

Research described in this chapter uses a novel way to process mouse kidney tissue samples, in combination with a specialized imaging technique, called multiphoton microscopy, to get a deep view into the kidney's internal structures. Left panel: This novel technique was used to visually reconstruct a section of the kidney with excellent detail and depth. Right panel: Scientists also used this novel technique to visualize kidney damage caused by the chemotherapy drug cisplatin. *Clockwise from left:* Visual reconstruction of a normal mouse nephron (the basic functional unit of the kidney) and of two nephrons damaged by cisplatin. Within each nephron, the glomerulus—the fundamental filtering apparatus in the kidney—is indicated by gray (normal covering) or green (missing normal covering); small blood vessels are indicated in red. Cisplatin was found to damage the outermost layer of cells that encapsulate the glomerulus, thus disconnecting the glomerulus from another part of the nephron called the proximal tubule. This new technique could shed light on ways to make cisplatin safer for people, as well as be used to study other forms of kidney damage.

*Images courtesy of Dr. Richard Torres and Dr. Robert Safirstein, Yale University School of Medicine. Republished with permission of the [Journal of the American Society of Nephrology](#), from: Three dimensional morphology by multiphoton microscopy with clearing in a model of cisplatin induced CKD, Torres R, Velazquez H, Chang JJ, Levene MJ, Moeckel G, Desir GV, Safirstein R. Copyright 2015; permission conveyed through Copyright Clearance Center, Inc.*

# 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, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, 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 increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.*

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, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life threatening condition.

It has been estimated that more than 20 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 2013, over 661,000 patients received treatment for ESRD: nearly 467,000 received either hemodialysis or peritoneal dialysis and over 193,000 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. African Americans are nearly four times more likely

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<sup>1</sup> Centers for Disease Control and Prevention (CDC). *National Chronic Kidney Disease Fact Sheet: General Information and National Estimates on Chronic Kidney Disease in the United States, 2014*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2014.

<sup>2</sup> U.S. Renal Data System, *USRDS 2015 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, 2015.

to develop kidney failure than are non-Hispanic Whites.<sup>2</sup> American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic Whites.<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 NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. 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. The NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; 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. To encourage testing practical, sustainable, acceptable, and cost-efficient adaptations of efficacious strategies or approaches to prevent and treat kidney disease, the Institute issued in 2015 a research solicitation entitled "Translational Research to Improve Outcomes in Kidney Diseases."

The NIDDK'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 CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and seeks to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

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 urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. In 2015, the NIDDK held a meeting to discuss and prioritize the clinical and basic urinary stone disease research needs and identify strategies and recommendations on how best to address those needs. In follow-up to the meeting, the NIDDK issued a solicitation entitled "Urinary Stone Disease Research Network" in 2015 (see feature later in this chapter for more on the meeting and initiative). 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 components 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 painful bladder syndrome.<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; this research network, described in a feature in this chapter, was recently renewed for an additional 5 years.

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women.<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 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 other lower urinary

tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes. To address this challenge, the NIDDK launched and recently expanded 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. In 2015, the NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health, established the Prevention of Lower Urinary tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal 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 reissued the "New Directions in Hematology Research (SHINE II)" initiative in 2015. New principal areas of research outlined in the initiative include the identification and characterization of the production of the various types of blood cells in the bone marrow.

The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research.

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<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> *Urological Diseases in America*. NIDDK, NIH Publication Number 12-7865, 2012.

## INSIGHTS INTO KIDNEY DISEASE AND DAMAGE

### Link Found Between Kidney and Bowel Diseases:

Researchers have found six new regions in the human genome that increase susceptibility to immunoglobulin A (IgA) nephropathy, a major cause of kidney failure worldwide. Using genome-wide screening, the scientists identified susceptibility genes in people of Asian and European ancestries that affect both the risk of developing IgA nephropathy and the age at which the disease develops. Along with nine previously reported genes, these genetic regions are also associated with the risk of developing inflammatory bowel disease. This observation suggests that IgA nephropathy may be part of a group of autoimmune and inflammatory disorders that share some risk genes.

