



Benign prostatic hyperplasia (BPH), which is often associated with a collection of lower urinary tract symptoms (LUTS), affects men of all races and ethnic groups and can progress in severity over time. If untreated, BPH can lead to significant consequences, such as acute urinary retention, incontinence, and urinary tract infection. Medical and surgical interventions, however, do not achieve symptom relief for all men with lower urinary tract dysfunction or may not provide a durable response. Research described in this chapter implicates fibrosis in the process of BPH/LUTS in men; specifically, men for whom current drug treatments do not work have greater amounts of fibrosis in their prostates. In this figure, prostate samples were obtained from men treated for BPH with a combination of doxazosin and finasteride and analyzed with two different forms of microscopy. Upper panels: prostate tissue from men whose BPH responded to the treatment. Bottom panels: prostate tissue from men who did experience clinical progression to increasingly severe BPH symptoms.

*Images courtesy of William Ricke, Ph.D., University of Wisconsin-Madison.*

# 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 affect millions of Americans, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new prevention and treatment strategies, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to improve the health of people who have or are at risk for kidney, urologic, and hematologic (blood) diseases.*

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, is a life-threatening condition.

It has been estimated that 37 million American adults have impaired kidney function—also called chronic kidney disease (CKD).<sup>1</sup> CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. CKD, 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 2016, over 726,000 patients received treatment for ESRD: over 511,000 received either hemodialysis or peritoneal dialysis, and over 215,000 were living with a kidney transplant.<sup>2</sup> Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives,

bear a disproportionate burden of CKD and ESRD. Compared to non-Hispanic Whites, ESRD prevalence in 2016 was about 3.7 times greater in African Americans, 1.3 times greater in Hispanics, 1.5 times greater in American Indians and Alaska Natives, 9.5 times greater in Native Hawaiians/Pacific Islanders, and 1.3 times greater in Asians.<sup>2</sup> In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The Institute supports a significant body of research aimed at understanding the biology underlying CKD and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. In addition to research on kidney disease related to diabetes and high blood pressure, the NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis;

---

<sup>1</sup> Centers for Disease Control and Prevention. *Chronic Kidney Disease in the United States, 2019*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2019.

<sup>2</sup> *United States Renal Data System. 2018 USRDS Annual Data Report: Epidemiology of kidney disease in the United States*. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2018.

and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. The Kidney Precision Medicine Project aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies.

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 interest include the causes of and treatments for urologic diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. To spur research in urinary stone disease, the Urinary Stone Disease Research Network (USDRN) is: a) conducting a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conducting clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) providing data and collecting biological samples from the studies to create a resource for future researchers.

Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important research topics of the NIDDK's urology program.

IC/BPS is a debilitating, chronic, and painful urologic disorder. Based on a recent large, national interview survey, it is estimated that among U.S. women 18 years old or older, 3.3 million (2.7 percent) have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/BPS.<sup>3</sup> Using a community-based epidemiologic

survey, researchers have estimated that among U.S. men ages 30 to 79 years old, 1.6 million (1.3 percent) have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with BPS.<sup>4</sup>

NIDDK-supported basic and clinical research on IC/BPS and on CP/CPPS is focused on elucidating the causes of these conditions, identifying important subsets of patients to aid diagnostic stratification, and improving treatment and interventions. One example of an ongoing study is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, which supports research designed to uncover the underlying causes of IC/BPS and CP/CPPS and to characterize the disease profiles in patients.

Based upon national public health surveys conducted over several years, it is estimated that about 54 percent of women (20 years and older) report urinary incontinence in the past 12 months. Urinary incontinence was self-reported by approximately 15 percent of men surveyed.<sup>5</sup> Many suffer in silence due to embarrassment and lack of knowledge about treatment 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 to provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to identify and understand the important subgroups of patients with lower urinary tract symptoms (LUTS) through improved measurement of patient experiences of LUTS in men and women. To address this challenge, the NIDDK supports the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health and Office of Behavioral and Social Sciences Research established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal

---

<sup>3</sup> Berry SH, et al. *J Urol* 186: 540-544, 2011.

<sup>4</sup> Link CL, et al. *J Urol* 180: 599-606, 2008.

<sup>5</sup> *Urologic Diseases in America, 2018 Addendum*. NIDDK, NIH Publication Number 12-7865, 2018.

or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

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 of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program and includes the following current research topic areas: regulation of blood (hematopoietic) stem cells, factors that play a role in the development of different types of blood cells, and red blood cell maturation. The Institute's SHINE II program seeks to further catalyze research in basic or pre-clinical, proof of principle research projects that are tightly focused and directed at validating novel concepts and approaches that promise to open up new pathways for discovery in benign hematology research. The NIDDK is also keenly interested in the basic biology of adult hematopoietic stem cells, which are used clinically in bone marrow transplants and may have broader application in gene therapy research.

## MODELING KIDNEY FUNCTION IN THE LABORATORY

**Streaming Fluid Across Kidney Organoids—Mini Kidney-like Structures—Grown on a Chip Drives Their Maturation:** Scientists found that streaming fluid across kidney organoids—engineered aggregates of kidney cells—prompts the organoids to develop blood vessels and to form natural tissue structures when grown on a chip, dramatically improving the extent to which they replicate normal kidney functions. Kidneys are highly complex organs in which systems of blood vessels intertwine with other structures to filter extra water and wastes out of the blood and make urine. Loss of kidney function can thus lead to build up of toxins in the blood and other problems, and total kidney failure is deadly without dialysis or a kidney transplant. Therefore, scientists have sought to develop treatments to repair, replace, or enhance lost kidney function. For many years, researchers have been improving

methods to use human stem cells in the laboratory to engineer kidney organoids, which are three-dimensional tissue constructs that can mimic kidney functions. However, it has been challenging to integrate blood vessels into growing organoids and coax stem cells to take on the required properties of kidney cells, and researchers have sought ways to overcome these technological hurdles to generate mature kidney organoids that fully replicate kidney function.

In a recent study, researchers attempted to recreate some of the environmental conditions under which kidneys normally develop in the body to see if these conditions would help organoids to mature properly. The scientists mounted organoids to small platforms, or “chips,” that can be modified to test various technological parameters. They reasoned that because developing kidneys normally are exposed to a flow of surrounding fluids, perhaps adding the stress of fluid flow to these chips could better mimic the natural environment. When the researchers grew chip-mounted kidney organoids in the presence of a high rate of fluid flow, they developed an array of blood vessels with varying diameters; by contrast, organoids exposed to low fluid rate or none at all had far fewer blood vessels. These blood vessels infiltrated the organoids and connected with internal tissue structures, as is required for normal kidney functions. The scientists found that under high-flow conditions, the developing blood vessels successfully transported fluids, and even assembled as networks connecting neighboring organoids. Organoids exposed to high flow also formed critical kidney tissue structures that closely resembled those found in normal kidneys. Together, these findings revealed that organoids grown on chips under high fluid flow conditions were far more physiologically mature than those under low or no flow.

The technological advances achieved in this study have boosted the ability of organoids on chips to mimic the natural physiological function of human kidneys. These conditions may help researchers utilize chips to test potential new drugs more quickly and accurately than has been possible. Improved kidney organoids also represent an important step toward the future development of functional, implantable structures that can enhance or replace lost kidney function in people.

