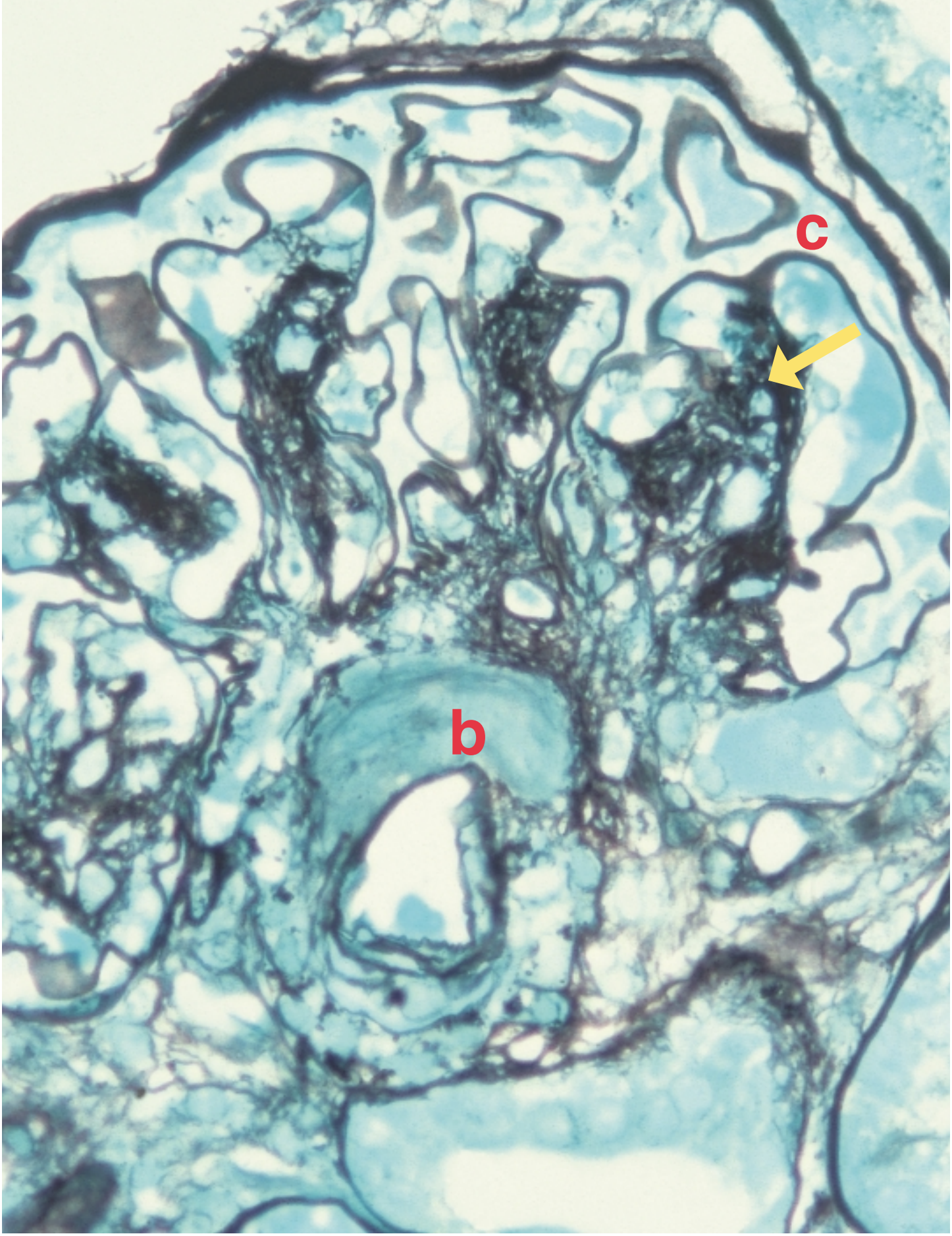

This photograph shows a cross-section of a glomerulus, the ball-shaped filtering unit of the kidney, taken from a patient with diabetic kidney disease. The tuft of capillary loops (c) surrounding one of the two central blood vessels (b) found in the glomerulus, has been extensively damaged (dark stain, as indicated by the yellow arrow). Both chronically elevated blood sugar levels and genetic susceptibility appear to contribute to this disease. Photo credit: Dr. Josephine Briggs, NIDDK, and Dr. Paul Killen, University of Michigan.

Kidney, Urologic and Blood Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health care problems in the U.S. They cause suffering and disability for millions of Americans, including children and young adults. The NIDDK is dedicated to research aimed at understanding, treating and preventing these diseases.

Chronic kidney disease is a growing epidemic in the U.S. It often progresses to irreversible kidney failure, which requires treatment with dialysis or kidney transplantation for patient survival. Presently, as many as 11 million individuals have substantially impaired kidney function. The two main causes of kidney disease, diabetes and hypertension, account for as much as 70 percent of all new cases of chronic kidney disease. The epidemic is due in large part to the increase of type 2 diabetes in the country.

The U.S. has seen an enormous increase in patients with end-stage renal disease (ESRD). In the year 2000, almost 100,000 people entered ESRD, with the result that a total population of about 300,000 were sustained on dialysis and 80,000 with functioning transplants. These numbers have doubled since 1990 and are expected to nearly double again by 2010. The cost of ESRD is high–almost $18 billion in 1999, as well as $2 billion to $4 billion of lost income for patients.

Ethnic minority populations, 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 and Hispanics are two times more likely to develop kidney failure than are whites.

The NIDDK devotes considerable resources to understanding the basic mechanisms underlying the causes and progression of kidney disease to end-stage kidney failure. The Institute's effort to combat ESRD includes research to reduce morbidity and mortality from bone, blood, nervous system, metabolic, gastrointestinal, cardiovascular, and endocrine abnormalities in ESRD; and to improve the effectiveness of dialysis and transplantation. Major areas of research focus include identification and testing of possible therapeutic interventions to prevent development or halt progression of kidney disease, and identification of the risk factors for ESRD and cardiovascular disease. A major outreach initiative is the National Kidney Disease Education Program.