IgA nephropathy is a kidney disorder that occurs when IgA—a protein that helps the body fight infections—forms aggregates in the kidneys, resulting in inflammation. After many years, the IgA deposits may damage the kidneys, causing them to leak blood and sometimes protein into the urine. Scientists do not know what causes IgA deposits to form in the kidneys; although, as with many common diseases, both genes and environmental factors are likely to play a role. In the current study, the researchers found that several of the genes that confer risk of developing IgA nephropathy may also provide protection against intestinal parasites, which may help explain why respiratory or digestive tract infections seem to be a trigger for IgA nephropathy in some individuals.

*Kirylyuk K, Li Y, Scolari F,...Gharavi AG. Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. Nat Genet 46: 1187-1196, 2014.*

### Genetic Variations in Young Patients with Chronic Kidney Disease May Be Risk Factors:

A fraction of children and adolescents enrolled in the Chronic Kidney Disease in Children Study (CKiD) have been found to have changes in their genetic material that may be important in the development of kidney disease and its complications.

CKiD is a study of pediatric patients with mild to moderate kidney disease. For most children enrolled in CKiD, the cause of their kidney disease has been identified; however, in others it has not. In the current study, researchers used chromosomal microarrays, which detect duplications or deletions of chromosomal regions called copy number variants (CNVs), to analyze the genetic material in volunteers whose kidney disease was of unknown origin. They found that, of the 419 children in this group, 31 (7.4 percent) had CNVs. Further analysis revealed that these CNVs involved changes in 10 genes previously shown to be associated with kidney disease and 12 others that are thought to be related to kidney disease based on their function. The identification of patients with these genomic changes may warrant more personalized clinical care and evaluation, because the genes in some of these regions are associated with a higher risk of certain complications, such as diabetes, heart disease, eye disease, or neurological problems. These conditions may result from the kidney disease, or, potentially in some cases, may be independent of the kidney disease but caused by the same CNVs.

In the United States, the main causes of chronic kidney disease in adults are diabetes and high blood pressure. In children and adolescents, the causes of kidney disease are quite different and less well understood. A better understanding of potential CNV-related causes of kidney disease in children,

especially when there is no other identifiable cause, could pave the way to earlier interventions to preserve kidney function and better monitoring and treatments for associated conditions.

Verbitsky M, Sanna-Cherchi S, Fasel DA,...Gharavi AG. Genomic imbalances in pediatric patients with chronic kidney disease. *J Clin Invest* 125: 2171-2178, 2015.

**Delving Deep into the Kidney with a Novel Imaging Technique:** Scientists have developed a new imaging technique that allows them to see deep into the kidney's internal structures and gain novel insights about cisplatin-induced chronic kidney disease (CKD) in mice. Cisplatin is a chemotherapy drug that is used to treat various types of cancer. However, some people taking the drug develop CKD, which limits the drug's usefulness. To investigate how cisplatin treatment causes kidney damage, researchers first generated a new mouse model of cisplatin-induced CKD by administering two doses of the drug to mice 2 weeks apart. The animals developed CKD similarly to what is seen in people taking the drug. To examine the resulting kidney damage in the mice, the researchers used a specialized imaging technique, called multiphoton microscopy. However, a major limitation to using this technique is that it only gives a shallow view of the organ. To overcome this barrier, the scientists used a novel way to process samples of kidney tissue before looking at them under the microscope—they used a “clearing” solution that replaced the water in the tissue with other chemicals. Use of this clearing solution greatly increased the imaging depth so that the microscope could produce high-resolution three-dimensional images deep inside the kidneys of the mice; this allowed the scientists to observe the damage caused by cisplatin. For example, they found that cisplatin treatment reduced the number of a type of cell (cuboidal cells) in the capsule of the

glomerulus—the fundamental filtering apparatus in the kidney. The loss of cuboidal cells corresponded, almost exactly, to reduced kidney function. The observations made in this study, as well as future research using this new approach, could provide novel insights about kidney damage caused by cisplatin and identify prevention targets to make the drug safer for people. Additionally, the imaging technique could be used to study other forms of kidney damage, such as kidney disease caused by diabetes or high blood pressure.

