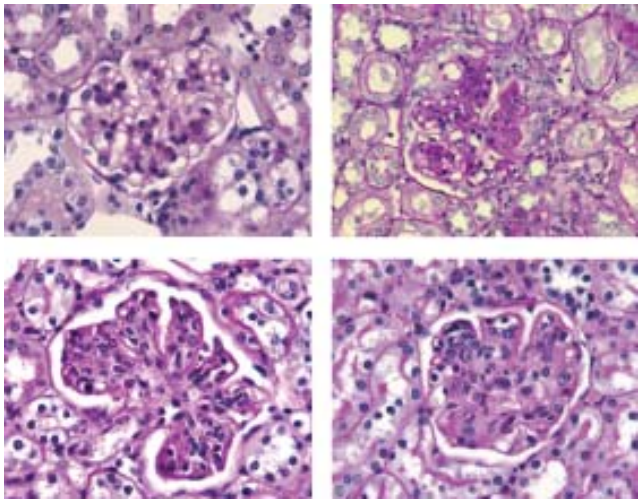
activity–targeting endocytosed proteins for intracellular degradation. This finding is consistent with other studies implicating the CD2AP podocyte protein in regulation of protein trafficking. From these results, it appears that this protein fulfills more than one role in mouse podocytes, and that its insufficient expression may increase vulnerability to glomerular injury over time.

Extending these observations to human glomerular disease, the investigators screened 45 persons suffering from idiopathic or HIV-associated focal segmental glomerulosclerosis (FSGS) for mutations in the human CD2AP gene that might alter protein levels. Among ten individuals, they identified six gene sequence variants that were not found in a control group. Two patients had a specific mutation in one of their two chromosomal copies of the CD2AP gene. This mutation was predicted to cause these patients to produce less of the normal protein. In fact, an easily grown test tissue from these individuals (B cells) showed reduced levels of CD2AP protein. These results suggest that variation in CD2AP levels may indeed contribute to human glomerular disease.

Collectively, glomerular diseases are a leading cause of kidney disease and kidney failure. These findings have provided researchers with new hypotheses to test regarding underlying mechanisms of glomerular injury. Furthermore, identifying factors such as CD2AP gene variants that may predispose individuals to glomerular injury could help clinicians develop tests to identify patients at higher risk for kidney disease.

Kim JM, Wu H, Green G, Winkler CA, Kopp JB, Miner JH, Unanue ER and Shaw AS: CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science* 300: 1298-300, 2003.

Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP and Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111: 649-658, 2003.



A mouse kidney glomerulus (upper left panel, center) becomes scarred and/or physically deformed in mice deficient in an important glomerular protein, CD2AP (other three panels). These findings suggest that deficiency in the protein may contribute to glomerular kidney disease. Photo: Dr. Jeong M. Kim and Dr. Andrey S. Shaw, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO. Reprinted with permission from Kim JM *et al, Science* 300:1298-300, 2003. © 2003 AAAS.

REGRESSION TO NORMAL KIDNEY FUNCTION IS COMMON IN TYPE 1 DIABETES PATIENTS

Type 1 diabetes patients are at increased risk for developing complications, such as kidney disease. Previous research had suggested that patients who secreted very slightly elevated levels of the protein albumin in their urine (microalbuminuria) had a very high risk of developing kidney damage. In a recent advance, researchers conducted a six-year study of nearly 400 type 1 diabetes patients, all of whom had microalbuminuria. Strikingly, they found that only 19 percent of these patients developed kidney disease, while approximately 60 percent underwent a regression to normal levels of urinary albumin. They found the regression to be dependent on factors such as age, cholesterol or triglyceride levels, blood pressure, and hemoglobin-A1c levels (a measure of long-term blood glucose control). Therefore, microalbuminuria in patients with type 1 diabetes does not always lead to diabetic kidney disease. The study also emphasizes that good control of blood pressure, lipids, and HbA1c levels may promote this beneficial regression.

The NIDDK is supporting many efforts aimed at reducing the onset or burden of kidney disease as a complication of diabetes. For example, the Institute is supporting the Mouse Models of Diabetes Complications Consortium. The Consortium is generating genetic mouse models to analyze the initiation and progression of diabetic complications, including kidney disease. Such accurate models of human diabetic kidney disease, once developed, will be especially valuable in uncovering the genes and cellular processes that confer disease susceptibility or resistance. Candidate methods for the prevention, detection, and treatment of diabetic kidney disease may also be effectively tested in these mouse models.

The Institute is also supporting studies of genetic influences on the development of kidney complications in diabetes. Families of patients with diabetic nephropathy have an increased prevalence of renal disease and certain populations appear to be more susceptible. Delineating the genetic loci associated with the development and progression of diabetic nephropathy could lead to improved outcomes; therefore, the NIDDK and the NIH National Center for Minority Health and Health Disparities have established the Family Investigation of Nephropathy of Diabetes (FIND) Consortium. FIND began in September 1999 and will conclude in September 2004. The overall goal of FIND is to identify genetic pathways that may be critical for the development of nephropathy, and that may lead to candidate genes or genetic pathways amenable to therapeutic strategies to prevent disease onset or progression.

To foster promising research more effectively on diabetes complications, including kidney complications, the NIDDK recently established an Institute Working Group for Diabetes Complications. The goals of this group are to provide seamless integration of NIDDK activities related to complications, including workshops, initiative planning and oversight of existing projects and trials; to establish liaison with other Institutes and to develop activities that will increase interest in diabetes complications in other scientific communities; and to lead future strategic planning activities on diabetes complications. Through these and other efforts, the NIDDK seeks to ensure continued progress in research leading toward improved clinical management of the kidney complications of diabetes.

Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH and Krolewski AS: Regression of microalbuminuria in type 1 diabetes. *N Engl J Med* 348: 2285-93, 2003.

ELIMINATING HEALTH DISPARITIES IN KIDNEY DISEASE

Chronic kidney disease and kidney failure disproportionately affect ethnic and racial minority populations in the U.S. The NIDDK is striving to reduce the burden of kidney disease in these populations through its support of focused clinical research efforts and initiatives. Steady progress is being made, as illustrated by the following recent advances.

Testing the Effects of Lowered Blood Pressure on Kidney Disease in African Americans: Within the general U.S. population, high blood pressure (hypertension) is a leading cause of end-stage renal disease (ESRD). However, African Americans have a particularly high risk for developing ESRD as a result of hypertension. The African American Study of Kidney Disease and Hypertension (AASK) was a clinical trial of over 1,000 African Americans with signs of hypertensive kidney disease. Participants received one of three medications–a beta-blocker, a calcium channel blocker, or an ACE inhibitor–and were treated with the goal of lowering blood pressure to either normal levels or lower-than-normal levels. During the trial, treatment with the calcium channel blocker was discontinued because it was less effective than either of the other two drugs at slowing the progression of kidney disease. Recent analysis of the data now reveals that there was no difference in rates of kidney disease progression between the groups that maintained either “normal” or “low” blood pressure.

However, kidney function in patients taking the ACE inhibitor was better than in those taking the other drugs. This study has important implications for the medical management of hypertensive kidney disease.

Nurse-Directed Diabetes Care Is Beneficial to Minorities with Diabetes: Most diabetes patients do not achieve the recommended strict control of their disease that decreases their risk for developing disease complications. This is a serious problem in minority populations, who are already at disproportionately increased risk for developing complications. A recent study determined that type 2 diabetes care directed by specially-trained nurses, who are under the direction of a physician, improved disease management in minority patients, compared to standard physician-only directed care. Nurse-directed care was already known to improve diabetes management in middle-class populations, and this study confirms the same benefits in a minority population of Hispanic- and African-American patients. Researchers studied several parameters of diabetes management, such as the number of times patients visited the clinic for routine diabetes monitoring. In nearly every parameter, patients under the care of a nurse had better management than patients under standard physician-directed care. Thus, nurse-directed care can improve disease management, which may have a dramatic effect in reducing morbidity and mortality in this high-risk population.

Encouraging Kidney Donation: Members of racial and ethnic minority groups, particularly African Americans, American Indians, Alaska Natives, and Hispanic Americans, are disproportionately afflicted with end-stage renal disease (ESRD). The most effective therapy for ESRD is kidney transplantation because it most improves patients' quality-of-life and survival rates. However, the number of organs and tissues donated by members of minority groups and other underserved populations is low; therefore, the likelihood is reduced for a good match between donor and recipient and, ultimately, survival of the transplanted organ. To address the present health disparities, the NIDDK, in collaboration with the NIH National Center on Minority Health and Health Disparities (NCMHD), established the Minority Organ and Tissue Donation Program. The program is expected to create an environment supportive of organ donation in racial and ethnic minority communities by increasing their exposure to organ donation messages and their opportunities to express donation commitments; evaluating the impact of increased support for living organ donation; increasing minority cadaveric and living organ donations; and increasing donation from non-traditional donors (for example, older donors and living donors). With more organs and tissues from minority groups in the donor pool, the survival rates and quality-of-life are expected to improve for ESRD patients from racial and ethnic minority groups.