Genetic Link Discovered for IgA Nephropathy

A principal cause of end-stage renal disease leading to kidney failure is inflammation of the glomeruli, specialized tufts of tiny blood vessels in the kidney that help clean the blood of waste and extra fluid. This inflammatory condition is known as glomerulonephritis. The most common cause of glomerulonephritis is IgA (immunoglobulin A) nephropathy, also called Berger's disease. About 100,000 Americans have IgA nephropathy. The disease may progress over a period of 10 to 20 years. About 30 percent of patients ultimately develop kidney failure. IgA nephropathy usually occurs in adolescents or young adults between the ages of 15 and 35. Males are affected two to three times more frequently than females.

Just why IgA deposits form is not known, although a variety of factors such as genetics and coincident infections seem to play important roles. There also is wide variation in incidence of IgA nephropathy in different parts of the world. IgA protein is a normal part of the body's immune system that protects against disease. In IgA nephropathy, however, IgA protein deposits in the glomeruli. The deposits lead to scarring of the kidneys, which interferes with the blood-cleansing process. The signs of disease are commonly revealed by the presence of blood and protein in the urine. IgA deposits are an immune system defect, hence IgA nephropathy is considered an autoimmune disease of the kidney.

Until recently, there was little reason to believe that IgA nephropathy would have a strong association with a single gene. Now, however, by studying ethnic groups

and families, researchers have located a gene area that is associated with IgA nephropathy. They collected 30 kindreds—24 in Italy and six in the United States—with 94 affected members, all ascertained via biopsy-documented cases. They performed a genome-wide analysis of linkage, searching for chromosome intervals showing linkage to the disease. Surprisingly, they found strong evidence for linkage of IgA nephropathy to chromosome 6q22–23. About 60 percent of kindreds with familial IgA nephropathy have disease attributable to inheritance at this genetic region.

The discovery that IgA nephropathy is influenced by a gene on chromosome 6 has opened the way to better understanding of the cause of IgA nephropathy, and to the possibility that treatment aimed at the molecular cause of IgA nephropathy may one day prevent kidney failure in patients with the disease. Researchers are working to identify the gene itself, a discovery that might yield clues to whether particular environmental influences trigger the disease. For example, the interaction between the gene and an environmental factor, such as an infectious agent, might explain why not everyone who inherits the IgA nephropathy gene develops the disease.

Gharavi AG, Yan Y, Scolari F, Schena FP, Frasca GM, Ghiggeri GM, Cooper K, Amoroso A, Viola BF, Battini G, Caridi G, Canova C, Farhi A, Subramanian V, Nelson-Williams C, Woodford S, Julian BA, Wyatt RJ, Lifton RP. IgA nephropathy, the most common cause of glomerulonephritis, is linked to 6q22-23. *Nat Genet* 26(3):354-357, 2000.

Genes and Technology Spur Advances in Polycystic Kidney Disease (PKD)

Polycystic kidney disease (PKD) is a genetic disease characterized by massive enlargement of the kidneys, caused by the growth of multiple fluid-filled cysts. It is estimated that PKD affects as many as 500,000 to 600,000 people in the U.S., and is the fourth leading cause of kidney failure.

A landmark advance in PKD research occurred when scientists discovered the genes, known as PKD1 and PKD2, which, when mutated, are responsible for the most prevalent form of PKD. Since discovery of these causative genes, investigators using three mouse models of the disease have made several advances in understanding PKD. They discovered a family of proteins produced by the PKD genes, called polycystins. In the most common form of PKD, the severity of the mutation was directly related to whether the animals died before birth or had decreased life spans. They concluded that the presence of polycystin-2 is essential for normal development of parts of the kidney, heart, and pancreas. A second research team examined kidney cysts from two patients and discovered that 71 percent of the cysts had mutations in the PKD2 gene, while a subset of cysts lacked those mutations but had mutations in the PKD1 gene. The findings suggest that PKD1 mutations may be modifiers of disease severity, and that independent disturbances in the production of the polycystin proteins by the PKD genes may be sufficiently disruptive to cause cyst formation.

Functions of PKD Genes: Before using this knowledge to develop PKD treatments, however, researchers need to know what these genes normally do within the kidney. Recent experiments suggest that polycystin-1 and -2 interact to form a channel, or opening, on the outer surface of cells that permits passage of calcium and other positively charged molecules. In the epithelial cells lining the kidney's filtering tubules, entry of calcium is thought to set off a chain of signals that controls cell growth and promotes normal structure and function of the tubules. Scientists have identified mutant versions of the polycystin-1 and -2 proteins in patients with PKD. One type of mutation prevents polycystin proteins from reaching the cell surface, so they are unable to form a channel. Without the channel, calcium can't enter the cells and signaling is disrupted. Disrupted signaling prevents normal maintenance of epithelial cell growth, and may result in generation of fluid-filled cysts. Another study suggests a second possible mutation that could result in signaling disruption. The end of the polycystin-1 protein that is located on the inside of kidney epithelial cells is called the C-terminus. The C-terminus of polycystin-1 must be intact in order for calcium and other positively-charged molecules to enter kidney epithelial cells. A mutation in PKD-1 that causes loss of the polycystin-1 protein C-terminus would prevent entry of calcium and other positively charged molecules into the cells, again causing signaling disruption and possibly resulting in cyst formation. The vital information provided by these and future studies could pave the way for the development of new and improved approaches for treating polycystic kidney disease.