Torres R, Velazquez H, Chang JJ,...Safirstein R. Three-dimensional morphology by multiphoton microscopy with clearing in a model of cisplatin-induced CKD. *J Am Soc Nephrol* 2015 Aug 24 pii: ASN.2015010079 [Epub ahead of print].

## KIDNEY DISEASE TREATMENT

**Single Drug As Effective As Pair in Treating Polycystic Kidney Disease:** Using two drugs is no more effective than a single drug in slowing disease progression in people with autosomal dominant polycystic kidney disease (ADPKD), according to two recent reports. One of the studies also showed that rigorous blood pressure treatment slowed growth of kidney cysts, a marker of ADPKD, but had little effect on kidney function compared to standard blood pressure treatment.

The HALT-PKD trials enrolled volunteers to test whether a combination of commonly used U.S. Food and Drug Administration (FDA)-approved drugs to treat high blood pressure, lisinopril and telmisartan, could shrink kidney cysts and therefore slow progression of ADPKD. One study examined 558 people with early-stage ADPKD and relatively healthy kidneys. Another study treated 486 people with more advanced disease and diminished kidney function. In each study, half of

the participants were randomly assigned to receive both drugs, while the other half received lisinopril plus a placebo. In both studies, adding the second drug did not change kidney function or rate of increase in kidney cyst size.

In the study of people with early ADPKD and healthier kidneys, researchers also tested whether decreasing blood pressure below usual targets would slow progression of ADPKD and preserve kidney function. One-half of the participants were assigned to a standard blood pressure group (between 120 to 130 over 70 to 80); the other was assigned to a lower blood pressure group (between 95 to 110 over 60 to 75), but still within the normal range. Participants in the lower blood pressure group, who took more medication to maintain the lower blood pressure, had a 14 percent decrease in kidney cyst size compared to those in the standard blood pressure group. However, kidney function—measured by estimated glomerular filtration rate (eGFR)—was about the same as the standard group at the end of the trial, yielding no clinical benefit.

ADPKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. Symptoms usually develop between the ages of 30 and 40, and many people with ADPKD experience a decline in their kidney function as the cysts grow. About one-half of them progress to kidney failure and require dialysis or a kidney transplant to live. High blood pressure and related cardiovascular complications, such as heart attacks and strokes, are common health problems for people with ADPKD. The results of the HALT-PKD studies demonstrate that more research is needed to better understand how ADPKD destroys kidney function over time, and to determine what combination of medications can most safely and effectively prevent or undo the damage caused by this serious condition.

Schrier RW, Abebe KZ, Perrone RD,...Chapman AB; for the HALT-PKD Trial Investigators. Blood pressure in early autosomal dominant polycystic kidney disease. *N Engl J Med* 371: 2255-2266, 2014.

Torres VE, Abebe KZ, Chapman AB,...Perrone RD; for the HALT-PKD Trial Investigators. Angiotensin blockade in late autosomal dominant polycystic kidney disease. *N Engl J Med* 371: 2267-2276, 2014.

**Living Kidney Donors Fare Well Over Time:** People who choose to donate one of their kidneys to someone with kidney failure remain relatively healthy 3 years after their donation. Relatively few studies have assessed the overall health, and specifically the kidney function, of living kidney donors over time after they donate one of their two kidneys compared to individuals who have not donated a kidney. In a recent study, researchers examined the health status of 182 kidney donors (who, after their donation, have only one kidney) and 173 non-donors (*i.e.*, people with two kidneys) over a 36-month period; 95 percent of the participants were white. Of those who did not donate a kidney, two clinical tests of kidney function—measured glomerular filtration rate (mGFR) and estimated glomerular filtration rate (eGFR)—declined over time, which is expected as people age. In the donor group, these measures were initially lower after donation but improved over time. Other health parameters were largely the same between the two groups. Blood pressure increased slightly over time but was similar in donors and non-donors. Another test of kidney function—protein excreted from the kidney into the urine—did not differ between donors and non-donors.

