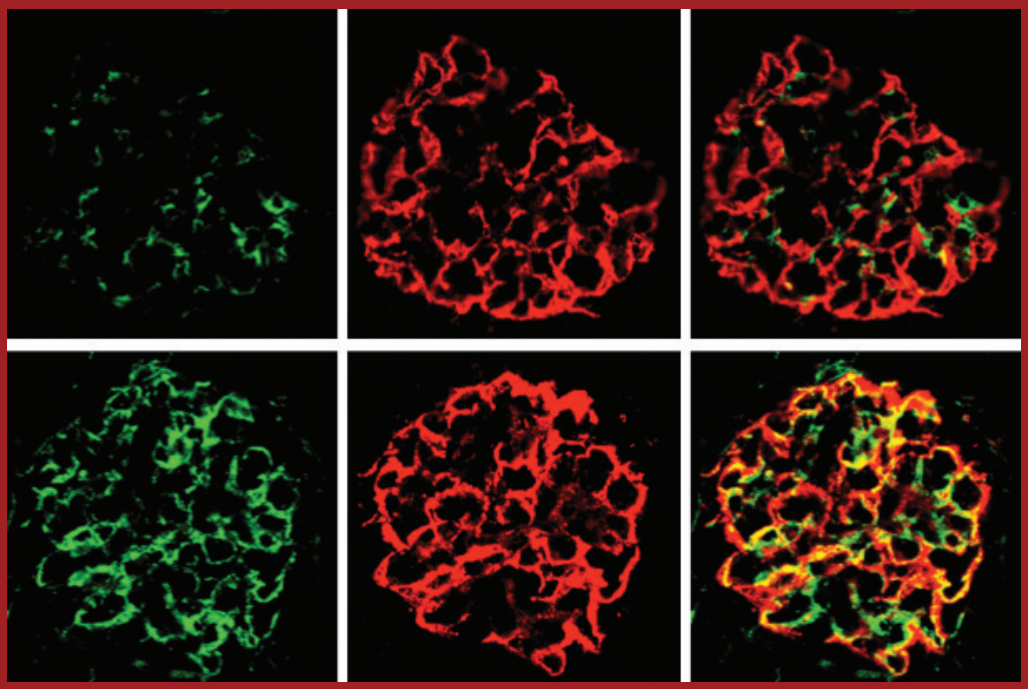

Elevated levels of the circulating protein suPAR have been identified as a potential mediator of the kidney disease focal segmental glomerulosclerosis. This figure shows sections of mouse kidney following injection of either a low dose (top row) or a 10-fold higher dose (bottom row) of suPAR. In the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

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Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans, including children and young adults. The NIDDK supports basic and clinical research studies of the kidney and urinary tract and disorders of the blood and blood-forming organs. The goal is to increase understanding of kidney, urologic, and hematologic diseases to enhance prevention and treatment strategies.

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about two quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, even for a short period of time or due to gradual deterioration, can result in life-threatening complications. Loss of kidney function, whether sudden or slow, represents an important health challenge.

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

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

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

The NIDDK supports a significant body of research aimed at understanding the biology underlying chronic kidney disease. The Institute's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading

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

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

[3] *U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2011.*

to progression of kidney disease to ESRD, and the identification and testing of possible treatments to prevent development or halt progression of kidney disease. In late 2011, the Institute provided funding for several planning grants for translating chronic kidney disease research into improved clinical outcomes. Also of interest are studies of inherited diseases such as polycystic kidney disease, congenital kidney disorders, and immune-related kidney diseases such as IgA nephropathy and hemolytic uremic syndrome. The Institute's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat chronic kidney disease and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of particular interest include the causes of and treatments for major adult urological diseases and disorders, such as benign prostatic hyperplasia, urinary incontinence and urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS) in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

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

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them women.[6] Many suffer in silence due to embarrassment and lack of knowledge about options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence, and the effect of different diagnostic tools and interventions on patient outcomes. For example, the utility of a common pre-surgical urinary test, called urodynamic testing, has not been proven to be helpful to women or their doctors. The NIDDK's Urinary Incontinence Treatment Network recently completed the Value of Urodynamic Evaluation clinical trial to clarify the necessity of urodynamic testing for a woman with stress urinary incontinence who is planning surgical treatment, and results are expected in 2012.

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

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

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

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. The Institute is also keenly interested in the basic biology of stem cells, including adult hematopoietic stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research. An additional priority of the Institute's hematology research program is the development of improved iron-chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

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

RESEARCH TOWARD IMPROVED TREATMENT OF KIDNEY DISEASE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INSIGHTS INTO THE CELLULAR MECHANISM OF KIDNEY REPAIR

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

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

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

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

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

UNDERSTANDING AND TREATING UROLOGICAL DISORDERS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DEVELOPING NEW THERAPIES FOR GENETIC BLOOD DISORDERS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Kao WHL, Klag MJ, Meoni LA, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. Nat Genet 40: 1185-1192, 2008.

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

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

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

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

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

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

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

[1] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 150: 604-612, 2009.
[2] U.S. Renal Data System, USRDS 2011 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2011.
[3] www.drherz.us/08ProviderTable.pdf

STORY OF DISCOVERY

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

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

The Kidneys, Nephrotic Syndrome, and Membranous Nephropathy

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

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

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

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

STORY OF DISCOVERY

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

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

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

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

The Identification of the Human Autoimmune Antigen

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

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

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

Looking Forward

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

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

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[1] In 1959, the NIH consisted of the National Institute of Health (singular) and the National Cancer Institute.

[2] Heymann W, Hackel DB, Harwood S, Wilson SG, and Hunter JL. Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. Proc Soc Exp Biol Med 100: 660-664, 1959.

[3] Kerjaschki D and Farquhar MG. The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. Proc Natl Acad Sci USA 79: 5557-5561, 1982.

[4] Beck LH Jr, Bonegio RGB, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med 361: 11-21, 2009.

[5] Beck LH Jr, Fervenza FC, Beck DM, et al. Rituximab-induced depletion of anti-PLA_2R autoantibodies predicts response in membranous nephropathy. J Am Soc Nephrol 22: 1543-1550, 2011.

Genetic Factors in Chronic Kidney Disease

Dr. John Sedor

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

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

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

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

The Intersection of Genes and Environment

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

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

FINDing a Clue to the Genetics of Kidney Disease

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

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

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

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

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

SCIENTIFIC PRESENTATION

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

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

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

Future Directions

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

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

SCIENTIFIC PRESENTATION

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

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PATIENT PROFILE

John Saul

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

John Saul
Photo credit: Mary Jo Peairs

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

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

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

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

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About AKI

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

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

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

There is no effective drug therapy to reverse AKI.

The ASSESS-AKI Study

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

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

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

PATIENT PROFILE

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

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

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

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

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