Use of CAT Scans To Monitor PKD: A crucial accompaniment to research efforts on the causes of PKD is the development of new technologies to assess its progression. Until recently, doctors have had no rigorous guidelines for judging whether or not a PKD patient is likely to develop kidney failure and how quickly the disease may progress. An NIDDK-supported study recently confirmed doctors' anecdotal observations that kidney enlargement due to increased number and size of cysts is an accurate marker of PKD progression to kidney failure. The study used computed tomography (CT) scans to visualize kidney size and monitor the number of kidney cysts over the course of several years. The rate of kidney enlargement varied widely from patient to patient, but it was directly linked to the number or size of kidney cysts. Patients whose kidneys became enlarged were more likely to develop kidney failure, and those whose kidneys remained small were more likely to maintain relatively normal kidney function. This study validated the use of CT scanning as a method for monitoring progression of PKD. Use of CT scanning will also enable doctors to judge how well potential treatments work by documenting whether kidneys and cysts grow, shrink, or remain the same in size. The NIDDK intends to intensify research in this area, in recognition of the enormous value of technologies that can both assess the progression of PKD and also aid in the evaluation of new therapeutic approaches.

Facilitating PKD Research: NIDDK support for PKD research is being strengthened by new work on mouse models and on basic cell biology, in order to understand the cause of disease and to facilitate testing new treatment interventions. Four centers for basic research on PKD have been established by the NIDDK. Another recent NIDDK initiative (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, or CRISP) has promoted the development and testing of improved state-of-the-art imaging methods for PKD, including the CT technology described previously; the NIDDK is now expanding its support for both technology development and improved image processing. Under a new initiative, the NIDDK also plans to implement a multi-center clinical trial to assess the best strategy for reducing morbidity and mortality in PKD. The trial will investigate the optimum target levels for blood pressure control for patients with PKD, and whether angiotensin-converting enzyme inhibitors offer superior benefit over other anti-hypertensive agents in slowing the progression of PKD. The hope is that results emerging from studies in these three areas—basic research, technology, and clinical trials of treatment—will complement each other to improve the lives of PKD patients as quickly as possible.

Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, and Germino GG: Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* 408(6815):990-994, 2000.

Sise C, Kusaka M, Wetzel LH, Winklhofer F, Cowley BD, Cook LT, Gordon M, and Grantham JJ: Volumetric determination of progression in autosomal dominant polycystic kidney disease by computed tomography. *Kidney Int* 58(6):2492-2501, 2000.

Vandorpe DH, Chernova MN, Jiang L, Sellin LK, Wilhelm S, Stuart-Tilley AK, Walz G, and Alper SL. The cytoplasmic C-terminal fragment of polycystin-1 regulates a Ca^{2+}-permeable cation channel. *J Biol Chem* 276(6):4093-4101, 2001.

Watnick T, He N, Wang K, Liang Y, Parfrey P, Hefferton D, St. George-Hyslop P, Germino G, and Pei Y. Mutations of PKD1 in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat Genetics* 25(2):143-144, 2000.

Wu G, Markowitz GS, Li L, D'Agati VD, Factor SM, Geng L, Tibara S, Tuchman J, Cai Y, Park JH, van Adelsberg J, Hou H Jr, Kucherlapati R, Edelmann W, and Somlo S. Cardiac defects and renal failure in mice with targeted mutations in PKD2. *Nat Genet* 24(1):75-78, 2000.

Kidney Disease and Diabetes

Kidney disease is the major cause of excess illness and premature death in people with type 1 diabetes. Studies have suggested that, although the prolonged high blood glucose levels present in type 1 diabetes play an important role, they do not act alone to cause kidney disease—genetic susceptibility is also required. Recently, NIDDK-supported researchers identified a variation in the apolipoprotein E gene in type 1 diabetics that is associated with a three-times greater risk of developing kidney disease. This association first was found by doing a large, case-controlled clinical study, and was extended to a family-based association study. The latter is perhaps the most reliable method for examining associations between DNA sequence differences and specific diseases. While several studies have yielded equivocal results about the association of this genetic variant with kidney disease, this study is perhaps the most definitive to date. The apolipoprotein E gene codes for a protein that plays an important role in cholesterol transport. The particular variant of the gene that the researchers identified is strongly

associated with the development of coronary artery disease. One important next step for this research is to determine the molecular mechanisms that underlie the risk for diabetic kidney disease that is caused by the apolipoprotein E gene variant.

Recent advances in genetic technology have made it theoretically possible to generate mice that will develop diabetic complications analogous to human diabetic kidney disease. To facilitate studies in this area, the NIDDK is currently sponsoring a research program, 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 susceptibility or provide resistance. Candidate methods for the prevention, detection, and treatment of diabetic kidney disease may also be effectively tested in these mouse models. Other related research programs sponsored by NIDDK include a clinical trials pilot program to identify the most promising interventions for preventing or slowing the progression of kidney disease in type 1 diabetic patients.

Araki S-I, Moczulski DK, Hanna L, Scott LJ, Warram JH, and Krolewski AS. APOE polymorphisms and the development of diabetic nephropathy in type 1 diabetes: Results of case-control and family-based studies. *Diabetes* 49(12):2190-2195, 2000.

Preventing Kidney Failure

Kidney disease exacts a heavy toll on the U.S. population, in terms of disease and death. To overcome this burden on the public's health and well-being, the NIDDK has developed and carried out a number of initiatives to identify the most appropriate treatments for kidney disease, the risk factors associated with complications, and the best ways to increase the public awareness of kidney disease, in order to prevent progression to irreversible kidney failure.

African American Study of Kidney Disease and Hypertension (AASK): African Americans constitute approximately 12 percent of the U.S. population but comprise 32 percent of the prevalent ESRD population. In African Americans, the racial disparity is most striking in younger people, where those between the ages of 25 and 44 are 20 times more likely to develop kidney failure caused by high blood pressure, or hypertension, than whites. While better management of hypertension has led to fewer strokes and heart disease, kidney failure is increasing.