Once kidney disease progresses to kidney failure, the only treatment is dialysis or transplant. As donor organs are in short supply, there has been interest in the use of living donor organ transplants. The current study suggests that living donor kidney

donation may represent a potential source of organs that would allow recipients to live healthy lives while not compromising the health of the donor, at least within the first 3 years after donation. Future studies could determine longer-term health outcomes for kidney donors.

*Kasike BL, Anderson-Haag T, Israni AK,...Weir MR. A prospective controlled study of living kidney donors: three-year follow-up. Am J Kidney Dis 66: 114-124, 2015.*

**Targeting Cells Involved in Fibrosis May Reduce Chronic Kidney Damage:** Researchers have discovered that members of the GLI protein family can promote kidney fibrosis and that inhibiting these proteins can reduce kidney damage. Fibrosis—the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney or from a slowly progressing, chronic condition. Extensive kidney fibrosis can cause irreversible organ damage and, in severe cases, lead to kidney failure.

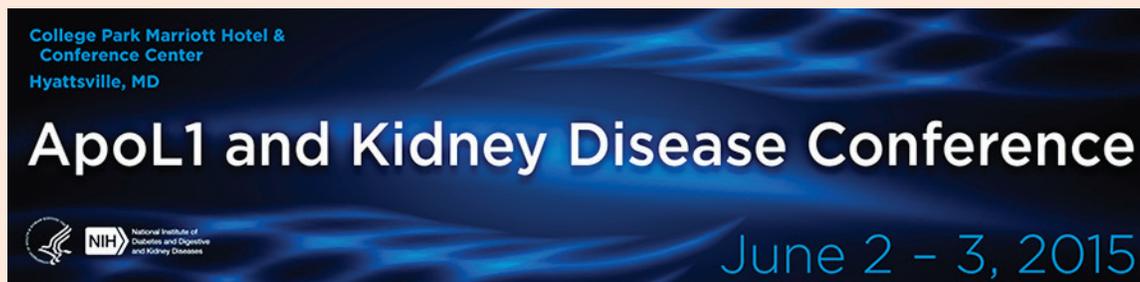
Cells called myofibroblasts are known to drive kidney fibrosis. Additionally, members of the GLI family of proteins have been found in precursor cells that become myofibroblasts, and these proteins become more active during kidney fibrosis. However, it was not known whether GLI protein activity, myofibroblast activity, and fibrosis are linked. Scientists have recently clarified this issue by asking whether GLI proteins regulate myofibroblast activity during kidney fibrosis.

To confirm that GLI proteins help control myofibroblast replication, the researchers first asked

what role GLI proteins play in cultured myofibroblast precursor cells in the laboratory. Reducing the levels of one of the GLI proteins, GLI2, caused the cells to stop replicating, suggesting that GLI2 was required for these cells to expand their numbers during organ injury. Male and female mice engineered to lack GLI2 also had less kidney fibrosis than their normal counterparts when kidney injury was induced, and this reduced fibrosis was due to myofibroblasts failing to replicate. These results suggested that GLI2 may be required for myofibroblasts to cause kidney fibrosis and that inhibiting GLI2 could be protective. To determine if GLI2 inhibition could protect against kidney fibrosis, researchers tested the effects of a GLI2 inhibitor drug called darinaparsin. In cultured myofibroblast precursor cells, darinaparsin reduced GLI2 protein levels and caused the cells to stop dividing. When male mice were exposed to kidney injury, darinaparsin again blocked the expected increase in GLI2 protein, significantly reducing kidney fibrosis compared to mice not given the drug. Importantly, darinaparsin reduced kidney fibrosis whether the mice were given the drug prior to kidney injury or after the fibrosis had already begun. Finally, the researchers found that the genes for GLI proteins were more active in kidneys from men and women who had severe kidney fibrosis than in people with less severe kidney fibrosis, though more research is needed to confirm that the GLI proteins work the same in people as they do in mice. Overall, these experiments demonstrate that GLI proteins contribute to kidney fibrosis through their control of myofibroblast replication. Furthermore, these findings suggest that GLI proteins such as GLI2 may be promising targets for developing kidney fibrosis treatments.