*Homan KA, Gupta N, Kroll KT,...Morizane R. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods 16: 255-262, 2019.*

## RESEARCH TO PROMOTE KIDNEY CELL REGENERATION

### Key Regulators of Kidney Regeneration Identified:

Scientists have determined that two cellular signaling pathways dynamically control kidney regeneration in a fish model system. Zebrafish are useful research models for studying kidney development and disease because they are transparent, genetically tractable, and easy to manipulate in the laboratory. Unlike humans, zebrafish have the ability to regenerate kidney tissue in response to injury through the formation of new nephrons—the tiny filtration units in the kidney that remove waste products and excess fluid from the blood. Upon kidney injury, nephron progenitors, or stem cells, quietly residing in the kidney start expanding (*i.e.*, increasing in cell number) and migrate to tubules, where they form aggregates that develop into new nephrons that directly connect to the internal plumbing system. However, the cellular signals induced by kidney injury have been unknown. A pair of recent studies have identified key cellular signaling pathways—referred to as the Wnt and FGF signaling pathways—regulating new nephron formation during kidney regeneration.

In one study, researchers chemically induced kidney injury to promote new nephron formation in adult zebrafish. At the site of the injury, several Wnt signaling pathway-related genes were turned on specifically in nephron progenitor cells. Treatment with inhibitors of Wnt signaling blocked these genes from being turned on and far fewer nephron aggregates formed as a result, demonstrating that active Wnt signaling was required for new nephron formation. In addition, when the gene *fzd9b*, which encodes a critical Wnt signaling component, was genetically deleted in the setting of kidney injury, the numbers of nephron aggregates were significantly reduced when compared with normal injured zebrafish. These results define a clear role for the Wnt signaling pathway in new nephron formation.

Researchers in another study reported a similar series of zebrafish experiments demonstrating that the FGF signaling pathway is activated upon kidney injury. Blocking the FGF signaling pathway, with chemical inhibitors or genetic ablation, completely disrupted nephron aggregate formation following kidney injury. Further analysis showed that inhibiting FGF signaling prevented nephron progenitor cells from migrating to the proper location and aggregating to initiate new nephron

formation. Furthermore, beads soaked in FGF proteins and implanted into uninjured zebrafish kidneys attracted nephron progenitor cells to migrate to those sites. Taken together, these findings suggest that FGF signaling is critical for inducing new nephron formation in regenerating kidneys.

These studies identify two vital signaling pathways that control new nephron formation during zebrafish kidney regeneration. New nephron formation does not occur in adult humans; nephrons are only formed prior to or shortly after birth. However, research has shown that Wnt and FGF signaling pathways play important roles in human kidney development and disease, suggesting some commonalities between fish and mammals. These findings could provide the foundation for research to develop therapeutic strategies for kidney regeneration.

*Gallegos TF, Kamei CN, Rohly M, and Drummond IA. Fibroblast growth factor signaling mediates progenitor cell aggregation and nephron regeneration in the adult zebrafish kidney. Dev Biol 454: 44-51, 2019.*

*Kamei CN, Gallegos TF, Liu Y, Hukriede N, and Drummond IA. Wnt signaling mediates new nephron formation during zebrafish kidney regeneration. Development 146: pii: dev168294, 2019.*

## CLINICAL RESEARCH ON KIDNEY DISEASE

### Lowering Blood Pressure Does Not Lead to Kidney

**Damage:** In contrast to previous reports, scientists have determined, upon further research, that intensive blood pressure control does not lead to kidney injury in people who do not have chronic kidney disease (CKD). Elevated blood pressure is relatively common in the U.S. population and is a risk factor for heart disease, stroke, and CKD. The Systolic Blood Pressure Intervention Trial (SPRINT) was designed to test whether using medications to reduce systolic blood pressure to a lower goal than currently recommended would reduce cardiovascular disease in people with high blood pressure but not diabetes. (“Systolic” refers to the higher of the two numbers in a blood pressure reading; it measures the pressure in the arteries when the heart beats. “Diastolic” refers to the lower of the two numbers and measures the blood pressure when the heart rests between beats.)

SPRINT researchers previously reported that, among the subset of study participants who did not have CKD at the start of the trial, those who received an

intensive blood pressure control regimen were at a slightly higher risk of developing CKD than those who received standard care. They defined new-onset CKD as a minimum 30 percent reduction in the rate at which kidneys filter blood (filtration rate) to a level considered less than normal. This elevated CKD risk was generally outweighed by a reduced likelihood of cardiovascular events and death. However, because kidney filtration rates are dependent on blood pressure, the observed reduction in kidney function in these participants could simply reflect changes in blood flow, not necessarily underlying kidney damage. To explore this possibility, SPRINT researchers tested urine samples from study participants for the presence of several molecules known to be directly associated with various types of kidney damage (also known as “biomarkers” of kidney damage). Surprisingly, after 1 year of intensive blood pressure control, participants who developed CKD exhibited greater reductions in some urinary biomarkers of kidney damage than those who did not develop CKD. In addition, urine from study participants who did not receive intensive blood pressure control but nonetheless developed CKD had elevated kidney damage biomarkers compared with those who received the intensive blood pressure regimen. These results suggest that a blood flow effect, rather than a bona fide kidney injury, led to the misclassification of CKD in these study participants. Patients and clinicians are now empowered by this new information to seek more intensive blood pressure control to reduce the risk of mortality.

*Zhang WR, Craven TE, Malhotra R, ...lx JH; for the SPRINT Research Group. Kidney damage biomarkers and incident chronic kidney disease during blood pressure reduction: A case-control study. Ann Intern Med 169: 610-618, 2018.*

**Treatment of Depression for People with End-stage Kidney Disease Undergoing Hemodialysis:** In a recent clinical trial testing therapies for depression in people undergoing hemodialysis for kidney failure, researchers found that an engagement interview had no effect on acceptance of depression treatment, while depression scores were modestly improved with the drug sertraline compared with cognitive behavioral therapy (CBT). Kidney disease can worsen over time and may lead to kidney failure. If less than 15 percent of the kidney is working normally, that’s considered kidney failure—also referred to as end-stage renal disease (ESRD). Hemodialysis is a treatment in which a machine

filters wastes and water from the blood, as the kidneys did when they were healthy; but it has limitations and does not totally replace the function of normal kidneys. Hemodialysis sessions usually last several hours each, on multiple days each week. A common condition associated with people on hemodialysis is depression. Depression is a serious mood disorder. It causes severe symptoms that affect how you feel, think, and handle daily activities, such as sleeping, eating, or working. Many people on hemodialysis do not receive treatment for depression, possibly because of their reluctance to accept a diagnosis for this condition and/or receive treatment. In addition, the effectiveness of antidepressant therapies in people undergoing hemodialysis has not been properly evaluated in clinical trials, making depression treatment in this patient population difficult.

A recent two-phase, randomized, controlled clinical trial assessed therapies for depression in women and men, ages 21 years and older, who had depression and ESRD and were receiving hemodialysis at any of 41 dialysis centers. The first phase of the trial investigated whether an engagement interview would increase the 184 participants’ willingness to accept the diagnosis of and treatment for depression. The engagement interview was conducted face to face by trained therapists while participants received their hemodialysis treatment. The participants in the engagement interview were also given a DVD to improve their understanding of depression and its treatment. The researchers’ findings indicated that the engagement interview had no effect on their acceptance of treatment for depression compared to a control group, which had a visit from a research team member during which they discussed the diagnosis of depression and associated treatment options. The 120 participants in the second phase of the trial were divided into 2 groups of 60 to receive either individual CBT or sertraline therapy. CBT teaches a person different ways of thinking, behaving, and reacting to situations. Participants in the CBT group were scheduled for 10 sessions of 60 minutes each over 12 weeks while undergoing hemodialysis. Sertraline is used to treat depression and is in a class of antidepressants called selective serotonin reuptake inhibitors. It works by increasing the amount of serotonin—a natural chemical in the brain that is implicated in the regulation of many behaviors, mental processes, and mood. After 12 weeks of treatment, depression scores were modestly better with sertraline treatment compared

to CBT. Sertraline use, however, was associated with mild to moderate adverse events related to the digestive, heart, and nervous systems.