Davidson MB: Effect of nurse-directed diabetes care in a minority population. *Diabetes Care* 26: 2281-7, 2003.

Wright JT, Jr., Bakris G, Greene T, Agodoa LY, Appel LJ, Charleston J, Cheek D, Douglas-Baltimore JG, Gassman J, Glassock R, Hebert L, Jamerson K, Lewis J, Phillips RA, Toto RD, Middleton JP and Rostand SG: Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: Results from the AASK trial. *JAMA* 288: 2421-31, 2002.

EFFORTS TO HALT PEDIATRIC RENAL DISEASE

Kidney disease in children has numerous causes and can have long-lasting effects on health and development. Acute renal (kidney) failure, though often transient, can be devastating in pediatric patients. Diarrhea-associated hemolytic uremic syndrome (HUS), although rare, is the most common cause of acute renal failure in previously healthy children in the U.S. The trigger in most cases is intestinal infection with certain strains of the bacteria, *Escherichia coli*. While *E. coli* is a normal component of the human gut flora, some strains, called STEC strains, produce "Shiga toxins." These toxins

are absorbed from the gastrointestinal tract and bind to the surface of cells lining the blood vessels, leading to diffuse vascular injury and organ failure. One example of a Shiga toxin *E. coli* strain is *E. coli* O157. STEC infection is commonly acquired through undercooked, contaminated meat, particularly beef. Because recent reports estimate that nearly 40 percent of pediatric cases of diarrhea-associated HUS require temporary dialysis and the mortality rate is 3 to 5 percent despite intensive supportive care, researchers are trying to identify effective interventions to prevent absorption and of the Shiga toxins.

In a recent randomized, controlled, double-blind clinical trial in nearly 150 children, researchers tested whether oral administration of a Shiga-toxin-binding agent diminishes the severity and improves the clinical course of diarrhea-associated HUS. The main outcome measures were the frequency of death or other serious extra-renal events and need for dialysis in the treated group as compared to the placebo group. Unfortunately, the researchers found that similar numbers of serious events occurred in the groups (18 and 20 percent, respectively) and there was a similar need for dialysis (42 and 39 percent, respectively). However, these results, although negative, are important, because they will help point researchers in other directions and reinforce the current efforts to prevent diarrhea-associated HUS through safer food handling and preparation.

Chronic kidney disease is another serious burden in children, with consequences for growth and development lying beyond the immediate reduction in kidney function. To stimulate research in understanding pediatric kidney disease and its complications, the NIDDK–in collaboration with the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute of Child Health and Human Development (NICHD)–has funded the Prospective Study of Chronic Kidney Disease in Children. The primary goals are to determine the risk factors for the decline in kidney function in these patients; the incidence of, and risk factors for, impaired neurocognitive development and function; and the prevalence of risk factors for cardiovascular disease; and long-term effects of growth failure and its treatment. The information obtained from this study will establish natural history and outcome measures for future intervention or prevention trials. In addition, the NIDDK is funding a study of focal segmental glomerulosclerosis (FSGS) in children and young adults that may yield further insights into the problem of kidney disease in young people. Finally, in 2004, the NIDDK is planning an initiative on vesicoureteral reflux (VUR), a urologic disease primarily affecting children that can lead to serious infection and kidney failure. These efforts are intended to lead to greater progress in reducing the serious effects of kidney disease in children.

Trachtman H, Cnaan A, Christen E, Gibbs K, Zhao S, Acheson DW, Weiss R, Kaskel FJ, Spitzer A and Hirschman GH: Effect of an oral Shiga toxin-binding agent on diarrhea-associated hemolytic uremic syndrome in children: A randomized controlled trial. *JAMA* 290: 1337-44, 2003.

IMPROVING OUTCOMES ON DIALYSIS

Kidney failure is a growing problem that can be prevented or slowed, but only a fraction of people who are at high risk are screened or managed appropriately. People with irreversible kidney failure, also known as end-stage renal disease (ESRD), require dialysis or a kidney transplant to survive. Patients, insurers, and the U.S. Government's Medicare program paid nearly $23 billion to treat nearly 400,000 people for kidney failure in 2001. Most are on dialysis, and most dialysis patients are on hemodialysis. Diabetes and hypertension are leading causes of kidney failure.

Although current standards for hemodialysis treatment are effective, some physicians believed that an increased dialysis dose or the removal of larger waste particles, using a high-flux dialysis filter, would improve survival. The NIDDK's Hemodialysis (HEMO) Study Group directly tested this hypothesis in a large-scale, randomized clinical trial. Over 1,800 patients received either standard or high-dose hemodialysis, through either a low- or high-flux

dialyzer, three times weekly. Patients were followed for an average of over three years. Researchers found that, overall, patients on the high-dose therapy, or using a high-flux dialyzer, did not experience any additional benefit over patients treated with standard therapy or a low-flux filter. These results suggest that physicians should continue to administer hemodialysis to their patients using current clinical practice guidelines.

The NIDDK continues to support studies to improve the dialysis process and the lives of patients on dialysis. The Institute is planning large clinical trials to examine whether there are health benefits to be gained from more frequent dialysis. It is expected that two trials will be initiated, one comparing short daily hemodialysis with conventional dialysis, and one comparing long nocturnal dialysis with conventional dialysis. Previous studies of increased dialysis frequency have reported good results, including reductions in blood pressure, serum phosphate levels, and requirements for erythropoietin (a factor critical for red blood cell formation). Improved patient well-being has also been reported. These observations, however, derive from small groups of selected patients in a few centers.

One of the major challenges in caring for the hemodialysis patient is maintenance of vascular access for hemodialysis. Access-related problems are among the most frequent reasons for hospitalization in the ESRD population, and the cost of vascular access placement and repair in the U.S. exceeds $700 million annually. In FY 2000, the NIDDK established the Dialysis Access Consortium to undertake interventional clinical trials to improve outcomes in patients with fistulas* and kidney transplants. Two clinical trials have now been designed and are recruiting patients. The first trial will evaluate the effects of the anti-platelet agent, clopidogrel, on prevention of early fistula failure. The second trial will study a drug combination (dipyridamole and aspirin), with the goal of preventing access stenosis (blood vessel narrowing) in hemodialysis patients who have received a kidney transplant.

Another clinical trial the Institute is supporting is entitled "Hypertension in Hemodialysis." This trial is determining how to diagnose high blood pressure in hemodialysis patients, and to treat it using angiotensin converting enzyme inhibitors or beta blockers.

The NIDDK also has implemented a new research initiative to study aspects of chronic kidney disease, including kidney dialysis. This initiative is soliciting exploratory and developmental grants that aim to assess dialysis therapy, dialysis access, anemia of kidney disease, and nutritional or cardiovascular aspects of ESRD.

Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, Allon M, Bailey J, Delmez JA, Depner TA, Dwyer JT, Levey AS, Levin NW, Milford E, Ornt DB, Rocco MV, Schulman G, Schwab SJ, Teehan BP, and Toto R: Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N Engl J Med* 347: 2010-19, 2002.

PROGRESS ON TREATING PROSTATE DISEASE

Benign prostatic hyperplasia (BPH) and prostatitis are the two leading benign prostate conditions affecting men across the lifespan. BPH affects an estimated 9 percent of men 30 years of age and older, but most cases occur in men 55 and older, with prevalence increasing with age. Prostatitis is inflammation of the prostate gland that accounts for a significant percentage of all office visits by young and middle-aged men for complaints involving the genital and urinary systems. Although termed "benign" because they are not cancerous or life-threatening, these prostate problems can have significant impact on men's quality of life. Prostate diseases can cause symptoms ranging from sensations of irritation and burning during urination to increased frequency or urgency of urination, weak streams of urine, urine leakage, and pain. Prostate diseases can also interfere with normal sexual function. While progress is being made in the under-

* Fistula: A surgically created communication between an artery and vein, usually performed in the forearm or leg of patients undergoing kidney dialysis.