*Kramann R, Fleig SV, Schneider RK,...Humphreys BD. Pharmacological GLI2 inhibition prevents myofibroblast cell-cycle progression and reduces kidney fibrosis. J Clin Invest 125: 2935-2951, 2015.*

# NIDDK ApoL1 and Kidney Disease Conference



The NIDDK held a conference entitled “ApoL1 and Kidney Disease Conference” in June 2015 to assess gaps in knowledge including the function of the ApoL1 protein and its role in kidney transplantation. Variants of the *APOL1* gene, which are found primarily in African Americans, are arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and among the only known genetic factors contributing to the well-appreciated health disparities in kidney diseases. These variants may explain 70 percent of the excess focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertensive kidney disease in African Americans.

The conference developed new ideas regarding how *APOL1* gene variants lead to disease susceptibility, what kidney and cardiovascular outcomes are associated with these variants,

which additional genetic variants or environmental factors play a role in differences in disease symptoms, and the possible roles of *APOL1* genotyping in guiding treatment as well as preventive strategies.

Experts were drawn from across multiple fields. They contributed a wealth of information from a variety of perspectives and identified a broad spectrum of areas in which additional information is needed to move the field forward. The input garnered included many suggestions for potential future research directions. Examples include: exploring the effects of *APOL1* variants on kidney transplantation outcomes in donors and recipients, identifying environmental and additional genetic factors that contribute to *APOL1*-related disease, and investigating how *APOL1* gene variants contribute to kidney disease.

# Scientific Workshop: Urinary Stone Disease— Research Challenges and Opportunities



To assess gaps in knowledge and evidence-based treatment, the NIDDK held a meeting titled “Urinary Stone Disease Research Challenges and Opportunities” in April 2015. Urinary stone disease (USD) is an important, increasingly common problem, with some people experiencing recurrent episodes. It is a costly condition and also carries the burdens of severe pain and loss of quality of life. Obesity and diabetes are associated with USD, and these conditions also are increasing in prevalence. USD is most common in Whites and in men, although USD prevalence is growing in all races and both sexes.

Despite the high prevalence and health and economic burden of the disease, little is known about how stones form or which are likely to be passed. Advances in treatments in the past 30 years have evolved from open surgery to remove large stones, to new technologies. Current treatments to prevent recurrence include increasing fluid intake, modifying diet, and

medications. These measures, however, have not decreased prevalence, suffering, recurrence rates, USD-related chronic kidney disease (CKD) incidence, or cost, representing limited progress in USD, especially compared to diseases such as cancer and cardiovascular disease. Few of the American Urological Association’s and American College of Physicians’ guidelines for USD treatment recommendations are based on level-one evidence; this level of evidence is obtained from randomized, well-controlled clinical trials.

The meeting’s objectives included presenting information on the epidemiology and pathophysiology of USD, as well as evidence for management strategies; discussing and prioritizing the areas in which additional USD research is needed, including studies to better understand the role of the microbiome, genetics, and exposome (defined as the measure of all the exposures—for example, diet, environmental conditions such as hot temperatures, constraints in many occupations

that limit urination frequency) of an individual in a lifetime and how those exposures relate to health; and providing input that could inform potential future NIDDK program initiatives, including input on the key question of the types of studies needed to inform management of USD.

Experts were drawn from across multiple fields—not just urology and nephrology, but also epidemiology, clinical trials, behavioral economics, and biology. Participants contributed a wealth of information from a variety of perspectives and identified a broad spectrum of areas in which additional information is needed to move the field forward. For example, the picture is incomplete regarding how genes and proteins cause USD. Disease gene identification could allow doctors to offer genetic diagnosis, provide novel insights into pathogenesis and physiology, and potentially permit personalized treatments. The lack of effective medications to prevent recurrence of USD was also highlighted, as well as side effects associated with the use of devices to pulverize stone(s) (e.g., bleeding and hospital re-admission).

Four break-out groups identified future research needs including: assessing how the diet and/or microbiome may affect USD, determining how genetic information can be used for personalized USD prevention, identifying strategies to prevent USD recurrences, and formulating the clinical studies needed to evaluate the impact of surgical stone removal therapies on stone clearance.