These results provide a foundation on which health care providers and patients on hemodialysis can enter into the shared decision-making process to choose between CBT and sertraline to treat depression. It may also pave the way for potential future research on other depression treatments for people on hemodialysis.

*Mehrotra R, Cukor D, Unruh M,...Hedayati SS. Comparative efficacy of therapies for treatment of depression for patients undergoing maintenance hemodialysis: A randomized clinical trial. Ann Intern Med 170: 369-379, 2019.*

**Gene Sequencing Can Help Tailor Treatments for Young People with Kidney Failure:** Sequencing portions of the genome could help diagnose the underlying cause of chronic kidney disease (CKD) in children and young adults receiving a kidney transplant, enabling clinicians to use precision medicine strategies to improve outcomes. When CKD worsens to kidney failure, transplantation is in many cases the preferable of the two main treatment options; the other option is dialysis. The underlying reasons that children and young adults develop CKD are different from older adults, and many of the gene variants that cause the different types of CKD have been determined over the past few years. Individual cases have demonstrated that knowing the genetic cause of CKD can help guide clinical decisions to improve outcomes in young patients who received a kidney transplant. Therefore, researchers in a recent study sought to systematically investigate whether sequencing portions of the genome previously linked to CKD could help diagnose the cause of kidney failure in kidney transplant recipients.

The scientists sequenced approximately 400 CKD-linked genes in 104 children and young adults who developed the disease under the age of 25, and who received a kidney transplant between 2007 and 2017. From their analyses, the scientists identified a genetic cause of CKD in 34 patients (32.7 percent). The likelihood of finding a genetic cause was highest in patients with certain types of CKD, such as conditions known as urinary stone disease and renal cystic ciliopathy. These results revealed that sequencing specific portions of the genome could help determine the type of CKD that led to kidney failure in about one-third of children and

young adults receiving a kidney transplant—findings that could be clinically useful because different types of CKD often require different patient care approaches (e.g., early screening for anticipated health problems; treatment strategies before and after transplantation). The researchers also point out that genetic analyses of potential living kidney donors may also help predict health outcomes and determine whether a kidney is suitable for donation; genetic testing of donors would be particularly important for close family members who are more likely to harbor the same genetic variants. Armed with this genetic knowledge, doctors, patients, and their families could make more informed clinical decisions and tailor treatment strategies to the patients' individual needs.

*Mann N, Braun DA, Amann K,...Hildebrandt F. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. J Am Soc Nephrol 30: 201-215, 2019.*

## RESEARCH ON LOWER URINARY TRACT SYMPTOMS AND DISORDERS

### **Achieving a Better Understanding of Symptom Flares in People with Urologic Pain Syndromes:**

Scientists studying urologic chronic pelvic pain syndromes (UCPPS) have recently reported on risk factors and variation regarding symptom flares in people—information that could help improve care in the future. People with UCPPS—interstitial cystitis/bladder pain syndrome (IC/BPS) or chronic prostatitis/chronic pelvic pain syndrome (CP/PPS, in men only)—experience pelvic pain and urologic symptoms, such as urinary frequency and urgency. When one or both sets of symptoms becomes worse for a period of time, this is called a flare. Understanding frequency, duration, and risk factors for flares over time could help in finding ways to manage or prevent them. In a study lasting nearly a year, researchers with the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network collected information about flares from nearly 400 participants. About 75 percent of participants reported having at least one flare, and the duration of flares could be as short as one day or as long as 150 days. Further analyses revealed that both urologic and pelvic pain symptoms worsened during flares, although the degree of worsening in each flare event varied not only between people but also within persons who had multiple flares. Risk of worse and/or longer flares was greater in women, in individuals who had greater than average UCPPS

symptoms overall when not having a flare, and in those with bladder pain associated with filling and/or urgency to urinate (bladder hypersensitivity). Finally, people with UCPPS commonly have co-occurring chronic pain conditions, such as irritable bowel syndrome (IBS), and having IBS or any such pain condition was also a risk factor for worse flares. Based upon their findings, the researchers also generated some new ideas for investigation regarding mechanisms underlying risk and extent of flares, such as the potential involvement of pain pathways both in the central nervous system and in peripheral organs and tissues. This newly acquired knowledge of UCPPS symptom fluctuation over time, risk factors, and variability in flare characteristics both within and between individuals helps to identify subgroups of people with UCPPS, and provides a foundation on which to research further flare management with the ultimate goal of flare prevention.

*Sutcliffe S, Gallop R, Henry Lai HH,....Landis JR. A longitudinal analysis of urological chronic pelvic pain syndrome flares in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. BJU Int 124: 522-531, 2019.*

### **Fibrosis Underlies Male Lower Urinary Tract**

**Symptoms:** Two recent studies have highlighted the importance of fibrosis in lower urinary tract symptoms (LUTS) in men and in mouse models. Fibrosis is the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage. The most common symptoms vary but LUTS can involve changes or problems with urination, such as a hesitant, interrupted, weak stream; urgency and leaking or dribbling; more frequent urination, especially at night; and urge incontinence. Previous studies have suggested that deposition of fibrosis in the prostate gland contributes to the development of LUTS/BPH (benign prostatic hyperplasia) in men. The extent to which fibrosis plays a role in the pathology of LUTS remains to be defined.

In 2003, NIDDK's Medical Therapy of Prostatic Symptoms (MTOPS) trial reported that combination therapy of two drugs (finasteride and doxazosin) is more effective than either drug singly in the treatment of BPH. Medical and surgical interventions, however, do not achieve symptom relief for all men with lower urinary tract dysfunction or may not provide a durable response. In one recent study, researchers examined tissue specimens

to test the hypothesis that fibrotic changes in the prostate may be associated with an increased risk for clinical progression to more severe symptoms among MTOPS participants. The researchers reported significant alterations in the architecture of collagen (*i.e.*, a surrogate marker for fibrosis) in prostate specimens from men who exhibited clinical progression compared to those who did not. This finding suggests that anti-fibrotic medications might offer another potential treatment option to men with BPH. Furthermore, men who had worsening symptoms were also found to have a high body mass index (a measure of weight relative to height).

In a second recent study, investigators sought to better understand the inflammation and pain associated with LUTS/BPH using a mouse model. The model is created by infection of the male mouse urethra with a strain of *Escherichia coli* (*E. coli*) bacteria called CP1; the mice then display symptomatic changes that mimic those observed in human LUTS/BPH. The investigators showed that *E. coli* CP1 infection increases the percentage of collagen within the prostate. Furthermore, the researchers identified that the immune system of a certain mouse strain increased production of two proteins called IL-4 and IL-13 in response to CP1 infection. These two proteins were shown to be associated with fibrosis in previous studies. Strategies that target IL-4 and IL-13 could thus be tested to see if they reduce the fibrosis and pain associated with LUTS/BPH in this model system.

Taken together, these studies report that fibrosis underlies LUTS/BPH in both humans and the CP1-induced mouse model. Further research will be required to determine whether anti-fibrotic medications can be of clinical benefit in this burdensome condition.

*Macoska JA, Uchtmann KS, Levenson GE, McVary KT, and Ricke WA. Prostate transition zone fibrosis is associated with clinical progression in the MTOPS Study. J Urol 202: 1-8, 2019.*

*Bell-Cohn A, Mazur DJ, Hall C, Schaeffer AJ, and Thumbikat P. Uropathogenic Escherichia coli-induced fibrosis, leading to lower urinary tract symptoms, is associated with type 2 cytokine signaling. Am J Physiol Renal Physiol 316: F682-F692, 2019.*

### **Genetic Risk Factor Associated with Erectile**

**Dysfunction:** For the first time, researchers have identified a genetic variant that increases the risk of erectile dysfunction (ED); the genetic variant is

near a gene called *SIM1* and may affect this gene's activity. ED is a condition in which one is unable to get or keep an erection firm enough for satisfactory sexual intercourse. Symptoms of ED include being able to get an erection sometimes, but not every time; being able to get an erection but not having it last long enough for sexual intercourse; and being unable to get an erection at any time. Several different diseases and conditions can lead to ED including type 2 diabetes, heart and blood vessel disease, and chronic kidney disease, among others. Although men are more likely to develop ED as they age, aging does not cause ED. A twin study of middle-aged males reported that about one-third of ED risk is heritable—meaning that there is a genetic component(s), but specific genetic variants had not been identified.