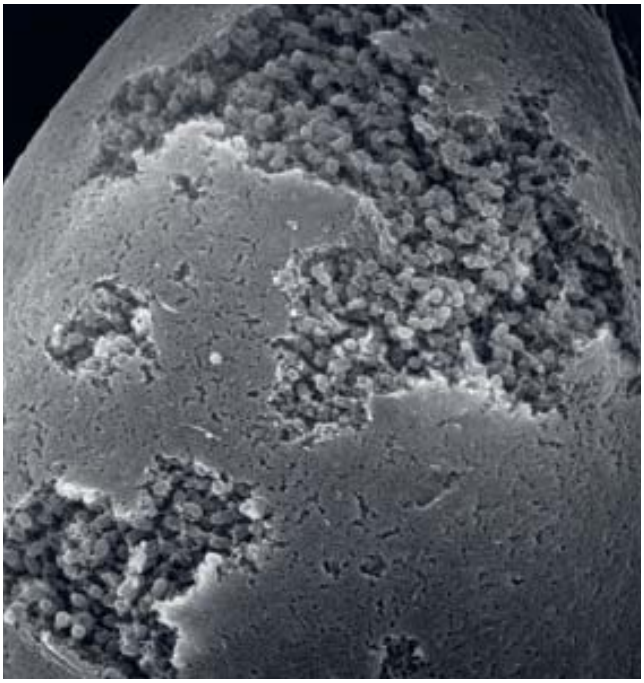
standing and treatment of prostate diseases, much work remains to be done to improve diagnosis and treatment, and possibly, to enable prevention.

Until the past 10 or 15 years, one type of surgical therapy, transurethral resection of the prostate (TURP), was the "gold standard" for treatment of symptomatic BPH. However, this is an invasive procedure that carries the inherent risks of surgery. New, less invasive approaches are rapidly being developed. Recently, investigators in the clinical trial, Medical Therapy of Prostate Symptoms (MTOPS), published results demonstrating that two drugs commonly used to treat BPH, finasteride and doxasozin, are significantly more effective at preventing symptomatic BPH incidence and progression when given in combination. Together, the drugs reduced overall risk of progression of BPH by 66 percent, versus 39 percent with doxasozin alone and 34 percent with finasteride alone. Importantly, the combination therapy and finasteride alone reduced the risk for invasive therapy by 67 percent and 64 percent, respectively. (Please see the "Story of Discovery," "Evolving Therapies for Benign Prostatic Hyperplasia.") Currently, the NIDDK is supporting two major clinical trials of alternative approaches to TURP for treating BPH. The Minimally Invasive Surgical Therapies (MIST) Treatment Consortium for BPH is designing trials to assess the safety and efficacy of new, less-invasive surgical treatments for BPH. The first trial to be conducted by this consortium is evaluating two surgical procedures, transurethral needle ablation (TUNA) and transurethral microwave therapy (TUMT), as well as a medical therapy similar to that used in the MTOPS study. Patient enrollment for this study began in 2003. A second trial, Complementary and Alternative Therapy for Benign Prostatic Hyperplasia (CAMUS), is a large clinical trial to examine the effects and efficacy of two commonly used, orally administered alternative therapies for BPH–so-called "phytotherapies"–saw palmetto and *Pygeum africanum*. CAMUS will use the same definition of clinical progression of BPH as used in the MTOPS trial.

Chronic prostatitis/chronic pelvic pain syndrome is a disabling condition of unknown origin which affects men of all ages and ethnic groups. Unlike prostatitis caused by a bacterial infection, chronic prostatitis cannot be cured with antibiotic treatment. Like interstitial cystitis (IC), another urologic disorder of unknown origin, one of the major symptoms associated with chronic prostatitis is pelvic pain. The NIDDK-supported Chronic Prostatitis Clinical Research Network (CPCRN) was established to perform a longitudinal study of a well-characterized group of patients with this disease, and to conduct trials of treatments for disease symptoms. The NIDDK has recently expanded the CPCRN to continue trials of therapies to alleviate symptoms of this disease, and to conduct ancillary research studies using data and samples collected from the cohort. Importantly, because of similar needs and approaches for the urologic conditions under study, investigators in the CPCRN will collaborate with researchers in the Interstitial Cystitis Clinical Research Network (ICCRN) in a "Urological Pelvic Pain Collaborative Research Network." This collaboration will improve the efficiency of protocol development, develop common definitions and criteria, and facilitate common data collection to permit comparisons between the clinical trials. Through support of these new and continued research efforts, the NIDDK is seeking to improve men's urologic health.

INVASION OF THE BLADDER SNATCHERS–BACTERIAL PODS IN ACUTE URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are extremely common in women, and many women suffer repeated infections. Most infections are caused by the common *Escherichia coli (E. coli)* bacteria. A UTI begins when bacteria attach to the cells lining the inside of the bladder. This adhesion provokes a defense response in the host, including activation of the immune system and sloughing off of bladder cells into the urine in the body's attempt to rid itself of offending bacteria. However, some strains of



A dense population of bacteria (grape-like objects) living in a "pod" at the surface of a mouse bladder cell is made visible through sophisticated imaging techniques. Photo: Dr. Joseph Palermo and Dr. Scott Hultgren, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO. Reprinted with permission from Anderson GG *et al, Science* 301: 105-7, 2003. © 2003 AAAS.

bacteria are able to persist in the bladder despite a vigorous host response and antibiotic treatment. One way bacteria avoid destruction is by invading the bladder cells, effectively "hiding out." This can give rise to a chronic, undetectable infection that can return at any time as a full-blown UTI.

To find out how bacteria can survive the onslaught of immune cells and other physiologic responses that follow infection–not to mention antibiotic therapy–researchers examined the bladders of immune-compromised mice inoculated with *E. coli*. Using scanning electron microscopy, they found that, while the inner surface of uninfected bladders was smooth, the surface of infected bladders was covered with pod-like protrusions. Closer examination revealed that the pods were filled with bacteria. The pods' contents resembled a "biofilm," a complex microbial community consisting of bacterial cells suspended in a matrix. Examples of biofilm in nature include dental plaque and the slimy coating on rocks in a stream. However, this biofilm-like structure was growing inside a living cell! Within a biofilm, bacteria located in different regions express different genes, which leads to distinct bacterial subpopulations within the larger community. Biofilms have previously been shown to protect bacteria from immune defenses and antibiotics. The growth of UTI-causing *E. coli* within a biofilm-like matrix in the bladder may make these bacteria more difficult to eliminate and infection more likely to recur.

These bacterial pods associated with an acute UTI represent a previously undescribed bacterial community. The protection presumably conferred on the bacteria by the biofilm-like structure, and the emergence of different subpopulations within the larger group, may afford a survival advantage. These structures may represent a mechanism through which the bacteria avoid being eliminated by host responses and survive to reinfect bladder cells over and over again. Finally, although these studies examined UTIs in mice, biofilm-like structures might prove to be important in many human conditions, such as pneumonia, tuberculosis, and cystic fibrosis, which involve bacterial infections as causes or complications.

The discovery of biofilms that house bacteria in the bladders of mice is just one example of basic research studies that are both fascinating and important for better understanding infectious and noninfectious diseases. The NIDDK is supporting both basic and clinical research efforts in bladder and other urologic diseases and disorders. The Institute recently launched an initiative to support studies of the basic biology of interstitial cystitis (IC), a debilitating bladder disease of unknown cause (please see sidebar, "Basic Research on Interstitial Cystitis–Advancing Toward Clinical Tools"). Support will also continue for the development of new research tools and innovative methods to study the cells of the bladder, prostate, and other organs of the genitourinary tract. The Institute is also co-supporting, with the NIH Office of Research on Women's Health, Specialized Centers of Research that have as their focus basic and clinical studies of UTIs, urinary

incontinence, and IC. Additionally, to ensure that advances in science are translated effectively to the public and to health care providers, the NIDDK plans to undertake a Women's Urologic Health Outreach Initiative in partnership with the American Urological Association and the Interstitial Cystitis Association. The program will be a campaign to increase awareness and knowledge among primary care physicians about current health care recommendations for women's urological problems, including interstitial cystitis, urinary incontinence, and UTIs.

Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J and Hultgren SJ: Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* 301: 105-7, 2003.

NITRITE IMPROVES BLOOD FLOW

A century ago, Alfred Nobel invented dynamite, with nitroglycerine as the active component. Nitroglycerine has an equally important and less-destructive aspect, however. During Nobel's lifetime, it was recognized that nitroglycerine could relieve chest pains caused by heart disease, and physicians began prescribing it for this purpose. Recently, scientists have uncovered the mechanisms behind nitroglycerine's ability to relieve cardiac pain: it generates nitric oxide (NO), a gaseous mediator of cell-to-cell communication and a potent dilator of blood vessels (vasodilator). In 1998, the Nobel Prize in Physiology or Medicine was given to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system.