To encourage research that addresses some of the research needs identified during the meeting, the NIDDK released Funding Opportunity Announcements to establish a “Urinary Stone Disease Research Network” in 2016, which will include several Clinical Centers and a Scientific Data Research Center. The Network will seek to 1) design and conduct 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, 2) conduct clinical research to understand and mitigate ureteral stent-related pain and symptoms, and 3) provide data and collect biological samples from the studies to create a resource for future researchers.

## ADVANCING UNDERSTANDING AND TREATMENT OF URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are common and occur more frequently in women, many of whom suffer repeated bouts of infection. Although treatable with antibiotics, the emergence of antibiotic-resistant microbes in UTIs, combined with the personal and medical costs of care, makes finding better therapeutic strategies a priority. The primary culprit in UTIs is the bacterium *Escherichia coli* (*E.coli*); UTI-causing *E. coli* bind to and invade the cells lining the inside of the bladder to initiate an infection. A better understanding of how microbes such as *E. coli* respond to and even manipulate host defenses to promote their own survival—and how hosts counter these activities—could help uncover new approaches to UTI treatment. Several studies, summarized below, have provided new insights into aspects of both bacterial and host biology that are advancing efforts in this area.

### Identifying Players in Urinary Tract Infections:

Two recent studies shed light on how the bacterium *E. coli* causes infection in the human urinary tract.

An acute UTI caused by *E. coli* begins when bacteria attach to and invade the cells lining the inside of the bladder. The UTI-causing *E. coli* form intracellular bacterial communities within the bladder that help to promote sustained infection. This invasion and proliferation provokes a defense response in the infected individual, including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the body of offending bacteria. While this exfoliation of infected bladder cells can help clear infection, it also presents an opportunity for bacteria to infect deeper cell layers of the bladder.

One group of researchers sought to investigate further the bacterial and host factors modulating the exfoliation of infected bladder cells. Previous research has found that almost half of *E. coli* obtained from patients with a UTI produce a molecule called  $\alpha$ -hemolysin (HlyA). HlyA damages bladder cells by causing holes or pores in the bladder cell membrane. The investigators determined that HlyA also activates a host cell death signaling program in infected bladder cells. Further, they found that *E. coli* controls its production of HlyA by a sensor system called CpxRA, a system that senses environmental cues such as changes in the makeup of membranes. Taken together, the results of this study provide valuable information showing that some UTI-causing *E. coli* can control their ability to cause disease (virulence) through regulation of HlyA by CpxRA, possibly using this system to manipulate the timing of exfoliation to optimize infection.

Using a molecular biology approach, a second group of investigators identified “fitness” genes expressed (turned on) by *E. coli* during UTI in women. In this context, fitness genes include genes that contribute to *E. coli* survival in the host environment. Among the nine fitness genes identified was a gene called *cus* that helps *E. coli* survive the toxic effect of copper that the body uses to fight infection. Strategies that target the protein products of *cus* and other genes that support bacterial survival may be an effective approach to combat bacterial infection.

These studies provide new insights into how bacteria such as *E. coli* use different yet critical processes to survive and promote UTI in humans. Strategies that target these bacterial processes may yield new therapeutic approaches for UTIs that are synergistic with, or even more useful than, current antibiotics.

Nagamatsu K, Hannan TJ, Guest RL,...Hultgren SJ. Dysregulation of *Escherichia coli*  $\alpha$ -hemolysin expression alters the course of acute and persistent urinary tract infection. *Proc Natl Acad Sci USA* 112: E871-E880, 2015.

Subashchandrabose S, Hazen TH, Brumbaugh AR,...Mobley HL. Host-specific induction of *Escherichia coli* fitness genes during human urinary tract infection. *Proc Natl Acad Sci USA* 111: 18327-18332, 2014.