Investigators recently undertook a genome-wide association study (GWAS) of erectile dysfunction in a racially diverse cohort of 36,649 men. GWAS can be used to search for rare or common susceptibility genes in large groups of individuals. Of the people studied, 14,215 men had reported ED symptoms, and these individuals were more likely to be older, have a slightly higher body mass index (a measure of weight relative to height), have diabetes, be smokers or former smokers, have a clinical diagnosis of ED, and have filled a prescription to treat ED, compared to the control population of 22,434 men. The researchers found that DNA sequence variations at a position in the genome (*i.e.*, genetic locus) near the *SIM1* gene are significantly associated with an approximate 26 percent increased risk of ED. The increased risk is independent of known risk factors such as higher body mass index. The DNA sequence variations were verified in a second cohort of 222,358 men. The researchers further showed a biological role for the implicated genetic locus. The locus resides within an “enhancer” element; enhancers are short DNA sequences that control the extent to which a gene is turned on or off. The researchers found that the variants they identified within the enhancer alter its ability to control the activity of the *SIM1* gene.

This study reveals a previously unknown mechanism associated with ED, and lays the foundation for efforts to develop approaches targeting *SIM1* to restore erectile function and, thus, help men achieve a healthy sex life.

Jorgenson E, Matharu N, Palmer MR, ...Van Den Eeden SK. Genetic variation in the *SIM1* locus is associated with erectile dysfunction. *Proc Natl Acad Sci USA* 115: 11018-11023, 2018.

## TREATING BLOOD DISORDERS

**Expanding Numbers of Blood Stem Cells Prior to Therapeutic Transplantation:** Two recent studies highlight potential strategies to expand blood (hematopoietic) stem cells *in vitro* (in a laboratory dish) in order to generate more of these rare cells prior to therapeutic transplantation in people. Hematopoietic stem cell (HSC) transplants can be life-saving for people with a number of conditions. When introduced into a donor, HSCs migrate to the bone marrow where they normally reside and renew—and when needed mature into all types of blood cells (*e.g.*, red cells, white cells, and platelets). However, it can be challenging to find HSCs in needed quantities from a donor whose cells are similar enough to a patient's cells to be a sufficient “match” for transplantation. Researchers supported by NIDDK continue studies to discover key factors that promote HSC expansion *in vitro*, and with these insights increase the potential availability of transplantation to benefit many more people.

In the search for ways to promote expansion of HSCs, one team of scientists focused on a protein called DEK. Previous research has shown that DEK is an abundant protein found in most human tissues, and that it may regulate blood cell development. In a recent study, the researchers found that a synthetically produced secreted form of the DEK protein was shown to greatly enhance expansion of mouse (both male and female) and human HSCs within 4 days *in vitro*. This finding is important as the ability to transplant increased numbers of HSCs might improve transplantation outcomes in the recipient.

Another research team sought to optimize the components of the liquid culture medium in which HSCs are expanded *in vitro*, and the surface that they are grown on. For example, serum albumin has long been used as part of the culture medium for the expansion of HSCs, but it contains a complex mixture of proteins, often inadequately characterized. In a recent study in mice, the researchers described the development of a defined culture system that includes a component called “polyvinyl alcohol” (PVA) as a substitute for serum albumin, and optimized levels of two

other components, thrombopoietin and stem cell factor. They also used another factor, fibronectin, to coat the dish on which the cells are expanded. The researchers found that this culture system supports long-term expansion of mouse HSCs. Both male and female mice were used in this study. This culture system facilitated expansion of HSCs between 236-fold and 899-fold during a 1-month timeframe. Furthermore, when transplanted into recipient mice, the expanded HSCs migrated to and engrafted into the bone marrow without the mice having to undergo standard, but toxic, pre-conditioning regimens.

Taken together, these research studies highlight potential new strategies to expand transplantable HSCs *ex vivo* prior to therapeutic transplantation. Future research could determine whether these strategies improve HSC transplantation in people.

Capitano ML, Mor-Vaknin N, Saha AK,...Broxmeyer HE. Secreted nuclear protein DEK regulates hematopoiesis through CXCR2 signaling. *J Clin Invest* 129: 2555-2570, 2019.

Wilkinson AC, Ishida R, Kikuchi M,...Yamazaki S. Long-term *ex vivo* haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature* 571: 117-121, 2019.

# Workshop Explores New Approaches for Improving Care for Patients After Acute Kidney Injury



On January 30-31, 2019, the NIDDK sponsored a multidisciplinary workshop in Bethesda, Maryland, focusing on the development of strategies to improve clinical outcomes among patients surviving an episode of acute kidney injury (AKI).

AKI, also called acute renal failure, is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. The resulting inability to excrete waste products and maintain fluid and salt balance poses urgent health problems for patients and their physicians. AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction caused by infection), decreased blood pressure, or kidney damage from drugs or other toxins. AKI is an increasingly common condition affecting hospitalized patients that is associated with future chronic kidney disease (CKD), cardiovascular events, diminished health care-related quality of life, and death. Multiple studies highlight rapid growth in the incidence of AKI, and with it, parallel increases in the number of survivors.

A proportion of patients without preexisting CKD experience significant loss of kidney function over the long term after an episode of AKI, resulting in the development of new CKD. Approximately 20 percent of such patients with AKI, usually of the greatest severity, will develop CKD over the course of a few years. Risk factors for the development of CKD after an AKI episode include older age, race (e.g., African Americans are at higher risk), and disease severity.

Although several therapeutic interventions for AKI during hospitalization have been tested, none is effective in changing the course of the disease. How patients should be treated—including medications and level of blood pressure control—in the post-AKI outpatient setting is unknown. Current clinical practice guidelines lack high-quality evidence to inform care recommendations.

This workshop brought together experts with the goals of: 1) facilitating the development of strategies to improve clinical outcomes among patients surviving an episode of AKI; 2) reviewing the current state-of-the-science of interventions following AKI and mechanisms that drive susceptibility to future CKD and cardiovascular events, thus identifying knowledge gaps that need to be addressed to better inform clinical care; and 3) identifying possible interventions, focusing on parameters of key interest for evaluation and feasibility of studies.

Through fruitful discussions, there emerged a broad consensus that post-AKI care interventions warrant testing in clinical trials. Given the challenges expressed by AKI survivors with the post-hospitalization transition, such studies should focus on intermediate (90- to 180-day) outcomes. Furthermore, the primary concerns reported by AKI survivors were not necessarily hard clinical outcomes (e.g., re-hospitalization), but rather symptoms and quality of life (e.g., anxiety/depression) during the post-AKI transition, and such concerns need to be incorporated into clinical trial design.

# Catheter-associated Urinary Tract Infections Technology Workshop



On March 11-12, 2019, the NIDDK sponsored a multidisciplinary workshop in Bethesda, Maryland, focused on the development of strategies to improve clinical outcomes among patients having an indwelling urinary catheter.

An indwelling urinary catheter is a thin, hollow tube inserted through the urethra into the urinary bladder to collect and drain urine. Hospitalized patients receive urinary catheters during their hospital stay for indications including voiding management for patients with urethral obstruction, and before and after certain surgical procedures. However, prolonged use of a urinary catheter is a risk factor for developing a catheter-associated urinary tract infection (CAUTI).