This past year, significant progress was made in identifying a treatment strategy for slowing the progression of kidney disease caused by hypertension. In 1994, the NIDDK began the largest U.S. clinical trial of kidney disease in African Americans, called the African American Study of Kidney Disease and Hypertension (AASK). It was hoped that this trial would determine whether doctors should treat patients with elevated blood pressure to a level lower than is usually practiced, whether a specific class of blood pressure drug is required, or whether both strategies are needed to slow or stop the progression of hypertension-related kidney disease in African Americans. Scientists working on this study recently showed that people with kidney disease caused by hypertension have a better chance of reducing the risk of kidney failure if they take an angiotensin-converting enzyme (ACE) inhibitor. They found that the ACE inhibitor ramipril slowed kidney disease by 36 percent and slashed the risk of kidney failure and death by 48 percent in patients who had at least one gram of protein in the urine. The drug was compared to the dihydropyridine calcium channel blocker amlodipine. Results were not related to blood pressure control, which was comparable between study groups.

ACE inhibitors have been the preferred treatment for kidney disease caused by diabetes since 1994. Now, AASK doctors are recommending it for kidney disease of hypertension, especially for people who also have protein in the urine. While calcium channel blockers help many patients, particularly African Americans, control blood pressure and reduce the risk of stroke and heart disease, patients may need an ACE inhibitor to protect the kidneys.

When the AASK trial concludes in 2002, the NIDDK will support investigations of the environmental, socioeconomic, genetic, physiologic, and other co-morbid factors that influence progression of kidney disease as part of the AASK Continuation Study.

Cohort Study of Chronic Renal Insufficiency (CRIC): The NIDDK is launching a major new clinical initiative to increase understanding of the risk factors associated with

progression of kidney disease and the development of cardiovascular disease and associated mortality. This initiative recognizes that cardiovascular disease is the leading cause of death in patients with ESRD, and that it is imperative to gain new knowledge about the relationship between ESRD and cardiovascular disease as a foundation for the development and evaluation of potential interventions. To this end, the NIDDK is initiating the "Cohort Study of Chronic Renal Insufficiency (CRIC)."

CRIC is a prospective longitudinal study that will examine genetic, environmental, behavioral, nutritional, quality of life, and health resource utilization factors in people with chronic kidney disease. CRIC also will determine the incidence of and risk factors for cardiovascular disease in these individuals. The participants in this study will reflect the racial, ethnic, and gender composition of the end-stage kidney disease patient population in the U.S., including an appropriate number of African Americans.

National Kidney Disease Education Program (NKDEP): Chronic kidney disease can be prevented in many populations at risk. Moreover, its progression can be slowed in those who already have the disease, as shown in the AASK trial and other studies. Despite these advances in treatment and prevention, data suggest that only a small fraction of people at serious risk for or with established but early kidney disease is receiving proper screening or treatment. To remedy this problem, the NIDDK recently initiated the National Kidney Disease Education Program (NKDEP), which will address the epidemic of kidney disease in the United States (see sidebar on page 66).

Agodoa, LY, Appel L, Bakris GL, Beck G, Bourgoignie J, Briggs JP, Charleston J, Cheek D, Cleveland W, Douglas JG, Douglas M, Dowie D, Faulkner M, Gabriel A, Gassman J, Greene T, Hall Y, Hebert L, Hiremath L, Jamerson K, Johnson CJ, Kopple J, Kusek J, Lash J, Lea J, Lewis JB, Lipkowitz M, Massry S, Middleton J, Miller ER III, Norris K, O'Connor D, Ojo A, Phillips RA, Pogue V, Rahman M, Randall OS, Rostand S, Schulman G, Smith W, Thornley-Brown D, Tisher CC, Toto RD, Wright JT Jr, Xu S; African American Study of Kidney Disease and Hypertension (AASK) Study Group. Effect of Ramipril vs. Amlodipine on renal outcomes in hypertensive nephrosclerosis: A randomized controlled trial. *JAMA* 285(21):2719-2728, 2001.

Understanding the Function, Structure, and Genetics of the Urinary Bladder

Through research, scientists are discovering that the urinary bladder is much more than a reservoir for liquid waste. Rather, it is a dynamic organ with many important structural and physiological properties. As the kidneys filter waste from the blood, they send it to the urinary bladder for collection as urine. When the bladder is full, nerve impulses sent to the brain signal that it is time to urinate, resulting in the sensation of "urgency." Proper function of the bladder is vital to rid the body of waste and to prevent infection. Unfortunately, millions of Americans suffer from disorders affecting bladder function, including temporary problems with urine retention; chronic and painful disorders such as interstitial cystitis, which can cause scarring and permanent bladder damage; urinary tract infections, which can move up through the bladder to endanger the kidneys; and urinary incontinence, resulting in uncontrolled urine flow from the bladder. Recent findings from NIDDK-supported research on bladder genes and proteins are providing insights into normal bladder function that could, in turn, lead to better tests, treatments, and prevention strategies for bladder disease.

Protecting the Bladder's Permeability Barrier: Recent studies have elucidated the functional importance of a class of four proteins, called uroplakins, found only in the urinary tract. Plaques of crystalline uroplakin particles almost entirely cover the bladder lining (urothelium). They are vital in the permeability barrier that protects the bladder from infectious agents and prevents leakage of waste products into the body. The plaques are also fairly dynamic structures that can break and re-fuse, which may be important in maintaining barrier flexibility as the bladder distends and retracts during filling and emptying.

Upon examining the interactions of the uroplakin proteins in animals, NIDDK-supported investigators found that two of the four proteins link specifically to the other two (uroplakin UPIa with UPII, and uroplakin UPIb with UPIII). The two pairs are present in all plaques, and all plaques have a similar uroplakin composition. It also appears that both uroplakin pairs are required for normal plaque formation, for when the same group of researchers removed the gene for uroplakin III in a mouse model, the bladder lining became permeable.