### Infected Bladder Cells Send Bacteria on a Trip via

**TRP:** A recent study reveals a mechanism used by infected bladder cells to rid themselves of invading bacteria. One common mechanism host cells use to purge themselves of invading pathogens is to round them up in cellular compartments called lysosomes, which are membrane-bound cell organelles containing digestive enzymes that break down excess or worn-out cell parts as well as invading viruses and bacteria. Researchers now have new insights into how bladder cells use lysosomes to combat *E. coli* infection. Previously, researchers found that bladder cells infected with *E. coli* expel the bacteria intact, rather than degrade them in lysosomes; now, they report the mechanism used by infected cells to accomplish this task. Through a series of experiments in mice and in human bladder cells, lysosomes filled with UTI-causing *E. coli* were found to have an altered pH—in this case a neutral pH rather than the acidic pH in which lysosomes exert their best degradative function. pH measures the acidity or alkalinity of a solution; for example, tomatoes are acidic with a pH of 4.6, ammonia has a pH of 11.6, and water is neutral with a pH of 7. It appears that the pathogenic *E. coli* cause the abnormal neutralization of lysosome pH. Lysosomes are unable to degrade the bacteria in neutral pH, so they activate a backup plan. The researchers found that a lysosomal membrane protein called mucolipin TRP channel 3 senses the altered pH and

sets in motion a series of events leading affected lysosomes to travel to and fuse with the bladder cell membrane, subsequently expelling the bacteria, wrapped up in small membrane-bound sacs, to the outside of the cell and into the urine.

These findings reveal a mechanism used by infected bladder cells to circumvent a malfunctioning lysosomal process and effectively rid the cell of bacterial pathogens. Molecules that simulate the activity of TRP and enhance bacterial expulsion from infected bladders may be an effective strategy to combat UTIs.

Miao Y, Li G, Zhang X, Xu H, and Abraham SN. A TRP channel senses lysosome neutralization by pathogens to trigger their expulsion. *Cell* 161: 1306-1319, 2015.

### Too Much of a Good Thing—Host Defense of the Bladder:

A recent study in humans and mice suggests that excessive activity by a cell of the immune system can predispose the bladder to recurrent UTI. Certain strains of *E. coli* can latch on to the epithelial cells lining the bladder wall and invade the bladder cells—establishing an intracellular reservoir leading to recurrent UTIs. Exactly how the host acts to rid itself of the infection and what factors may predict susceptibility to recurrence are poorly understood, although activation of the immune system—the “defense complex” of cells and molecules used by many organisms to ward off dangerous microbes and other threats—appears to play a role in both.

Researchers sought to determine whether a biomarker for susceptibility to recurrent UTI (e.g., an easily detectable and measurable molecule associated with this condition) could be identified in blood samples from women who sought treatment for a UTI less than 7 days in duration, and who were then followed for 3 months to

determine recurrence of UTI. Several molecules were found to be increased in women who developed recurrent UTI compared to those who did not experience a recurrence. These molecules are known to be produced in different types of immune system cells—neutrophils, monocytes, and macrophages—all of which can play a role in promoting inflammation. Acting on this finding, the researchers interrogated the role of these three immune system cells in a female mouse model of recurrent UTI through experiments that revealed an unknown contribution by the neutrophil. When the neutrophil population was depleted in the bladder, the mice developed severe infection and chronic UTI. However, when the neutrophil pro-inflammatory response was robust in the bladder, the mice were also susceptible to chronic UTI. In contrast, moderating this neutrophil response by partially reducing the abundance of neutrophils in the bladder led to a reduced incidence of chronic UTI. The investigators concluded that, while neutrophils are necessary to prevent severe bladder infection, an excessive neutrophil response damages the bladder lining and predisposes the bladder to chronic UTI.

Drilling down further, the researchers found that both neutrophils and cells lining the bladder produce the pro-inflammatory molecule COX-2 during infection. Notably, COX-2 is a target of non-steroidal anti-inflammatory drugs, such as ibuprofen, as well as other medications. While COX-2 is not detectable in the uninfected bladder, it is found in women with UTI. Similarly, the *Cox-2* gene is barely “turned on” in the uninfected mouse bladder but increases 50-fold in bladders infected by *E. coli*. Importantly, blocking COX-2 activity with a specific inhibitor reduced the severity of bladder inflammation and protected mice from chronic UTI—apparently, at least in part, by helping to prevent entry of neutrophils into the bladder.