CAUTIs are the most common health care-associated infection and are responsible for increased morbidity and mortality (*e.g.*, due to bloodstream infections), excess length of stay, increased cost, and unnecessary antimicrobial use. In addition to implementing clinical best practices

to reduce CAUTIs, research toward development of new catheter technologies is expected to have a significant clinical impact.

The purpose of this workshop was to promote the development of new or improved technologies to reduce the incidence and severity of CAUTIs. As a baseline, the workshop attendees reviewed the state-of-the-science in this research area. The workshop afforded a venue for regulators to provide guidance to innovators on the catheter development and device approval path. Attendees also identified common hurdles, and discussed potential proactive solutions to these difficulties.

Challenges remain, however, including why some patients have bacteria in their urine without symptoms or other signs of an infection, while others progress to CAUTI. Additional research could address this and other issues, such as how to overcome obstacles to conducting clinical investigations in this area.

# STORY OF DISCOVERY

## Engineered Kidney Tissues and Organoids: Tools for Improved Disease Modeling and Development of Therapies

Chronic kidney disease (CKD) is a major public health problem in the United States. The impact of CKD is substantial and includes increased risk of death, diminished quality of life, numerous co-associated diseases and conditions, such as cardiovascular disease, and significantly increased risk of progression to kidney failure (end-stage renal disease). As symptoms are few or non-existent, most people are unaware that they have CKD until most kidney function has been lost. Development of drugs for CKD has been hampered by non-predictive animal models, the inability to identify and prioritize molecular factors in human kidneys that could be targeted with medication, and an underlying poor understanding of human CKD. Therefore, CKD research, as well as research on other kidney-related conditions (e.g., acute kidney injury, drug toxicity, cancer), would benefit greatly from the development of improved laboratory tools to model human kidney structure and function.

Kidneys are highly complex, bean-shaped organs that cleanse metabolic waste products from the blood and maintain proper salt and mineral balance and fluid volume in the body. Each human kidney contains about 1 million individual filtration units, called nephrons, in which blood vessels intertwine with other structures to achieve these remarkable functions. Kidney formation in the developing embryo is a dynamic, orchestrated process; clusters of cells move and interact, and various cells undergo tightly regulated molecular and physical changes that ultimately drive them to mature into functional nephrons. Due to this complexity, for many years, scientists struggled to develop models that accurately recapitulate human kidney structures and function, and the lack of such human kidney models has limited the ability to develop new drugs to treat or prevent CKD. However, due to rapid technological

advances made over the past few years, engineered kidney tissues and organoids—self-organizing, three-dimensional kidney assemblies often derived from adult human stem cells—have emerged as promising tools to accelerate CKD research.

### KIDNEY ORGANOIDS— A LEAP FORWARD

Over the course of many years, scientists painstakingly defined a number of laboratory conditions under which human pluripotent stem cells (hPSCs)—cells that are able to become any type of cell in the body—could be coaxed to develop into individual cell types found in the nephron. However, researchers faced the challenge of assembling the different kidney cell types together into three-dimensional structures that can recapitulate human kidney functions. Between 2013 and 2015, several research groups reported important technological breakthroughs that defined conditions under which hPSCs could develop into higher-order kidney-like structures. For example, in 2014, scientists in Japan used mouse models to carefully define a series of molecules that act sequentially to induce the stages of normal kidney development over time. The researchers then applied this knowledge from mice to a human system by adding these molecular signals step-wise to hPSCs growing in culture, coaxing the cells to grow in number, aggregate, and mature into organoids that recapitulate complex kidney structures. Other research groups also used a variety of methodologies to identify molecules and processes in normal kidney development that, when applied to cultured hPSCs, improve their ability to self-organize into organoids.

# STORY OF DISCOVERY

Kidneys filter the blood through interactions between very thin blood vessels (capillaries) and specialized kidney cells in the nephron. The incorporation of capillaries in developing kidney structures is a process called “vascularization,” and is essential for kidney function. Therefore, vascularization of kidney organoids in culture is critical, but has been a technological hurdle for researchers. In 2018, a team of scientists, supported in part by NIDDK, made a leap forward when they showed that hPSC-derived kidney organoids, when grown in certain culture conditions and transplanted into mice, could recruit the host mouse’s blood vessels into the organoids’ budding blood vessels. They observed over time that the organoids grew in size, formed critical structures such as filtration membranes between the mouse blood vessels and kidney cells, and developed vascular connections that were fully functional. These findings demonstrated that hPSC-derived organoids exposed to a physiological environment similar to that in the body (in this case, when transplanted into a mouse) are poised to vascularize and mature.

These experiments generated a relatively small number of organoids that could, under specific conditions, mimic kidney structures and functions. But another obstacle to translating this research to wider use in the laboratory, and potentially the clinic, is scalability—the amount of hPSC-derived kidney organoids will have to be increased. To address this need, researchers, supported in part by NIDDK, modified culture conditions to optimize the yield of kidney structures that could be produced in the laboratory. In 2019, they published their finding that certain conditions favored the generation of large quantities of kidney tissue in the form of much smaller organoids, which they term “micro-organoids,” than previous studies had reported. The cellular compositions and maturity levels of the micro-organoids were similar to those of standard organoids, but these laboratory conditions generated 3 to 4 times the total amount of kidney tissue from the same number of starting cells, with a 75 percent reduction in cost.

## MODELING BIOLOGICAL FUNCTIONS BY MOUNTING KIDNEY TISSUES TO PLATFORMS

While the laboratory protocols for growing organoids in culture have made impressive strides in producing tissue structures that resemble the kidney, kidney development and maturation has not been fully replicated under simple culture conditions. As an example, organoids in the previously described study required grafting into mice to achieve vascularization. To more closely replicate the conditions of normal kidney development, researchers have turned to “microfluidic” platforms, which are devices on which kidney cells or organoids can be mounted and exposed to tiny, controllable volumes of liquid. These modifiable platforms, or “chips,” can therefore be used to test various culture conditions with extraordinary precision to identify factors that promote maturation of kidney cells and organoids.

In recent studies in 2018 and 2019, two teams of NIDDK-funded scientists sought to use chips to improve functional models of tubules, which are specific portions of the nephron where various molecules (e.g., water, proteins, salts, sugars) are exchanged between the nephron and surrounding capillaries to achieve proper balance in the body. One research team developed a chip-mounted renal vascular-tubular unit (hRVTU), consisting of a permeable membrane with vascular (capillary) components on one side and tubule cells on the other. Over time, molecules produced by the cells “remodeled” the membrane between the two compartments to closely resemble the normal interface between tubules and capillaries in human kidneys. Moreover, proteins and sugars that normally pass between tubules and capillaries could flow between these two compartments, but others could not; this selectivity revealed the great extent to which the hRVTU could replicate kidney function.

The other group of scientists used “3-D bioprinting” technology to create chips on which three-dimensional tubules and capillaries were

# STORY OF DISCOVERY

“printed” directly adjacent to one another and are embedded in an engineered matrix that resembles the environmental conditions surrounding these structures within nephrons in the body. By measuring the contents within the two compartments, they observed the exchange of molecules between the two structures, demonstrating that the chip modeled normal tubule function. The scientists then exposed the chips to hyperglycemic (excess sugar in the fluid) conditions to model the effects of diabetes on nephron tubules and capillaries. Hyperglycemia led to cellular damage and dysfunction in the capillary and tubular cells, effects that were prevented by treating the chips with a drug that reduces the transport of sugar between the compartments. Thus, the chips proved useful for accurately modeling tubule-capillary dynamics in both normal conditions and immediately following high sugar exposure; future studies are needed to determine whether these chips could be useful for modeling the effects of long-term, chronic high sugar exposure characteristic of diabetes.