Upon examining the remaining uroplakins, the researchers found several abnormalities in UPIII's partner protein, UPIb. The end result was that only small patches of urothelial plaque were formed, causing the permeability defect. Just as importantly, knocking out UPIII also caused the mouth of the tubes (ureters) connecting the kidneys to the bladder to become larger, resulting in vesicoureteral reflux, in which urine flows back from the bladder into the kidneys. In humans, vesicoureteral reflux is a hereditary condition that affects about one percent of pregnancies and represents a leading cause of renal failure in infants. Until now, there was no known genetic basis for this disease. Although reflux is likely caused by more than one gene, these new data now suggest that the absence of uroplakin III can cause this defect, opening up avenues for future therapies.

Dynamic Role of Bladder in Releasing Proteins into the Urine: Further dismantling the image of the bladder as primarily a "receptive" organ, NIDDK-supported researchers have also found that the bladder can secrete a number of different proteins that go directly into the urine rather than forming part of the bladder lining. Depending upon the animal, proteins previously identified in the urine come either directly from the kidneys, or from the liver *via* the kidneys. The proteins secreted into the urine by the bladder itself, which include both enzymes and enzyme inhibitors, may have important physiological or protective functions in the lower urinary tract. Importantly, this finding also suggests that the bladder may play a more dynamic role in responding to its environment. For example, if bladder proteins are inappropriately secreted in response to factors encountered in the urine, this could act as a trigger for bladder dysfunction.

These new findings are providing great momentum to bladder research at the cellular level. By exploiting these discoveries, scientists can propel bladder research even further to reveal, with even greater precision, how the bladder functions, and how bladder diseases can be optimally treated and prevented.

In addition to supporting basic and applied research in bladder disease, the NIDDK seeks to enhance progress in bladder disease research efforts by engaging in strategic research planning with scientific leaders in the external research community. Consistent with this goal, the NIDDK convened the Bladder Research Progress Review Group (Bladder Research PRG) meeting in the summer of 2001. This group was composed of representatives from professional and patient organizations and experts in specific bladder diseases from a broad range of research disciplines. These experts evaluated the bladder research portfolios of NIDDK and NIH, identified research opportunities, and defined unmet needs in bladder research. A strategic plan to redefine bladder research and to target specific areas for expansion is being formulated by the Bladder Research PRG. The report from this meeting will be invaluable in guiding future NIDDK efforts in bladder disease research.

Deng F-M, Ding M, Lavker RM, Sun T-T. Urothelial function reconsidered: A role in urinary protein secretion. *Proc Natl Acad Sci USA* 98(1):154-159, 2001.

Hu P, Deng F-M, Liang F-X, Hu C-M, Auerbach AB, Shapiro E, Wu X-R, Kachar B, and Sun T-T: Ablation of uroplakin III gene results in small urothelial plaques, urothelial leakage, and vesicouretral reflux. *J Cell Biol* 151(5):961-971, 2000.

Liang F-X, Riedel I, Deng F-M, Zhou G, Xu C, Wu X-R, Kong X-P, Moll R, Sun T-T: Organization of uroplakin subunits: transmembrane topology, pair formation and plaque composition. *Biochem J* 355(Pt1):13-18, 2001.

Bladder Cancer Diagnosed by Simple Urine Test

NIDDK-supported researchers recently developed a urine test that could lead to an easier, less invasive way to detect bladder cancer. This new test has enormous therapeutic implications, as it may help patients avoid partial or total loss of the bladder to cancer, which can affect quality of life, even beyond the inability to urinate normally. In both men and women, the proximity of internal sexual organs to the bladder means that they may have to be removed in cases of invasive bladder cancer. As with all cancers, early detection of bladder cancer is vital to avoid such drastic surgery. Bladder cancer is the fourth most common type of cancer in men and the eighth most common in women. Right now, there is no simple, approved test for bladder cancer.

The simple, noninvasive test screens for a protein called survivin, which is undetectable in most normal adult tissues, but is prominently expressed in common human cancers. Survivin is an inhibitor of programmed, or "natural," cell death. When the survivin gene is switched on, subsequent production of survivin permits mutated cells to survive. Switching off the survivin gene

stops the progression of cancer. The new test uses an antibody to detect survivin in urine samples.

In a series of experiments, survivin was found in all (46) urine samples of patients with new or recurrent bladder cancer, but not in the urine of any of the healthy volunteers (17) or patients with other urologic cancers, i.e., kidney, prostate, cervical, or vaginal cancer (30). The results of these and other experiments indicate that the sensitivity of the urine survivin test for new or recurrent bladder cancer was 100 percent, and its specificity for other noncancerous and benign genitourinary tract disease was 95 percent. The researchers suggest that their simple, noninvasive, urine survivin antibody test would be a useful complement to other diagnostic markers to identify new bladder cancers early, to monitor bladder cancer patients more effectively, and to identify recurrences early. With this impressive research advance, it is hoped that a urine test for bladder cancer could become as routine as tests used at other regular check-ups, such as prostate specific antigen (PSA) tests for prostate cancer.

Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, and Altieri DC. Urine detection of survivin and diagnosis of bladder cancer. *JAMA* 285(3):324-328, 2001.

Urinary Tract Infections: Insights into Causes and Treatments

Urinary tract infections (UTIs) are among the most common infectious diseases acquired by humans; in fact, only respiratory infections occur more often. Women are especially prone to UTIs, in part, it is believed, because women have shorter urethras than men. Urine flows out from the bladder through the urethra during urination, and this "tube" is the primary site of UTIs. Most UTI's arise from one type of bacteria, *Escherichia coli (E. coli)*, that normally lives in the colon. If the infection is not treated promptly, bacteria may travel to the bladder, which can lead to a relatively serious infection of the kidneys, a condition known as pyelonephritis. UTIs are treated with antibacterial drugs. The choice of drug and length of treatment depend on the patient's history and the urine tests that identify the offending bacteria and its relative sensitivity to various drugs.