Research scientists continue to investigate the molecular characteristics of the NO molecule and the mechanisms involved in its biological activities. One goal researchers have been pursuing is to identify molecules in the body's network of blood vessels (vasculature) that can be rapidly converted to NO to cause vasodilation. Identifying such molecules could have important therapeutic implications for diseases and conditions involving vascular blockage or constriction. In a recent advance, researchers have found that nitrite–a molecule present in high concentrations in the plasma and vasculature–is converted to NO by hemoglobin and causes vasodilation. The researchers found that when they injected sodium nitrite into arteries in the forearms of 18 human study participants, the participants' blood flow increased by an average of 175 percent over resting levels and their systemic blood pressure was reduced. When they investigated the underlying mechanism of the nitrite-induced vasodilation in experiments *in vivo* and *in vitro*, the researchers found that hemoglobin–the molecule in red blood cells that transports oxygen–was facilitating the conversion of sodium nitrite into NO. Specifically, it was the deoxygenated form of hemoglobin that was interacting with nitrite to produce NO-modified hemoglobin molecules. Based upon their data, the researchers propose that, as hemoglobin releases oxygen in body tissues with low oxygen, the resulting deoxygenated hemoglobin can then convert nitrite to NO, causing vasodilation and enhancing blood flow to–and, hence, oxygenation of–the surrounding tissue.

These research findings hold the potential for far-reaching therapeutic applications for acute and chronic conditions involving restricted blood flow. Nitrite could represent a potential new treatment for conditions such as high blood pressure, heart attacks, sickle cell disease, and leg vascular problems. Such promising applications await the clinical testing of the safety of nitrite when administered at the concentrations necessary to induce its positive effects on the vasculature of patients.

Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, III, and Gladwin MT: Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med.* 9: 1498-505, 2003.

SEEKING NEW WAYS TO MEASURE BODILY IRON

Although many people suffer from a lack of iron in the diet, too much iron can be equally unhealthy. The body has no way to get rid of iron in excess of need. Over time, chronic excess can lead to a toxic buildup of iron in organs and tissues, especially the heart, liver, and brain. When a disease or disease therapy causes so-called "iron overload," patients may need to be monitored for iron accumulation and also take drugs, called chelators, to help get rid of excess iron. This is the case with Cooley's anemia, a blood disorder that requires lifelong blood transfusions that result in the accumulation of excess iron (see "Patient Profile," "Living with Cooley's Anemia"). The NIDDK supports research on both better tools to measure the body's iron stores and on improvements to current chelator therapy.

Currently, the only non-invasive means to measure body iron that has been calibrated and validated for clinical use is a device known as a Superconducting Quantum Interference Device (SQUID). However, this device is not widely available, and is very costly to run. The NIDDK and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) have been collaborating to solicit new research grants for projects that may improve the utility of magnetic resonance imaging (MRI) as a method for quantitative determinations of body iron. MRI potentially provides a useful and widely available technique for monitoring excess iron in the body in conditions of iron overload, such as found in Cooley's anemia and sickle cell disease patients. The NIDDK and the NIBIB recently funded an initiative supporting projects that have the potential to improve the utility of MRI as a method for quantitative determinations of tissue iron, especially in the liver, heart, and brain.

The NIDDK also continues to support research to find effective and less burdensome alternatives to the injected iron chelating drug, desferrioxamine (Desferal® or DFO). One drug that is moving into clinical trials, HBED, appears to be a more effective chelator than DFO, indicating that it may need to be used less frequently and for shorter periods of time, which would be a great relief for patients. However, as with DFO, HBED still must be injected, so the NIDDK continues to search for better iron chelating drugs. Already, these studies have resulted in successful preclinical evaluation of a re-engineered version of the oral chelator desferrithiocin, and this new compound has recently been approved by the Food and Drug Administration for use in clinical studies. The NIDDK is planning additional studies on related chelators that may be even more effective.

STORY OF DISCOVERY

Evolving Therapies for Benign Prostatic Hyperplasia

If you are a man, chances are that someday you will have an enlarged prostate. Benign prostatic hyperplasia, or BPH, is a condition that affects an estimated 9 percent of men 30 years of age and older, even if they are unaware of it. Prevalence increases significantly in middle age, with the majority of cases reported in men age 55 and older. Not everyone with BPH will develop symptoms, but–for those who do–until recently, surgery provided the only relief. Now, new medical approaches to the treatment of BPH, built on basic research stretching back over 50 years, offer an effective, non-surgical option. For men who still require surgery, less invasive techniques may provide effective, less traumatic alternatives.

The walnut-sized prostate gland is located below the bladder and surrounds the urethra as it leaves the bladder. The urethra and the fluids it transports pass through the prostate. One important function of the prostate gland is to release seminal fluid into the urethra at sexual climax to provide a vehicle for sperm.

The prostate gland undergoes two phases of growth. The first, during puberty, results in a relatively rapid doubling in size of the gland. The second, slower phase begins at around age 25 and continues throughout a man's life. As the region of the prostate immediately surrounding the urethra grows, it can, in some men, compress the urethra and inhibit the flow of urine. As a result, the bladder does not empty completely during urination. The narrowing of the urethra and partial emptying of the bladder are the cause of the complaints most commonly associated with BPH, such as frequent urination, inability to urinate, and urinary tract infections. Thus, while prostatic hyperplasia is "benign" in the sense that it is not cancerous, it can nevertheless cause serious health problems.

The male sex hormone testosterone plays an important role in a wide range of biological and physiological processes, ranging from development of the male urogenital tract during embryogenesis to the emergence of secondary sexual characteristics, such as facial and body hair, in the post-pubescent adult. In the late 1950s, researchers working with rats knew that experimental administration of testosterone resulted in an accumulation of protein in the male urogenital tract. By the mid-1960s, scientists could purify the hormone from these cells, but found–to their great surprise–not testosterone, which had been given to the animals, but a closely-related derivative: dihydrotestosterone, also known as DHT. Previously, it had been widely thought that DHT was a relatively unimportant breakdown product of testosterone. However, the finding of DHT in testosterone's target cells caused researchers to take a second look at this molecule.

An important role for DHT in male urogenital development was postulated in the mid-1970s from studies of a rare inherited form of hermaphroditism. Individuals with this condition are genetically male but appear female at birth, insofar as they lack external male genitalia and a prostate gland. Oddly, blood tests of these individuals found normal circulating levels of testosterone. However, closer analysis revealed markedly reduced levels of DHT. This finding suggested that the genitalia and prostate require

STORY OF DISCOVERY

DHT to develop, because these features were absent in affected individuals, while other components of the male urogenital tract, which were present, do not. Years later, in the 1990s, sophisticated molecular analysis would reveal that the cause of this condition is a mutation in one of the proteins that converts testosterone to DHT, an enzyme known as 5-alpha reductase.

In the late 1970s, scientists had developed a model system in which they could induce prostatic hyperplasia in dogs by administering male sex hormones. Furthermore, they could block or reverse it by administering agents that inhibited 5-alpha reductase. This observation suggested that it was DHT that was responsible for the continued growth in the prostate in the adult, and stimulated pharmaceutical firms to pursue drugs that target the 5-alpha reductase enzyme as possible therapies for BPH. In 1992, the Food and Drug Administration approved for use in humans the drug finasteride–the first drug to block the conversion of testosterone to DHT by inhibiting 5-alpha reductase.

In the early 1990s, treatment of BPH generally involved a surgical procedure called Transurethral Resection of the Prostate, or TURP. As its name implies, TURP involves inserting a small scope up the urethra to the prostate and cutting away the obstructing tissue, thereby restoring normal urine flow. While TURP is an excellent treatment for BPH, it is expensive, requires several weeks of convalescence during recovery, and carries risks inherent in any surgical procedure. Because of these drawbacks, as an alternative to surgery some physicians began using finasteride to inhibit DHT production and thereby shrink the size of the prostate. Others used alpha blockers, a class of drugs that relax the smooth muscle in the prostate and bladder neck, and thus allow urine to flow more easily. However, urologists were not sure whether medical therapy was truly treating the BPH or was only relieving the symptoms while the underlying disease silently progressed.

To help answer this question, the NIH launched the Medical Therapy of Prostatic Symptoms (MTOPS) trial in 1994. A large, randomized clinical trial to assess the overall effectiveness of medical therapy on BPH symptoms and progression, the MTOPS trial would ultimately enroll over 3,000 men with BPH. Participants received one of four interventions: placebo (sugar pill), the 5-alpha reductase inhibitor finasteride, the alpha blocker doxazosin, or finasteride and doxazosin together. The study followed participants for an average of five years and monitored them for signs of BPH progression such as an inability to urinate, incontinence, or recurrent urinary tract infections. The results of the MTOPS trial were announced in 2002 and published in 2003, and were unequivocal: combination therapy, consisting of finasteride and doxazosin together, reduced the risk of BPH progression by 66 percent compared to placebo. Each drug was also effective when used alone: the risk of progression was reduced by 39 percent with doxazosin and by 34 percent with finasteride. However, the results with combination therapy surprised all involved and will likely lead to important changes in the way BPH is treated.