While these studies focused on creating chips modeling particular parts of the nephron, other researchers have sought to mount and develop entire organoids on microfluidic platforms. In a recent study published in 2019, NIDDK-supported scientists sought to overcome the barrier of organoid vascularization by reasoning that because developing kidneys normally are exposed to fluid flow, perhaps adding the stress of fluid flow to chips could mimic the natural environment. When the researchers grew chip-mounted kidney organoids in the presence of a high rate of fluid flow, they developed an array of blood vessels with varying diameters; by contrast, organoids exposed to low fluid rate or none at all had far fewer blood vessels. Under high flow conditions, the developing blood vessels successfully transported fluids and even assembled as networks connecting neighboring organoids, demonstrating that they were physiologically mature. (For more details, please see the advance summary earlier in this chapter.)

## ONGOING AND FUTURE RESEARCH EFFORTS

Tools developed through these advances are already proving their utility for accelerating CKD research. For example, one study used hPSC-derived kidney organoids to help overcome a long-standing technological roadblock. Previously, researchers had tried to treat damaged kidney cells with viral gene therapy, but research protocols to deliver genes to appropriate human cells were unsuccessful. NIDDK-supported scientists recently identified a specific subtype of virus that could successfully deliver gene therapy and prevent damage to kidney cells in mice. However, it is well known that many discoveries in mice cannot be translated to humans due to inter-species differences between kidneys. Therefore, the scientists tested the virus in hPSC-derived organoids, determining that the gene delivery vehicle could successfully work in these human cell-derived structures and providing a foundation for potential future therapeutic studies. This study exemplifies how using organoids that faithfully mimic human kidney structures and physiology can help predict drug or other treatment effectiveness and toxicity at a relatively low cost.

NIH and the International Space Station U.S. National Laboratory are currently collaborating on another fascinating use for kidney chips, as well as chips modeling other organs and diseases. Researchers will use the tissue chips in space to study aging and certain disease states that appear to be accelerated in microgravity and then later to test the potential effects of drugs on those tissues. The projects aim to provide insights that will speed the development of treatments for kidney stones, arthritis, and other conditions that affect us here on Earth.

Research to develop engineered kidney tissues and organoids have taken extraordinary leaps over the past few years. As technology continues to improve, these laboratory tools will undoubtedly play central roles in understanding human kidney development, modeling disease, accelerating drug discovery, and catalyzing innovation in renal replacement therapy.

# SCIENTIFIC PRESENTATION

## Dr. Lisa M. Guay-Woodford—Autosomal Recessive Polycystic Kidney Disease: New Insights Reveal Provocative Complexities

*Dr. Lisa M. Guay-Woodford is Professor of Pediatrics at George Washington University (GWU) School of Medicine and Health Science, and the McGehee Joyce Professor of Pediatrics at Children's National Research Institute (CNRI). She serves as the Associate Vice President for Clinical and Translational Research at GWU. Dr. Guay-Woodford leads the Clinical and Translational Science Institute, which is funded by the NIH Clinical and Translational Science Awards program and is a partnership between CNRI and GWU. A graduate of the College of the Holy Cross, she earned her M.D. degree from Harvard Medical School, and then completed pediatric training and a pediatric nephrology fellowship at Boston Children's Hospital.*

*Dr. Guay-Woodford is an internationally recognized pediatric nephrologist with a research program focused on identifying clinical and genetic factors involved in the pathogenesis of inherited kidney disorders, most notably autosomal recessive polycystic kidney disease (ARPKD). Her laboratory has identified disease-causing genes in several experimental models of ARPKD, and her research group participated in the identification of the human ARPKD gene as part of an international consortium. For her contributions to the field, Dr. Guay-Woodford was awarded the Lillian Jean Kaplan International Prize for Advancement in the Understanding of Polycystic Kidney Disease. Dr. Guay-Woodford has established and directed national and international collaborative research groups, as well as assumed numerous elected leadership positions, including: President of the Society for Pediatric Research, Councilor for the International Pediatric Nephrology Association, Chair of the NIH Cellular and Molecular Biology of the Kidney Study Section, Board of Trustee member for the Polycystic Kidney Disease Foundation, and Board member for the Association of Clinical and Translational Science. She currently serves*

*as a member of the National Diabetes and Digestive and Kidney Diseases Advisory Council, and at its May 2019 meeting, Dr. Guay-Woodford presented a lecture on the provocative complexities of ARPKD.*

Polycystic kidney disease (PKD) is a genetic disorder that results in the growth of multiple fluid-filled cysts in the kidneys. ARPKD is a rare and severe form of PKD that usually becomes apparent around the time of birth or in early childhood, and many affected patients do not survive this time period. Ninety-nine percent of people with ARPKD have been shown to have mutations in the *PKHD1* gene; researchers have found a wide variety of different mutations in this gene that are associated with the disease. As with most genes, people have two copies of *PKHD1*, and the disease results when there are mutations in both. ARPKD is characterized by fluid-filled kidney cysts and connective tissue build-up in the liver (i.e., hepatic fibrosis). The greatly enlarged kidneys have reduced kidney function. *PKHD1* mutations affect kidney development starting before birth, and can be detected first in the proximal tubule of the kidney, which reabsorbs water and salts, and then in the kidney's collecting ducts, which form a drainage system that opens into the tubes that carry urine from the kidney to the bladder. The kidney cysts in ARPKD involve dilatation or widening of virtually all of the collecting ducts. Dr. Guay-Woodford's research group has led efforts to understand the origin of ARPKD in order to establish a foundation on which to develop effective treatment strategies for this intractable disease.

The *PKHD1* gene encodes a protein called FPC that sits in the cell membrane. Working with NIDDK Deputy Director Dr. Gregory Germino's laboratory, Dr. Guay-Woodford and her team showed that FPC undergoes a processing event in which one end of the protein,

# SCIENTIFIC PRESENTATION

called the carboxyl-terminus, is cleaved off and travels to the nucleus of the cell, where the DNA is housed.

To better understand the function of FPC, researchers have genetically engineered mouse models for study. As there are hundreds of different strains of mice that differ in their genetic makeup, researchers must take into account that these differences could modify how the gene under study may function. Dr. Guay-Woodford showed results from several mouse models that were generated having different mutations in the mouse *Pkhd1* gene—including mixed-strain mice and inbred mice. Mice with a “mixed” genetic background and two mutated *Pkhd1* gene copies develop cystic kidney disease—primarily in the proximal tubule or collecting duct—as well as liver disease. By contrast, researchers found that an inbred strain of mice with these same mutations does not have kidney disease, but does still have liver disease. A research team led by Dr. Terry Watnick developed a mouse model with a specific mutation in the *Pkhd1* gene that results in the FPC protein missing most of the carboxyl-terminus. These mice did not have kidney or liver disease. Furthermore, when Dr. Germino’s research team completely knocked out the *Pkhd1* gene, resulting in no FPC protein being produced, they found that the mouse model did not have kidney disease but did have liver disease. From these investigations, it is clear that these mutations in *Pkhd1* in mice fail to consistently model human ARPKD.

To further study the disease, Dr. Guay-Woodford and her colleagues analyzed the *PKHD1* gene in 36 Afrikaner families with ARPKD, and found that a majority of them had the same *PKHD1* mutation. As a way to gain further insight into this disease, Dr. Guay-Woodford’s team has generated an analogous mutation in mice, which they are currently studying.

## ATYPICAL KIDNEYS IN PARENTS OF CHILDREN WITH ARPKD

Dr. Guay-Woodford presented findings from another research group that performed ultrasound evaluations on 62 parents from ARPKD families.

Ultrasound identified six parents—who have just one copy of the mutated gene—as having atypical kidney images called medullary echogenicity that resembled cysts—and intriguingly they were all female. Further research is needed to understand how the presence of one copy of the mutated gene may play a role in the development of the kidney cyst lesions in the six female parents.