Asymptomatic UTIs: Scientists and physicians have begun to realize that often, a woman may have bacteria in her urine but not have symptoms of a UTI. These types of infections are called "asymptomatic" because the patient does not display any physical signs of a UTI. A recent study demonstrated that asypmtomatic infections are relatively common and rarely persist for a long period of time. Thus, bacteria in the urine do not automatically lead to clinically overt UTIs. However, such asymptomatic infections are strong predictors of subsequent, symptomatic UTIs.

Recurrent UTIs: Many women suffer from frequent UTIs. Nearly 20 percent of women who have a UTI will have another, and 30 percent of those will have yet another. Of the last group, 80 percent will have recurrences. Usually, the latest infection stems from a strain or type of bacteria that is different from the infection before it, indicating a separate infection. Even when several UTIs in a row are due to *E. coli*, slight differences in the bacteria indicate distinct infections.

Because UTIs are a recurrent problem for a large number of women, there has been interest over the years in determining whether it may be beneficial for women to self-diagnose and self-medicate with antibiotics as a valid approach to treating these chronic, recurrent infections. In order to determine the effectiveness of this strategy, researchers studied 172 women in a university-based primary health care clinic. Within this group, 88 women diagnosed a total of 172 UTIs, about 94 percent of which were subsequently confirmed by laboratory evaluation, and treated themselves with the antibiotics ofloxacin or levofloxacin. Self-treatment of uncomplicated recurrent UTIs was very effective in curing infection in this study, as the cure rate exceeded 90 percent.

While self-diagnosis followed by self-treatment simplifies the care of women with recurrent UTIs, it will nevertheless still be important to involve physicians and other health care professionals in the management of these infections, especially in light of the growing problem of drug-resistant bacteria, described below.

Antibiotic Resistance: The use of antibiotics to combat UTIs needs to be tempered with caution in selecting the appropriate drug. Because some strains of bacteria are resistant to certain drugs, it is important to choose agents that will be effective against a given strain of *E. coli*. For example, a recent study showed that empirical treatment of UTIs with trimethoprim-sulfamethoxazole (TMP-SMX),

initiated before the results of microbiological tests were known, led to lower cure rates in individuals who were subsequently found to be infected with organisms resistant to this drug.

In many cases, the current drug of choice for treatment of uncomplicated UTIs is TMP-SMX, though many other antibiotics are used. However, the frequency of UTIs caused by bacteria that are resistant to TMP-SMX is increasing. The widespread overuse of antibiotic drugs has led to increased incidence of multiple-drug resistance in *E. coli*, including those strains that can cause UTIs. The appearance of antibiotic-resistant bacteria is worrisome because it eliminates that drug as a treatment option. Multiple-drug resistance, therefore, severely limits the efficacy of existing antibiotics to treat infection.

Responding to a sharp increase in drug-resistant UTIs, researchers recently identified a new strain of *E. coli*, which they called clonal group A, in urine samples from women with UTIs in California, Michigan, and Minnesota. In this study, scientists examined *E. coli* isolated from young women in a university community in California who had uncomplicated urinary tract infections that were resistant to TMP-SMX. The results of the analyses were compared with bacteria isolated and analyzed from similar patients in Michigan and Minnesota. The bacteria isolated from these patients were tested for a number of molecular markers and for antibiotic susceptibility. In the California group, over one half of the urine samples tested positive for *E. coli*, and nearly one-quarter of these consisted of bacteria that were resistant to TMP-SMX as well as other antibiotics. Half of the antibiotic-resistant samples of *E. coli* displayed a common DNA fingerprint, suggesting that the bacteria isolates belonged to the same genetic strain, dubbed "Clonal group A" by the investigators. Similar findings were noted in the analysis of patients from Michigan and Minnesota. This prevalence of Clonal group A *E. coli* is surprisingly high for a single strain. Importantly, the distinct geographic clusters of the patient groups studied suggest a common route of dissemination of Clonal group A, possibly through contaminated food. This finding indicates that molecular typing of the *E. coli* causing drug-resistant UTIs may provide important information about the origins and spread of these bacteria within communities, enhancing opportunities to prevent further transmission of drug-resistant infections.

Gupta K, Hooton TM, Roberts PL, Stamm WE. Patient-initiated treatment of uncomplicated recurrent urinary tract infections in young women. *Ann Intern Med* 135(1):9-16, 2001.

Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Ann Intern Med* 135(1):41-50, 2001.

Hooton TM, Scholes D, Stapleton AE, Roberts PL, Winter C, Gupta K, Samadpour M, Stamm WE. A prospective study of asymptomatic bacteriuria in sexually active young women. *N Eng J Med* 343(14):992-997, 2000.

Manges AR, Johnson JR, Foxman B, O'Bryan TT, Fullerton KE, Riley LW. Widespread distribution of urinary tract infections caused by a multidrug-resistant Escherichia coli clonal group. *N Eng J Med* 345(14):1007-1013, 2001.

Prostate Disease: New Clinical Efforts

Diseases of the prostate, including benign prostatic hyperplasia (BPH) and chronic prostatitis, are a major health care burden for men. BPH affects more than 50 percent of men past the age of 50. If left untreated, BPH can lead to urinary tract infections, urinary retention, and in occasional cases, kidney disease. Chronic prostatitis is a chronic, disabling condition in which pelvic pain is the most common symptom. Such pain is followed by various voiding symptoms, such as waking up at night to urinate; frequent, urgent urination; incomplete voiding; decreased force or intermittency of the urinary stream; and/or a need to push or strain to fully empty the bladder. Because these diseases of the prostrate represent a potential burden to men of all ages and ethnic backgrounds, the NIDDK has undertaken vigorous efforts to study these problems, to identify their underlying causes and search for new potential therapies.