Even with improvements in drug therapy, a fraction of men will ultimately require a surgical procedure to alleviate symptoms of BPH. While TURP remains the “gold standard,” a number of new surgical treatments for BPH have been developed over the past decade. These procedures aim to achieve the same long-term outcomes of TURP, but to do so with lower costs, more rapid recovery, and less risk. However, the relative effectiveness and long-term safety of these new surgical approaches is unknown.

To bridge this gap in knowledge, the newly-launched Minimally Invasive Surgical Therapies (MIST) clinical trial will compare two new, less invasive surgical approaches, Transurethral Needle Ablation (TUNA) and Transurethral Microwave Therapy (TUMT), with combination medical therapy in men with BPH. The results of this trial are expected to further expand treatment options for men with BPH, to provide both physicians and patients with valuable information, and to give all involved the knowledge needed to make the most appropriate choices for long-term management of BPH.

The National Kidney Disease Education Program

Early in 2002, the NIDDK launched the initial efforts of the National Kidney Disease Education Program (NKDEP). The mission of this pilot public education program is to raise awareness about the seriousness of kidney disease, the importance of testing, and the availability of treatment to slow or prevent kidney failure. An estimated 7.4 million Americans currently suffer from kidney damage, also called chronic kidney disease, and, each year, nearly 400,000 must have either dialysis or a kidney transplant to stay alive. The number of people developing irreversible kidney failure, also called end-stage renal disease (ESRD), has doubled each decade for the last two decades, and disease statistics indicate that this trend is likely to continue. The leading causes of kidney disease are diabetes and hypertension (high blood pressure). If current trends continue, the recent increases in obesity and type 2 diabetes in the U.S. will have grave implications, as more and more people develop kidney complications related to diabetes. For example, the current public and private costs of treating ESRD were estimated at $23 billion in 2001.

Fortunately, chronic kidney disease can be slowed, if not prevented, provided it is detected early enough. Good control of blood sugar and blood pressure can reduce the risk of developing kidney disease. Diets low in protein can also slow kidney disease progression. In spite of these advances in treatment and prevention, only a small number of those who most need proper screening or treatment receive it. The NKDEP will strive to disseminate information on prevention and treatment to physicians and patients who can most benefit from it.

Racial and ethnic minorities suffer a far higher incidence and prevalence of irreversible kidney failure than Caucasians. Rates of ESRD are disproportionately greater in African Americans, American Indian and Alaska Natives, Native Hawaiians and other Pacific Islanders, and Hispanic Americans. Diabetic kidney disease is the most common cause of ESRD in all of the aforementioned minority groups except for African-Americans, in whom high blood pressure-induced kidney damage is also a major cause.

The ultimate goal of the NKDEP is to reduce complications and death due to kidney disease and kidney failure among all Americans. Currently in its early, pilot phase, the NKDEP is targeting primary care providers and people at high risk for kidney disease–particularly African Americans with diabetes, hypertension, and/or a family history of kidney failure–in four pilot sites: Atlanta, GA; Baltimore, MD; Cleveland, OH; and Jackson, MS. In April 2003, the four pilot sites launched the campaign, “You Have the Power to Prevent Kidney Disease,” which emphasizes three key messages:

- Early detection is important. If you are at risk due to diabetes, hypertension or a family history of kidney failure, talk to your doctor about having your kidneys checked.
- Effective treatment can prevent or slow kidney damage.
- Left undiagnosed and untreated, kidney disease can lead to kidney failure.

Prior to launching the pilot site activities, the NKDEP conducted two baseline surveys. The first survey assessed African-American adults' knowledge, attitudes, and behaviors related to kidney disease. Ten-minute telephone interviews were conducted with 400 randomly sampled African-American adults over the age of 30 in each of the four pilot sites and in a composite control site, for a total of 2,000 respondents. The second survey was conducted with primary care physicians. Surveys were faxed to 100 primary care providers in each of the four pilot sites and 200 in a composite control site, for a total of 600 respondents.

After one year, follow-up surveys will be conducted to determine the extent to which the pilot interventions achieved their goals of increasing awareness about the seriousness of kidney disease and the availability of effective treatments to prevent or slow kidney failure. The follow-up survey also will evaluate which program activities were most related to changes in the awareness, attitudes, and behaviors of the target audiences. Successful strategies identified through the pilot sites will be used to develop a broader national campaign, which is planned to launch in June 2004.

In addition to public awareness activities, the NKDEP has several Work Groups that are working on removing specific barriers to better kidney disease awareness and care. The membership of these groups is drawn from the professional partnership network of the NKDEP, which includes non-profit groups, industry, and government. The NKDEP Laboratory Work Group has made efforts to encourage improvement and standardization of the serum creatinine assay–which is used to estimate how well the kidneys are functioning–in order to address issues of inter-laboratory variation in this assay. The Group has also begun efforts to encourage laboratories to report glomerular filtration rate (GFR) estimates as soon as possible in adults with low GFRs, to enable physicians to quickly identify individuals with impaired kidney function. The NKDEP Quality Indicators Working Group, in partnership with the Centers for Medicare and Medicaid Services (CMS), is undertaking a pilot project to spur the development of quality indicators of care for chronic kidney disease among Medicare beneficiaries hospitalized for cardiovascular disease.

Through all of its efforts, the NKDEP is striving to become a positive force in helping to reduce the burden of kidney disease in the U.S.

Basic Research on Interstitial Cystitis– Advancing Toward Clinical Tools

One of the most painful bladder experiences most people may ever have is waiting for the next rest stop on a crowded interstate. However, patients with interstitial cystitis (IC) live each day in constant awareness of their bladders. Interstitial cystitis is a chronic bladder disease characterized by pelvic pain (pain in the area below the navel) and increased frequency and urgency in urination. These symptoms can be quite debilitating, interfering with a patient's ability to work, go out, and enjoy family life. While the precise number of persons affected is unknown, as many as 870,000 American men, women, and children may suffer from IC; however, 90 percent of reported cases occur in women. The cause(s) of IC is as yet unknown. Current treatments for symptoms are not effective in all patients, and there is no cure. The NIDDK is supporting clinical and basic research investigations on several fronts to understand the cause(s) of IC, to develop and test more effective treatments, to develop better diagnostic tools, and, ultimately, to develop a cure for this disease.

Investigation of the fundamental mechanisms underlying the initiation and development of IC is critical to understanding the disease. When physicians examine the inside of the bladder of an IC patient using a cystoscope*, they often find that it is marked by glomerulations (hemorrhages) and/or deep ulcers. Most consistently, defects such as tears and thinning in the layer of epithelial cell tissue that lines the inside of the bladder are observed. This layer of cells normally expands as the bladder fills, and it protects the underlying nerves, blood vessels, and muscle of the bladder wall from toxic components of urine. Based upon investigations of the physical signs and symptoms associated with IC, there are several hypotheses regarding its cause(s) and development, including defects in nerve signals traveling to and from the bladder; autoimmune or other "immunogenic" disease; and intrinsic or induced defects in the protective lining of epithelial cells within the bladder.

While researchers are trying to investigate the cause(s) of IC, a major stumbling block is the lack of a specific biological marker (biomarker) for the disease. Biomarkers–which can be genes, proteins, or other molecules–help researchers distinguish one disease from another, ideally using minimally invasive or non-invasive techniques. Biomarkers may also provide clues to the stage of a disease, and, in some cases, can be used to assess whether or not a candidate disease therapy is working. Finally, in addition to their high value in diagnosing disease and gauging response to therapy, biomarkers may also simultaneously yield clues about a fundamental disease process. Because of their importance, research studies of biomarkers were solicited in a recent NIDDK-supported basic research initiative in IC, and emphasized at a recent scientific meeting on IC co-sponsored by the NIDDK and the Interstitial Cystitis Association.

Currently, an especially promising biomarker for IC is "anti-proliferative factor," or APF. One team of researchers has worked on the hypothesis that the damage to the bladder epithelium in IC is a distinguishing characteristic of the disease, and that it might be due to a toxic entity present in the urine of IC patients. Several years ago, Dr. Susan Keay and her colleagues at the University of Maryland first demonstrated that an anti-proliferative activity was present significantly

* A thin, lighted (usually fiber optic) instrument used to look inside the bladder and remove tissue samples (biopsy) or small tumors.

more often in the urine of IC patients than in control samples. This activity was measured by how much the growth of normal human bladder epithelial cells was inhibited when they were incubated in a solution containing urine from IC patients. This detection strategy for a biological activity is called a functional assay. The activity identified in the functional assay appeared to be linked to a small, heat-stable peptide (tiny protein) that was either "activated" in the urine or added to it when it reached the urinary bladder.