## KIDNEY CYSTIC DISEASE AND THE C-MYC PROTEIN IN HUMANS AND MOUSE MODELS

While reflecting on the biological pathways that had been identified as important in kidney cyst formation, Dr. Guay-Woodford and her colleagues focused on a protein called c-Myc, which regulates gene activity. Dr. Marie Trudel and other investigators have shown that c-Myc protein levels are increased in patients with another form of PKD, autosomal dominant polycystic kidney disease (ADPKD). ADPKD is the most common form of the disease, and people are usually diagnosed between the ages of 30 and 50. Using different approaches to study c-Myc, research teams led by Dr. Trudel and Dr. Vincent Gattone had discovered that increased levels of this protein correlate with cystic kidney disease in both ARPKD and ADPKD mouse models, and that experimentally decreasing levels of this protein in mice could reduce kidney cysts and improve kidney function. More recently, Dr. Trudel and her colleagues showed that c-Myc regulates a gene called *Pkd*; mutations in this gene cause ADPKD. Dr. Guay-Woodford concluded her presentation by suggesting that c-Myc could be part of a molecular mechanism for PKD susceptibility, which could be targeted for the development of drugs to attenuate the disease in people.

# PERSONAL PERSPECTIVES

## SHAREing Their Experiences with Bladder and Urinary Tract Health: Five Women Talk About Their Participation in the PLUS Research Consortium

At some point in their lives, the majority of women will face at least one health issue having to do with the bladder and/or urination. Such issues range from acute infections to chronic, sometimes painful conditions. These urologic health challenges can have far-reaching negative effects on a woman's health and well-being. NIDDK-supported scientists with the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium have embarked upon a journey to improve both bladder health and overall health for women, with the help of hundreds of research volunteers across the country—including five women featured here who discussed their experiences participating in a foundational study called SHARE (see insets).

### BLADDER ISSUES: FROM HEALTH TO HEALTH BURDEN

Urinating is a way to rid waste products of daily metabolism that would otherwise build up in the bloodstream. These toxic substances, once filtered from the blood, are sent to the bladder for storage in the form of urine. Periodically, urine is voided from the bladder out of the body through a tube-like structure called the urethra.

However, many problems can affect the bladder and urethra, resulting in symptoms that disrupt normal voiding and overall health. These problems, such as urinary incontinence (UI), urinary tract infections (UTIs), overactive bladder (OAB), and interstitial cystitis/bladder pain syndrome (IC/BPS), occur much more frequently in women than in men. Bothersome lower urinary tract symptoms, or LUTS, can also be caused by behaviors such as drinking too much

fluid. Whatever the cause, LUTS can exacerbate or contribute to other chronic health problems in women,



### ADINA

With over 26 years of experience under her belt, 53-year-old Adina is in contact with people in her community every day as a workforce development specialist focused on helping

students prepare for employment. Outside of work, Adina spends time with her fiancée and young adult daughter and son, enjoying dancing, bike riding, and reading. Adina decided to join a SHARE focus group because she has a close relative with bladder health issues, and she also wanted to know more about these issues herself as she is getting older. In describing her experience, Adina observes that her group was very enthusiastic and supportive of women's health, and that she feels the organizers made the setting safe and comfortable to "ask uncomfortable questions" and "share openly, without being judged." Adina says she is glad to have participated in SHARE because when she thinks about bladder health she feels "people really don't talk about that" and are less aware of it compared to other medical conditions. "I don't come across a lot of advertisement or marketing for specifically bladder issues," she notes, adding that, especially in light of her relative's bladder problems, she is very eager to know "is there something I can do to be proactive and to prevent this?" Ultimately, Adina feels her SHARE group was both "wonderful" and "important."

# PERSONAL PERSPECTIVES

including obesity, diabetes, and depression, by creating barriers to engaging in physical and social activities (e.g., fear of embarrassment, risk of leaking urine, and need to maintain easy access to bathrooms).

Up to this point, almost all research on bladder conditions has been aimed at determining their causes and testing treatments. An understanding of healthy bladder function across the lifespan is still somewhat elusive. Such an understanding would help scientists and clinicians set goals for research to promote bladder health and develop prevention as well as improved treatment strategies for different lower urinary tract problems and symptoms.

## THE PREVENTION OF LOWER URINARY TRACT SYMPTOMS (PLUS) RESEARCH CONSORTIUM

In 2015, the NIDDK, in collaboration with other NIH components, established the PLUS Research Consortium. The overarching goal of this Consortium is to establish the scientific basis for future intervention studies that can promote bladder health and prevent LUTS and associated bladder conditions in girls and women.

PLUS is using several strategies to accomplish its mission: a transdisciplinary approach (i.e., one that harnesses multiple research disciplines to tackle important, complex questions); a research structure and research techniques to study not just biological factors, but also mental health, social and behavioral factors, and the impact of many other potentially important factors in a woman's "environment" (e.g., home life, school, and work) that may contribute to bladder health; and the inclusion of the experiences and perspectives of adolescents and women.

PLUS investigators decided early on that defining and measuring the state of bladder health was a necessary component of the evidence for future prevention efforts. In one step toward reaching this objective, PLUS recently developed and published a novel, multi-faceted research definition of bladder health

that can inform approaches for evaluation of bladder health promotion and prevention of LUTS both in research and in public health initiatives. Informed by the World Health Organization's definition of health, the PLUS Consortium defined bladder health as "a complete state of physical, mental, and social well-being related to bladder function, and not merely the absence of LUTS. Healthy bladder function permits daily activities, adapts to short-term physical or environmental stressors, and allows optimal well-



### ROXANNE

A self-described "Midwestern gal all around" whose attitude is to "keep moving and grooving" despite serious health challenges, 60-year-old Roxanne is a retired social worker who directed projects for

several different urban, community-based programs, including one that promoted career mentoring for high school girls. She participates in clinical studies regularly, especially those focused on women's health, as a way to "meet people ... and to give something back," as well as to benefit her health as she gets older. SHARE was of interest to Roxanne because of this drive and her own experience with a bladder condition. Describing her SHARE focus group experience, she recalls that a key thing she took away from it was validation—"it's not just me." Roxanne also participated in a second SHARE focus group, which brought together one participant from each of the focus groups at her research center. Recalling that group, she remembers sharing both information and personal stories, such as triggers that affect her bladder—"every time I go to the grocery store I have to pee ... it's like a cue in my brain!" she says. When asked if she would participate in a similar focus group and whether she would recommend it to other women, Roxanne's response is a simple one: a resounding "Yes!"

# PERSONAL PERSPECTIVES

being (e.g., travel, exercise, social, occupational, or other activities).” In a related effort, PLUS scientists analyzed comprehensive data on LUTS and LUTS-specific interference with physical and social activities from an existing community-based study. As a result, they estimate that only about one in five women ages 30 to 80 years old has optimal bladder health—underscoring the need for LUTS prevention and bladder health promotion.

Additionally, PLUS scientists have shed light on suspected associations between occupation, industry, and work environment and the risk of LUTS in women. They found that there was insufficient data from past studies to evaluate LUTS by occupation types. This represents a profound gap in knowledge and indicates that future studies should characterize factors such as voiding frequency and toilet access in a consistent manner by occupation and explore their relationships to LUTS development.