Developing Biologic Markers and Genetic Tests: The NIDDK is seeking better biologic markers for BPH, as well as genetic tests to identify patients at high risk for early disease, for rapidly progressing disease, and for prostate cancer. Genetic tests could also be used to determine which patients are likely to respond to various forms of therapy. It is likely that such important tools for aiding diagnosis and treatment may be developed from biologic materials that have been accumulated as part of the NIDDK's ongoing clinical trial, the "Medical Therapy of Prostatic Symptoms" (MTOPS). Nearing completion, the MTOPS trial is testing two different

drug regimens for BPH. One drug, finasteride, inhibits formation of a hormone involved in prostate enlargement, while the other, doxazosin, relaxes the muscle of the prostate and bladder neck to improve urine flow and reduce obstructions.

New biologic and genetic tools may be developed by further study of the serum samples of the 4,000 patients who participated in the MTOPS clinical trial, as well as the tissue from prostate biopsies of approximately 900 patients. The availability of these biologic materials presents an extraordinary opportunity to develop and evaluate markers that will further our understanding of fundamental processes contributing to BPH and prostate cancer, or related to response to therapy of BPH. The large number of well-characterized patients in the MTOPS consortium should enable the identification and testing of such biomarkers. To take advantage of this valuable resource, the NIDDK intends to assemble a cross-disciplinary, multi-institutional consortium with a range of expertise to perform cooperative studies. This consortium will use the MTOPS material to evaluate genetic, immunologic, or biochemical biomarkers relevant to the progression of BPH, response to therapy, and the concurrent development of prostate cancer.

Another recent and complementary NIDDK initiative will expand the cadre of prostate researchers and increase the use of novel technologies and innovative approaches in prostate research as part of the Institute's Prostate Research Novel Exploratory Teams (Prostate Research NET). This initiative is the result of several information-gathering and planning meetings conducted in coordination with other NIH institutes and centers, including the National Cancer Institute-sponsored Prostate Research Progress Review Group, April 1997; the International Symposium on Prostate Growth, March 1998; and the Symposium on Prostate Growth and Aging, September 2000.

Assessing New, "Minimally Invasive" Surgical Treatments: Research funded by the NIDDK has contributed to the development of new "minimally invasive" surgical treatments for BPH, which will now be assessed for long-term safety and efficacy. For many years, transurethral resection of the prostate (TURP) has been the standard of surgical therapy for treatment of symptomatic BPH. With TURP, an instrument called a resectoscope is inserted up the urethra through the penis. The resectoscope has a wire loop at its end that the physician uses to remove the tissue obstructing the urethra. Transurethral procedures are less traumatic than open forms of surgery and require a shorter recovery period, but are not ideal.

Technical innovations over the past decade have furthered the development of more advantageous surgical treatments. These new "minimally invasive" surgical treatments aim to achieve the same long-term outcomes of TURP, but with the benefits of lower costs, shorter length of hospital stay, and more rapid recovery. These approaches include laser therapy, transurethral electrovaporization, microwave therapy, and transurethral needle ablation.

To assess the quality of outcomes of these new therapies, the NIDDK has formed a group of collaborative Prostate Evaluation and Treatment Centers and a Biostatistical Coordinating Center. These centers will develop and conduct randomized, controlled clinical trials of the long-term efficacy and safety of the major "minimally-invasive" approaches for the treatment of symptomatic BPH. Through carefully designed and controlled clinical trials, a clearer picture of the benefits and risks of these methods will become available, thus aiding the physician and patient in making the most appropriate treatment choices.

Chronic Prostatitis Collaborative Research Network: The NIDDK's Chronic Prostatitis Collaborative Research Network has developed and validated a questionnaire being used by the wider research community to provide accurate assessments of symptom severity and quality of life. The Network is documenting symptoms, possible risk factors, medical histories, treatments, and the results of blood, prostate fluid, semen, and urine tests. Three avenues of study are being pursued through the Network: basic laboratory investigation, a longitudinal cohort study, and a randomized clinical trial. The Network recently began its first clinical study by comparing the effects of two drugs with placebo. The drugs are: (1) tamsulosin hydrochloride, which may increase the flow of urine and decrease pelvic pain, and (2) ciprofloxacin, an antibiotic that may reduce inflammation. Two additional clinical sites have been added to facilitate recruitment of minority study participants, and full-scale implementation of trials with all sites recruiting patients is slated to occur in 2002.

Stem Cell Approaches for Blood Disorders

Sickle cell anemia is an inherited, chronic blood disease in which the red blood cells become altered and function abnormally. The disease is caused by a change in the chemical composition of the protein, hemoglobin, which carries the oxygen inside red blood cells. This abnormal hemoglobin, called "hemoglobin S," causes the shape of the molecules to change under certain conditions and chemically link to each other, creating chains of molecules called polymers. Elongated hemoglobin S polymer structures distort the shape of the whole red blood cell, which interferes with red blood cell movement through blood vessels. Damage results to the vessels around the distorted cells and the tissues that depend on the vessels for oxygen and nourishment. Sickle cell anemia can become life-threatening when the red blood cells break down or the bone marrow fails to produce blood cells during periodic sickle cell "crises." Repeated episodes such as these can lead to damage of the kidneys, lungs, bone, liver, and central nervous system. Sickle cell disease is a serious disease in the African American community.