Although APF is not the only factor altered in the urine of IC patients, it is the one that, to date, appears to be most effective for distinguishing between IC patients and patients with different urogenital conditions or no disease at all. Researchers have reported that, in blinded studies, 86 to 94 percent of patients diagnosed with IC have significant APF activity in their urine, while only 9 percent of asymptomatic patients and 0 to 18 percent of patients with other urogenital disorders have significant APF activity. Based on the functional assay, the researchers reported that APF has a sensitivity of 94 percent, and a specificity of 95 percent, as a detection tool for IC. Furthermore, of 14 candidate factors for biomarkers detectable in the urine of IC patients, APF had the least overlap between IC patients and controls. Although these results need to be tested further in larger populations and in patients diagnosed with IC by using less restrictive criteria than those used for the initial clinical research studies, they offer a compelling rationale for possible clinical test development and validation of APF as a biomarker for IC.

Moreover, APF is not just a biomarker candidate, but may also be central to some or all of the pathology of IC. While working on validating APF as a biomarker, the research team led by Dr. Keay has continued to explore the hypothesis that bladder epithelial cell abnormalities lie at the root of the disease–and that APF might be directly involved in the disease by affecting the body's ability to regenerate damaged bladder epithelium. They first showed that the source of APF in the urine of IC patients is most likely the epithelial cells of the bladder. Using the APF functional assay, they detected the same APF activity in the growth medium from bladder epithelial cells biopsied and cultured from IC patients as they had found in patients' urine. Through biochemical purification steps, they obtained highly enriched APF from both sources, narrowing it to a small, heat-stable peptide. In contrast, identical purification steps performed with non-IC urine samples yielded no APF activity. These results indicate that the APF is produced by the affected epithelial cells themselves–which suggests that the pathology of IC may be due in part to an intrinsic abnormality in these cells.

The team has also observed that several molecules, called growth factors, are produced in different amounts in the urine of IC patients as compared to patients without IC. Growth factors can stimulate or inhibit the proliferation of target cells. Two of the molecules, epidermal growth factor (EGF) and heparin-binding epidermal growth-factor-like growth factor (HB-EGF), show significant changes in the urine of IC patients. EGF is significantly increased, and HB-EGF is significantly decreased. Notably, the growth of IC bladder epithelial cells is markedly slower than the growth of normalbladder epithelial cells when they are grown under identical conditions in the laboratory. Both the altered growth factor production and the growth defect in the IC bladder appear to be linked to APF activity. When APF enriched from IC bladder epithelial cells or from IC patient urine was applied to normal bladder epithelial cells, the cells released less HB-EGF and more EGF into their growth medium and ceased to proliferate. Furthermore, the results of other experiments *in vitro* suggest that bladder cells from IC patients can "recover" from the growth inhibition induced by exposure to APF by treating them with HB-EGF, further implicating HB-EGF as a primary target in APF-induced growth inhibition. Importantly, the cells' ability to recover from APF exposure in these experiments also firms up hope that the bladder epithelial cell damage observed in IC is reversible–which, in turn, may help reverse the course of the disease.

Most recently, Dr. Keay and her colleagues have delved even deeper into the biology of APF and examined its effect on bladder epithelial cell gene expression. In a study using DNA microarray technology, they looked for differences in gene expression between normal bladder epithelial cells and cells from IC patients, as well as between "mock-treated" normal bladder epithelial cells and cells treated with purified APF. They demonstrated for the first time that APF can alter expression of a number of genes in bladder epithelial cells. As might be expected, many of the genes that showed *decreased* expression in IC cells and APF-treated cells normally promote cell proliferation, and many of the genes that showed *increased* expression are associated with growth inhibition. Thus, the research team has hypothesized that not only might APF be interfering with the release of factors necessary for appropriate cellular growth, such as HB-EGF, but that it might also be "reprogramming" bladder cells to terminate their growth potential prematurely. These interesting results and their significance in the IC disease process await further confirmation in future studies.

The increasing wealth of data about APF is providing compelling support for one hypothesis regarding IC, which suggests that the disease may develop in susceptible individuals because of an inability to regenerate bladder epithelium after an assault that damages this tissue (for example, a bladder infection). This would leave the deeper layers of the bladder vulnerable to the toxic components of the urine, thus contributing to the observed hemorrhages, ulcerations, and inflammation. More knowledge of APF–such as the gene encoding it, APF's chemical sequence and structure, and the trigger(s) for its production–may explain both its role in IC and its presence in patients with other bladder diseases, and, if it turns out to be a risk factor for IC, why seemingly asymptomatic patients have shown APF activity.

At the same time as studies of APF are progressing, fundamental investigations of other hypotheses about the pathology of IC are ongoing. For example, there is evidence suggesting that sensory nerve cells in the bladders of IC patients may be hypersensitive to normal stimuli, which, in turn, may play a role in the initiation and/or progression of IC. Researchers are continuing to study bladder innervation and alterations in nerve function, especially in order to better understand the pain-receptor-pathways in the bladder that likely contribute to the pain experienced by IC patients. Another hypothesis is that neurogenic inflammation and mast cell activation are responsible for IC symptoms. Mast cells normally function in allergic and inflammatory reactions, and have been observed in bladder tissue of IC patients, where they may be activated by nearby nerves to induce bladder inflammation. Along those lines, investigators are also learning more about how the epithelial cells of the bladder produce signaling molecules, called chemokines and cytokines, that may participate in the initiation or progression of inappropriate signals that result in pain and/or inflammation. These and other promising investigative routes are, like studies of APF, important to elucidating the mechanisms underlying IC and its symptoms.

The study of APF illustrates that the feedback loop between basic and clinical research is bidirectional and dynamic. While basic research often paves the way to clinical progress, in this case, translating clinical research observations into fundamental investigations shed further light on a disease process. The importance of complementary fundamental research in IC has been further highlighted in a basic research initiative recently launched by the NIDDK. Through this initiative, the Institute is supporting over twenty large-scale research projects and smaller, exploratory projects that will test the viability of specific new research directions in IC. Research studies ranging from harnessing cutting-edge proteomics technology for IC research, to studying the role of immune system cells in the disease, to studying the genetics of IC–as well as studies of other candidate biomarkers for IC–are being supported through this initiative. Importantly, the IC investigators leading these studies will meet regularly to discuss their findings, thereby pooling resources and strengthening efforts to uncover the cause(s) and develop better treatments, preventive therapies, and strategies to cure this disease.

Recent Advances

Erickson DR, Xie SX, Bhavanandan VP, Wheeler MA, Hurst RE, Demers LM, Kushner L and Keay SK: A comparison of multiple urine markers for interstitial cystitis. *J Urol* 167: 2461-9, 2002.

Keay S, Seillier-Moiseiwitsch F, Zhang CO, Chai TC and Zhang J: Changes in human bladder epithelial cell gene expression associated with interstitial cystitis or antiproliferative factor treatment. *Physiol Genomics* 14: 107-15, 2003.

Keay S, Zhang CO, Shoenfelt JL and Chai TC: Decreased in vitro proliferation of bladder epithelial cells from patients with interstitial cystitis. *Urology* 61: 1278-84, 2003.

Keay SK, Zhang CO, Shoenfelt J, Erickson DR, Whitmore K, Warren JW, Marvel R and Chai T: Sensitivity and specificity of antiproliferative factor, heparin-binding epidermal growth factor-like growth factor, and epidermal growth factor as urine markers for interstitial cystitis. *Urology* 57: 9-14, 2001.

PATIENT PROFILE

Alicia Somma

Living with Cooley's Anemia

For most people, having to undergo a blood transfusion every two weeks would be difficult enough. But for 18-year-old Alicia Somma, who was diagnosed in infancy with Cooley's anemia, that's the relatively easy part of living with this life-threatening disease.

Cooley's anemia is an inherited blood disorder that results in failure to produce sufficient hemoglobin, the blood's oxygen-carrying component. This necessitates the "somewhat painful and unpleasant" blood transfusions, to treat the severe anemia. But, although these transfusions are life-saving today, they lead to an insidious buildup of excess iron, particularly in her heart and liver. Unfortunately, the body has no natural way of removing excess iron. So, in addition to bi-weekly blood transfusions, Alicia must painstakingly inject herself most evenings with a drug called a "chelator" that binds excess iron to itself and removes it from her body via her urine–a procedure that sounds a lot easier than it is.

Removing, or chelating, the excess iron requires several steps. First, Alicia must mix two parts of a powdery drug, called deferoxamine, with eight parts water. Next, she draws the dissolved drug into a syringe, places the syringe into an infusion pump, swabs with alcohol a place on her stomach or leg that is not already swollen or inflamed from countless other injections, and creates a fold in her skin into which she can insert the needle. She then adjusts the needle just beneath the surface of her skin and secures it by taping it down. Finally, she goes to bed, while the pump continually injects the life-prolonging chelating drug into her body for 10 to 12 hours.