## PAVING THE WAY TO PREVENTION: PLUS CLINICAL STUDIES

To obtain the evidence base for intervention studies to prevent LUTS and promote bladder health, PLUS scientists identified the need for a nationally representative long-term study in women and adolescent girls—one that would determine the distribution of bladder health conditions and expand our understanding of factors that promote bladder health or contribute to bladder conditions. As a result of its novel and visionary approach, PLUS developed a multi-pronged research strategy to lay the foundation for such a study. This strategy included talking to adolescent and adult women; scientific literature reviews; analyses of existing databases; and identification, development, and validation of an array of items (questions) to obtain measurable information about a person’s bladder health and about novel factors that contribute to bladder conditions, such as how much adolescent and adult women know about their bladders. Key studies that depended upon human volunteers are known by the acronyms SHARE, CLEAR, and VIEW.

**SHARE:** Every journey begins with a step, and in the case of the PLUS, a key first step was the Study of Habits, Attitudes, Realities and Experiences, or SHARE.

From its inception, PLUS made inclusion of the voices of women who are not researchers or clinicians a priority. Using principles of community engagement, PLUS quickly developed a plan for gaining broader community input on each step of the process in developing a definition of bladder health and the elements of a research method (tool) to measure bladder health. This plan resulted in SHARE.

## MAXINE

A nurse for over 40 years, 74-year-old Maxine is retired now but still attends classes to keep up her license and stay current on what is happening in nursing, research, and patient care—particularly the impact of electronic health records, which she finds very exciting. She also spends a great deal of time focused on her family, especially her three great-grandchildren. Maxine’s reasons for joining a SHARE group included past bladder issues of her own and experience teaching students about bowel and bladder issues during one phase of her nursing career. In describing her SHARE experience, Maxine recalls the group being asked about the influence of TV and other media on thinking and decisions affecting bladder health, such as products they might buy, or treatments they might request from their physicians—the topic “sparked a pretty interesting conversation,” she says. Maxine also recalls the group discussing whether their mothers influenced their perception of bladder issues and how to talk about these conditions. This question resonated with Maxine as she recalled her own early life experiences and also brought up “responses [that] were interesting to me” from the other women, she says. The SHARE group was the first medically oriented focus group Maxine had participated in, and she says she would “definitely” recommend participation in such a group to others. “I’m looking forward to seeing the results of the entire study,” she adds.

# PERSONAL PERSPECTIVES

A large, multi-center, focus-group based, qualitative study, SHARE was conducted in 2017 to understand how women conceive of and talk about their bladders and bladder health. PLUS held 44 focus groups, with more than 300 women and girls 11 to 80 years of age from racially, ethnically, and sociodemographically diverse backgrounds. Many participants had bladder health issues, while others did not. This differentiated SHARE from earlier studies of this kind, which focused on people with particular bladder conditions. Participants met in groups of approximately 5 to 10 persons of similar ages, together with a discussion facilitator and a study coordinator from the local PLUS study site.

Although participants were provided some basic information about the bladder, the focus groups were not meant to be classes—rather, they were forums for women and girls to talk about the bladder and bladder problems, how they monitor their own bladder behavior, what bladder health “looks like” to them, their experiences with navigating toilet access in public spaces such as workplaces and schools, and a variety of other topics. For many participants, the focus groups represented a venue in which to discuss issues or problems that are often swept under the rug due to embarrassment or cultural norms. Five of the participating women from sites across the country discussed their experiences in their respective SHARE focus groups for this feature (see insets).

PLUS scientists are in the process of interpreting the findings and publishing the results from the SHARE study. The information garnered from SHARE participants has been instrumental to the development of a multi-component measure of bladder health that will ultimately be used in the long-term observational study, and is informing other PLUS activities as well. For example, the focus groups provided key insights into the terms and language used by a very diverse group of women and girls to discuss bladder health and issues, as well as the emotional and social aspects of such discussions. Also, because the participants were grouped by age, how women’s experiences and perspectives differed across the lifespan could be more easily explored, analyzed, and incorporated into

PLUS research efforts. Without SHARE, many of the other studies developed by PLUS would not have been possible.

**CLEAR and VIEW:** In preparation for its large-scale study, PLUS also needed to work methodically to identify the factors most likely to influence risk for or protection from bladder problems; develop ways to measure knowledge, attitudes, and beliefs about bladder function and health; and ultimately to draft research questions for the study. To put the draft questions through an initial test for usability, PLUS developed the Clarification of Language, Evaluation And Refinement, or CLEAR, study—a way to determine whether the questions are easily



## Laura

Between working toward a Ph.D. in clinical psychology, running, and taking time to read and bake with her young daughter, 30-year-old Laura is super busy.

However, she was very excited to take time to participate in the SHARE focus group because of her general interest in women’s health and because of her own specific bladder health challenge—a diagnosis of interstitial cystitis when she was 19 years old, which she happily says is currently under control with medication. In talking about her SHARE experience, Laura notes that there was a point when the discussion turned to whether participants felt like bladder health was common knowledge, and that “I just remember being, like, yeah, we don’t think about women’s bladder health, and ... that struck me.” When asked what she thought was the best part of being in SHARE, Laura says that “considering that I’ve been struggling with this since I was a pre-teen, to have a group that focused on bladder health felt almost empowering.” Also, Laura notes she is aware that research is being done on bladder health, and as she puts it, “to be part of it felt encouraging to me.”

# PERSONAL PERSPECTIVES

understood or need to be further refined. Candidate questions for a “Bladder Health Instrument,” will undergo full validation in the Validation of Bladder Health Instrument for Evaluation in Women, or VIEW, study. Validated and age- and culturally-appropriate instruments will be developed for both English- and Spanish-speaking women. The validation process will reduce the number of items needed to determine the state of bladder health and the final instrument will be known as the Bladder Health Scale. PLUS researchers will then use the Bladder Health Scale to obtain the main results about bladder health and dysfunction in the planned long-term study in women and girls.

## GOING FORWARD IN PLUS

The PLUS Research Consortium is expected to enter its second 5-year phase later in 2020, during which recruitment of participants for the national, long-term study will begin. It is anticipated that this study will determine the state of bladder health in adolescent and adult women in the United States and monitor changes in bladder health over time. These assessments, together with additional medical

and other data gathered from a subset of study volunteers, will also expand the understanding of risk and protective factors to identify targets for future intervention studies.

Because of the suspected influence of access to toilets and the toilet environment on lower urinary tract symptoms, PLUS has also been developing a smartphone app called “Where I Go” to capture real time assessment of toilet access, safety, and cleanliness, and decision-making about when and where to go. PLUS researchers anticipate using this app in the long-term study. Finally, in anticipation of future efforts to promote bladder health and prevention strategies, PLUS will work on expanding its community engagement strategy to facilitate community-based participatory intervention and implementation research.

In all of its efforts, PLUS will continue to depend upon the individuals who contribute their time and experience to participate in clinical research that could benefit women and girls across the Nation and potentially even around the world.

*For more information, visit the PLUS website: <https://plusconsortium.umn.edu/>.*



## CINDI

A marketing professional for over 25 years, 49-year-old Cindi spends her time outside of work focused on the arts, music, volunteering, socializing with friends, and exercise—especially highly aerobic aqua exercise. She also has been managing bladder challenges since her youth, and thus was especially interested in the SHARE focus group. Cindi, who contends with other chronic health conditions as well, notes that her current bladder issues have led her to avoid some activities, such as long road trips where she might not be able to get to a bathroom easily. Cindi also says bluntly that she’s “happy that I’m not having major accidents, but it is embarrassing—I feel like I can’t go very long without having to go to the bathroom.” When asked what part of SHARE stuck with her most, Cindi notes that the diversity of perspectives, norms, habits, and personal experiences the women described was “incredibly eye opening.” She adds that her SHARE group experience “sort of normalized” her situation for her—recalling the stories she heard in the group, Cindi observes that, “What I’m dealing with is hard, but what everybody is dealing with is hard ... it made me feel like at least I’m not the only one out there who’s struggling.” In addition to spurring her to follow up with a specialist for her own bladder health, Cindi says that she felt SHARE was “a safe place” and “a positive experience.”