The abnormal hemoglobin S is inherited as an autosomal recessive trait; this means that it must be inherited from both parents for a person to contract sickle cell anemia. If hemoglobin S is inherited from only one parent, the offspring will "carry" the sickle cell trait, but are usually without symptoms. No cure is available for sickle cell anemia and the current objective of therapy is the comprehensive management and control of symptoms relating to sickle cell crises. However, even with treatment of symptoms, patients with sickle cell anemia usually die from organ failure between the ages of 20 and 40. New research in therapies for sickle cell anemia capitalizes on the molecular basis for the disease, which makes it a good target for genetic correction at the stem cell level. Current research is under way in the NIDDK intramural program to pursue stem cell-based approaches to this disease, successful completion of which would eliminate all symptoms and complications of patients with sickle cell anemia. Researchers in the NIDDK's Molecular and Clinical Hematology Branch are currently investigating stem cell-based approaches to sickle cell anemia; two of these approaches are allogeneic bone marrow transplantation and autologous stem cell gene therapy.

Allogeneic Bone Marrow Transplantation: The first approach under study is stem cell replacement by allogeneic bone marrow transplantation, in which the donor, either a relative or an unrelated individual from a registry, is genetically similar to the patient. Bone marrow contains the stem cells responsible for producing red blood cells and transplantation has become an accepted curative therapy for a broad range of diseases, including malignant diseases such as leukemias and lymphomas, as well as non-malignant and inherited diseases such as sickle cell anemia.

For effective transplantation, high doses of chemotherapy and/or radiation must be given to the patient in order to destroy the defective bone marrow and suppress the recipient's immune system to decrease the chance of graft rejection. This process is called myeloablative conditioning. The normal marrow obtained from the donor is delivered intravenously to the patient and the stem cells find their way to the bone marrow to produce new, normal blood cells. This ablative conditioning, however, can be toxic to the patient, causing significant acute side effects including hair loss, vomiting, disease of the heart muscle, and acute kidney failure. NIDDK researchers are now aiming to develop a nonmyeloablative conditioning regimen in which both the donor's and the patient's stem cells would contribute to normal blood formation. In this scenario, although the patient's defective stem cells would be present, the normal donor stem cells should have a selective advantage over the defective ones, thus favoring normal red blood cell formation.

Using a non-myeloablative conditioning regimen, NIDDK researchers have shown that bone marrow transplants into mice with sickle cell anemia result in complete replacement by donor hemoglobin, reflecting the selective advantage conferred upon the normal hemoglobin-containing red blood cells. With this success, plans are under way at NIDDK to pursue a non-myeloablative bone marrow transplant clinical trial for patients with sickle cell anemia.

Autologous Stem Cell Gene Therapy Approaches: A second type of stem cell-based correction is also being pursued because many sickle cell patients lack a suitable matched donor for bone marrow transplantation and even those with a donor are at risk of developing graft-versus-host disease (GVHD). About half the patients undergoing bone marrow transplants develop GVHD, which results

when the donor's bone marrow attacks the patient's organs and tissue. This attack happens because the donor's T-lymphocytes, a type of white blood cell, recognize the patient as being foreign. In most cases GVHD is mild, but it can be life-threatening. For these patients, autologous stem cell gene therapy may be useful. It is a process by which the patient's own stem cells are removed from the bone marrow, genetically corrected, and then reinfused into the patient to contribute to normal red blood cell formation and permanent correction of the sickle cell defect. Gene transfer generally involves the identification of a therapeutic gene or other nucleic acid (for example, an RNA molecule or a synthetic nucleic acid piece), a vector that allows delivery of the therapeutic nucleic acid to the appropriate cell, and a device (for example, a catheter, syringe, or stent) to deliver the gene/vector combination to the appropriate tissue *in vivo*.

Gene transfer can also be achieved through the use of a modified retrovirus or adenovirus vector to enhance gene delivery to certain cells. Using a modified retroviral vector, researchers have demonstrated gene transfer rates of greater than 10 percent in the rhesus monkey, a primate with stem cell biology similar to humans. This is the first time results such as these have been seen in a large animal and they mark a major advance for the field. Also, persistent, successful engraftment was seen in the nonhuman primates after less toxic, low dose irradiation. The NIDDK intramural research team continues efforts to refine and optimize techniques for gene delivery. They have begun to develop a preclinical nonhuman primate model for gene transfer, which could soon become a viable form of therapy for patients with sickle cell disease.

Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, Read EJ, Tisdale J, Dunbar C, Linehan WM, Young NS, and Barrett AJ. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *New Engl J Med* 343(11):750-758, 2001.

Jackson KA, Mi T, Goodell MA. Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* 96(25):14482-14486, 1999.

Kang E, Giri N, Wu T, Sellers S, Kirby M, Hanazono Y, Tisdale J, and Dunbar CE. In vivo persistence of retrovirally transduced murine long-term repopulating cells is not limited by expression of foreign gene products in the fully or minimally myeloablated setting. *Hum Gene Ther* 12(13):1663-1672, 2001.

Kang EM, Hanazano Y, Frare P, Vanin EF, DeWitte M, Metzger M, Liu JM and Tisdale JF. Persistent low-level engraftment of rhesus peripheral blood progenitor cells transduced with the Fanconi anemia C gene after conditioning with low-dose irradiation. *Mol Ther* 3(6):911-919, 2001.

Kim HJ, Tisdale JF, Wu T, Takatoku M, Sellers SE, Zickler P, Metzger ME, Agricola BA, Malley JD, Kato I, Donahue RE, Brown KE, and Dunbar CE. Many multipotential gene-marked progenitor or stem cell clones contribute to hematopoiesis in nonhuman primates. *Blood* 96(1):1-8, 2000.

Wu T, Kim HJ, Sellers SE, Meade KE, Agricola BA, Metzger ME, Kato I, Donahue RE, Dunbar CE, and Tisdale JF. Prolonged high-level detection of retrovirally marked hematopoietic cells in nonhuman primates after transduction of CD34+ progenitors using clinically feasible methods. *Mol Ther* 1(3):285-293, 2000.