Alicia Somma

Aside from being enormously time consuming and terribly inconvenient, "It's really painful when you hit a sore spot with the needle," says Alicia. Extremely mature and articulate, Alicia is an ardent spokesperson on behalf of those with Cooley's anemia. "But it's something I've been doing about five times a week for the last 16 years." Without this chelating treatment, Alicia's heart and liver would rapidly fail. Even under the best of circumstances, these organs become damaged by excess iron in Cooley's anemia patients. In many cases, heart failure often occurs between the ages of 20 and 30. But close adherence to the chelating treatment could extend Alicia's life well into her 40s. Although researchers have discovered the genes that cause Cooley's anemia and other forms of thalassemia, to date there is no cure for this debilitating and deadly disease.

About Cooley's Anemia

Once believed to be common only to people of the Mediterranean region, Cooley's anemia is a one of a group of genetic blood disorders, called thalassemia, found also in many Asian and African populations. The most common disorder is "beta thalassemia trait" (also called beta thalassemia minor), which means that an individual carries the genetic trait for beta thalassemia. Today, because of the migration and intermarriage of different ethnic populations, the trait for beta thalassemia can be found in people with no obvious ethnic connection to the disorder. Except in extremely rare cases, this genetic trait causes no symptoms and requires no treatment. However, parents who both carry the same kind of genetic trait, as do Alicia's, have a one-in-four chance with each pregnancy of having a child with a serious form of beta thalassemia–the most serious being Cooley's anemia. Alicia's 20-year old sister, Christine, is thalassemia-free.

An estimated 2 million Americans carry the genetic trait for thalassemia.

Bone marrow transplantation from a perfectly matched donor is the only available cure for thalassemia at this time. However, of patients who have a matched donor and low risk factors, only a small percentage (estimated at 30 percent) can undergo the procedure. Bone marrow transplantation for thalassemia also has to be considered carefully because of the procedure's inherent risks. Overall, younger patients and those lacking the complications of the disease or its treatment have the best outcomes.

Living with Cooley's Anemia

It's been said that life isn't a matter of holding good cards, but rather playing well the cards you hold. Alicia is playing her cards as well as she can. "Shortly after I was diagnosed with Cooley's anemia at eight-months-old, a doctor told my parents that I wouldn't be able to participate in sports or do anything too strenuous, and that I wouldn't be smart," says Alicia. "But I've been taking dance lessons ever since I was three, and I was in gifted and talented classes from 1st to 6th grade," she adds with a hint of an adolescent "I-gotcha" giggle. While in high school, Alicia was extremely active in theater and, as a senior, played the leading role in her high school's production of a Broadway musical. Now, she's attending a community college. "I love to be on stage, to act and to sing," she says in a voice full of *joie de vivre*, but adds that she's not sure she's ready for the auditions and everything else that goes along with making acting a career.

Cooley's anemia is usually diagnosed within the first year of life. Most children with Cooley's anemia are relatively inactive and are unable to keep up with their playmates. In fact, listlessness, fatigue, shortness of breath and jaundice are symptoms of the disease.

But all this belies how difficult it's been for Alicia–and her family–to live with Cooley's anemia. Reflecting back nearly 18 years, Alicia's father, Frank Somma, who is president of the Cooley's Anemia Foundation (CAF), says with traces of pain in his voice, "To try to find a vein on an eight-month-old baby is difficult. But then to have to hold Alicia down while she stared back at us with this look of, 'how can you forsake me like this?,' is a lot for loving parents to bear."

Alicia can't remember as far back as being eight months old, but she does remember her parents sticking her with a syringe to inject her with deferoxamine. "I have a scar on my lower lip because at the age of two I tried to run from them (her parents) and hit my face on the side of the fireplace," says Alicia. But soon the situation changed. By age eight, she was mixing her chelating medicine and injecting

the needle into herself. Even when it came to blood transfusions, "I remember when I was five years old telling my nurse, who did my IV, where the best place was to put the needle….I was a very mellow kid," she adds. Mellow–and very honest with herself and others about the disease her body harbors. "I'm aware of what it means to have Cooley's anemia. Some days I have to wear the [deferoxamine] pump to school because the night before I forgot to push the start button or the needle fell out. I'm not embarrassed to be wearing this box on the side of my hip.I sometimes even carry it in my hand. Telling other people makes life so much easier. All my friends know I have Cooley's anemia."

As a result of the disease, Alicia is also experiencing early onset of osteoporosis–a condition which predominantly affects post-menopausal women. Says Mr. Somma, "Osteoporosis isn't a disease 18 year-old girls should be concerned about."

Both Alicia and her father have testified before Members of the U.S. Congress to advocate for intensified research programs benefiting thal assemia patients, as well as for increased blood safety monitoring. Because of their continual need for transfusions, patients with Cooley's anemia are highly vulnerable to blood-borne diseases. For example, before the availability of specific blood screening tests, the viruses causing HIV/AIDS and hepatitis C posed serious threats to people with Cooley's anemia.

Alicia also has appeared on national TV and radio to talk about Cooley's anemia and thalassemia. Her main message to others with Cooley's anemia is: Do the pump! In other words, comply with the chelating treatment. "Except for the transfusions and chelating treatments, people with Cooley's anemia are in no pain," says Mr. Somma, who, in Alicia's words, is a "real stickler" about her taking her injections as often as possible. He likens this insidious disease to smoking. "People who smoke don't realize the negative impact it's having on them until they're diagnosed later in life with a serious illness related to their habit," he says. "Well, the same holds true for people with Cooley's anemia. As unpleasant as it is to inject the chelating drug five to six nights a week, not taking the drug doesn't make them feel bad, but eventually the noncompliance will result in a much earlier onset of heart and liver disease."

Research Efforts in Cooley's Anemia

The NIDDK, along with the National Heart, Lung, and Blood Institute (NHLBI), is committed to fostering basic and clinical research that will lead to more effective treatments, and ultimately a cure, for Cooley's anemia.

NIDDK ongoing research is devoted to studying both the causative gene and genes that influence disease severity of Cooley's anemia through studies undertaken by NIDDK intramural researchers and through funding of projects at academic and other research institutions. For example, one research group recently discovered a protein in red blood cells that interacts with a subunit of hemoglobin to stabilize its structure. Researchers may now study the human gene encoding this protein for variations that could modify disease severity when present in Cooley's anemia patients. The hope is that greater knowledge of natural genetic modifiers of Cooley's anemia and other forms of thalassemia will help researchers devise new therapies to reduce disease burden.

The NIDDK also supports research related to more efficient and less painful ways of measuring iron in the body, less burdensome iron removal regimens, as well as to the development of safer and more cost-effective transfusion therapies.

In fact, NIDDK-supported research led to the development of the only non-invasive method for measurement of tissue iron stores that has been calibrated, validated, and used in clinical studies. Ongoing and newly launched efforts supported by the NIDDK include:

- A large-scale NIH Bioengineering Research Partnership that is supporting efforts to advance the "SQUID" (superconducting quantum interference device) technology that is at the core of the currentmethod of non-invasive iron measurement. The goal of this project, which is also supported by the NHLBI, is to modify the SQUID technology to make it more accessible and ready for more widespread clinical use, thus providing a viable alternative to liver biopsy.

- A Magnetic Resonance Imaging Technology (MRI) initiative. In response to a solicitation designed to encourage research in this area, the NIDDK and the National Institute of Biomedical Imaging and Bioengineering (NIBIB) are funding several studies exploring ways to adapt the relatively inexpensive MRI technology already in widespread clinical use for application to the clinically useful measurement of iron stores.

- Basic and preclinical research studies to identify and evaluate oral drugs for removing toxic iron from the body as an alternative to injected drugs.

Although researchers are making progress, to date there remains no cure for Cooley's anemia. Alicia's ace in the hole, however, is her courage and determination. She says she will continue to be vigilant with her iron removal treatment and live the best life that she can. "I understand it could be fatal if you don't take care of yourself," she says, "but as far back as I can remember my parents have told me 'this is what you have and this is what you've got to do to get through it.' When I get married, I hope I raise my children with the same strength my parents raised me."

PATIENT PROFILE

Jennifer Klann

Life With Polycystic Kidney Disease: Experiencing Hope Through Research

Jennifer Klann was 18 years old and a college student when she began experiencing severe pelvic pain. Her physician thought it might be from cysts on her ovaries. To confirm the diagnosis, he recommended that Jennifer undergo an ultrasound exam. During the exam, Jennifer–who is now 32, married, and the mother of a 3-year-old son–vividly recalls hearing the attending nurse say, "Oh my, I've got to get the doctor." It turns out that Jennifer does indeed have cysts, but not on her ovaries–on her kidneys.

Jennifer Klann and son, Dylan

Jennifer was diagnosed with polycystic kidney disease, or PKD, an inherited disorder that could result in chronic renal (kidney) disease and, ultimately, end-stage renal disease, or kidney failure. "I was in tears when I called my dad to tell him my results," says Jennifer, whose father had PKD, and whom Jennifer believes later died prematurely as a result. "He consoled me and said I was going to be just fine," says Jennifer, "but I'd seen him suffer so much from PKD that I knew the potentially life-threatening impact this disease could have on me." Although there are things Jennifer can do to keep herself as healthy as possible–and research on potential therapeutic strategies is progressing rapidly–to date, there is no treatment or cure for PKD directed at the basic mechanism of the disease.

PKD is the fourth leading cause of kidney failure in the United States.

Jennifer is currently taking part in an NIDDK-supported study, called the Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, or CRISP. The consortium consists of four participating clinical centers and a data-coordinating and imaging-analysis center located in various parts of the country. It is testing whether imaging techniques can help determine the progression of renal disease in patients with PKD.

Meanwhile, cysts continue to grow on Jennifer's kidneys, which she says scares her. But, she is grateful to be part of the CRISP study and is hopeful that her son, Dylan, is not genetically predisposed to develop the disease. PKD symptoms are usually manifested in mid-life. If Dylan eventually is diagnosed with PKD, Jennifer is counting on researchers by then to have discovered an effective treatment for the disease, or perhaps even a cure.

About Polycystic Kidney Disease

Jennifer is one of many people in the U.S. and worldwide who suffer from PKD. PKD is a genetic disease characterized by fluid-filled cysts that, over time, multiply and enlarge both kidneys. These cysts, which range in size from a pinhead to a grapefruit, eventually can take over and destroy functioning kidney tissue, which may result in chronic renal failure and end-stage renal disease. A normal kidney weighs approximately 8 ounces and is the size of a human fist. Jennifer's kidneys are already twice as large. Depending upon the severity of the disease, these growing cysts can result in kidneys that are the size of a football or larger and can weigh as much as 22 pounds each. Currently, Jennifer says her kidneys are functioning normally, "but I worry about my enlarged kidneys pushing on other organs," she says, "and the older I get the more concerned I become. My kidneys aren't getting any smaller."

There are two forms of inherited PKD. Autosomal dominant PKD (ADPKD) is the most common of all life-threatening genetic diseases and is indiscriminate, meaning it equally affects people regardless of sex, race, age or ethnic origin. Autosomal recessive PKD (ARPKD) is relatively rare and often causes significant mortality in the first month of life. PKD can be passed on from one generation to the next by an affected parent, and does not "skip" a generation as do some genetic diseases. Jennifer's father and grandfather both had ADPKD, as did a number of her father's cousins. Jennifer's son has a 50 percent chance of having the ADPKD gene.

Even if someone carries the gene, there is no way of knowing how adversely he or she will be affected by the disease. PKD can progress silently, for many years, without detectable cysts or symptoms. Although individuals with the ADPKD gene eventually develop cysts on their kidneys, not all will experience kidney failure, and if they do, it is rarely before the age of 40.

Currently there is no known treatment or cure for PKD. However, some people whose kidneys begin to fail may be fortunate enough to receive a kidney transplant. Jennifer's father received a transplant at age 55, after being on dialysis for only two weeks. "He went through a couple of bouts of organ rejection before he stabilized and started to regain his energy," says Jennifer. He died at age 66 from cancer. But Jennifer contends that the life-saving immunosuppressant drugs he was taking to ward off rejection of his kidney transplant simultaneously made it hard for him to fight other illnesses and diseases. "I often wonder if my father died prematurely due to his PKD," says Jennifer.

Living with PKD

Because the disease results in abnormally large kidneys, living with PKD can be quite painful. People with PKD commonly suffer from chronic pain in the flanks (the area between the ribs and hips) and back, and may also experience more infections and have high blood pressure. Of even greater concern are complications from the disease, which can include lethal brain aneurysms, cardiac abnormalities, and polycystic ovaries or testes.

Fortunately for Jennifer, she does not currently suffer from any chronic pain, nor does she normally need to take time off from her job selling real estate. But in 1998, Jennifer was admitted into the hospital with a 104 degree fever, flank pain and blood in her urine. One of the cysts on her kidneys had suddenly burst, which caused a serious infection. As a result, she was hospitalized for three days. Last year, although she doesn't know whether they were directly related to her having PKD, she passed 10 uric acid kidney stones and was hospitalized for two weeks.

Jennifer is keenly aware that her PKD may never result in complete kidney failure. "Just because my dad's disease progressed to the point where he needed a kidney transplant doesn't mean that mine will do the same." With that hope in mind, she does

all she can to take care of herself. She takes medication to control her high blood pressure; maintains a balanced, low-protein, low-salt diet; doesn't drink soda or coffee; and is actively involved with the PKD Foundation, where her husband, Scott, is the Development Director.

CRISP Study

Ultrasound is an imaging technique that clinicians currently use to diagnose PKD. Ideally, clinicians would like to expand the use of ultrasound and other non-invasive or minimally invasive imaging techniques to enable them to mark the progression of PKD, predict the course of the disease in individual patients, and detect positive responses of patients to new treatments. The CRISP study, in which Jennifer is participating, is an NIDDK-sponsored initiative designed to find ways, through imaging techniques, to monitor changes in the kidneys and in kidney cyst size in patients with PKD. The goal is to improve clinicians' ability to monitor the progression of kidney disease in these patients, in order to assess possible strategies for clinical intervention.

Overseen by NIDDK's Division of Kidney, Urologic and Hematologic Diseases, CRISP has been following more than 200 participants since March of 1999. All those enrolled in the study were very early in the course of the disease so that structural changes in their kidneys and cysts could be tracked through two widely used imaging techniques magnetic resonance imaging (MRI) and ultrasound.

For the study, Jennifer keeps a log of any illnesses and hospitalizations she may experience, as well as a record of her medications. A nurse periodically reviews her log with her by phone. Once a year, she receives a glomerular filtration rate (GFR) test to help establish the rate of progressive loss of her renal function, undergoes an MRI and/or ultrasound, and has a blood workup that includes a measurement of her creatinine levels. Creatinine is a waste product of muscle metabolism that is removed from the blood by the kidneys. As kidney disease progresses, the level of creatinine in the blood increases.

Researchers are gathering an incredible amount of information about PKD from CRISP and similar studies and are hopeful that, within five or ten years, they will achieve major strides in combating the disease.

In the meantime, Jennifer remains concerned that her cysts are gradually increasing in size. "It's hard for me to believe something like that is growing inside my body," she says. But she's forever hopeful. "With all the research they're doing, and how much they're discovering, I truly believe there will be some kind of treatment for PKD in the near future. It is fear, denial and ignorance of the disease that have become our greatest obstacles, so it is important we play an active role in managing our own healthcare. It is my vision and hope that one day no one will suffer the full effects of PKD." The many other people with PKD echo that same hope.

The CRISP study is a powerful effort to harness technology to enhance monitoring, treatment, and intervention in PKD. As CRISP moves forward, the NIDDK has also recently launched an interventional clinical initiative to study protective therapies for patients with PKD. The Polycystic Kidney Disease Clinical Trials Network was established in 2001 to design and implement clinical trials of treatments that will slow the progressive loss of renal function in PKD. It will also conduct one or more pilot and feasibility studies examining innovative strategies for slowing progression of PKD, and collect and store patient specimen samples and data for future research by PKD investigators. Four clinical centers from across the country, as well as a data-coordinating center, are currently collaborating on the first large interventional clinical trial conducted by this network. The HALT-PKD trial will be a randomized, controlled trial of the efficacy of anti-hypertensive agents that block the renin-angiotensin system on slowing the rate of decline of kidney function in patients with high blood pressure and PKD. Agents that block this system have demonstrated an advantage over other blood-pressure-lowering medications in reducing the loss of kidney function in patients with other types of kidney disease. The commonly used "ACE inhibitors" target the renin-angiotensin system, and an ACE inhibitor will be one of the drugs tested in this trial. It is expected that patient recruitment will be initiated in 2004.

ACKNOWLEDGEMENTS

Printed February 2004
For Administrative Use

Research:

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

Writing and Production:

Patient Profiles:
Larry Checco, Checco Communications

Highlights of Research Advances and Opportunities:
Staff of the NIDDK Office of Scientific Program and Policy Analysis

Editorial Production:
Eleanor F. Hoff, PhD,
NIDDK Office of Scientific Program and Policy Analysis

Design:
Elizabeth D. Gemignani, the Hill Group

Publication Coordination:
the Hill Group

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