These findings in humans and mice provide new information about the role of the immune system in promoting susceptibility to recurrent UTIs. Although more research is needed, they also suggest that inhibition of COX-2 may have beneficial effects in women by preventing recurrent UTI.

Hannan TJ, Roberts PL, Riehl TE,...Hultgren SJ. Inhibition of cyclooxygenase-2 prevents chronic and recurrent cystitis. *EBioMedicine* 1: 46-57, 2014.

### Fighting Fire with Fire To Treat Urinary Tract

**Infections:** A new study in mice suggests that bacteria that do not cause symptomatic UTIs may be an effective therapy against ones that do. Some *E. coli* strains can invade and multiply in the human urinary tract without causing the symptoms of pelvic pain and urinary urgency normally associated with UTIs. Recent evidence suggests that this may be due to differences between these asymptomatic bacteriuria (ASB) *E. coli* and uropathogenic *E. coli* (UPEC) in certain bacterial surface molecules, with consequent differences in host responses. A number of studies in humans and animal models have also suggested that ASB *E. coli* may prevent or disrupt infection by UPEC, and, furthermore, may have pain-relieving, or analgesic, properties.

Working in female mice, scientists recently tested whether delivering live ASB *E. coli* into the bladder could be used to both reduce the total number of UPEC and reduce UTI pain symptoms. They found that one time administration of a commonly used ASB *E. coli* strain both reduced bacterial numbers and reduced measures of pelvic pain in mice infected with UPEC. Additional tests showed that pain relief provided by ASB *E. coli* 24 hours after administration was similar to that seen after 1 hour treatment with a pain-relieving medication called lidocaine. They then tested other ASB *E. coli* strains isolated from humans for their analgesic

properties and found a range of efficacy; one strain in particular, termed isolate 2-12, was even more effective than the initial strain at reducing pelvic pain in mice infected with UPEC. But how well does the ASB *E. coli* treatment approach compare to antibiotic treatment? Initial tests with the original ASB *E. coli* strain suggested that its ability to reduce the total number of UPEC during infection is comparable to a 3-day course of the antibiotic ciprofloxacin—the clinical standard for treatment of human UTIs. More significantly, however, experiments using the highly analgesic 2-12 strain demonstrated that it provided dramatic and early relief of pain symptoms, whereas ciprofloxacin did not. Furthermore, isolate 2-12 was not only superior to ciprofloxacin in relieving pain induced by UPEC, but also pain induced by three other, non-*E. coli* UTI-causing bacterial species.

While further research is needed, a “probiotic” strategy using ASB *E. coli* to interfere with UTI-causing bacteria and the pain they cause may prove to be a safe and effective treatment strategy for women and men with UTIs, and may even prove useful in treating pelvic pain from other causes.

Rudick CN, Taylor AK, Yaggie RE, Schaeffer AJ, and Klump DJ. Asymptomatic bacteriuria *Escherichia coli* are live biotherapeutics for UTI. *PLoS One* 9: e109321, 2014.

## EVALUATING PAIN IN WOMEN AND MEN

**What's the Difference? New Insights into Pain in Women and Men:** Research has revealed significant differences in how women and men experience pain. Many chronic pain conditions, such as the pelvic pain conditions interstitial cystitis/bladder pain syndrome (IC/BPS) and irritable bowel syndrome (IBS), are much more

prevalent in women than in men, and women with these conditions tend to report more severe, frequent, and longer-lasting pain than their male counterparts. Evidence suggests that individual experiences of pain can be affected both by pain sensitivity—*i.e.*, the threshold at which a person detects a stimulus, such as heat or cold, as painful—and by complex, “top-down” neural pathways running from the brain to the body that can diminish (inhibit) a person’s response to such a stimulus. While additional studies have suggested that there are sex differences in these two factors affecting pain, results have been somewhat mixed. A research team set out to clarify whether being male or female can influence pain sensitivity and/or pain inhibition.

For the study, data were collected from 24 women and 24 men between the ages of 19 and 45 (average age of about 22) who were healthy and free of chronic pain conditions. Each study participant completed a series of tests to measure pain experience. The initial test measured pain sensitivity: Participants were asked to conduct exercises with one hand while blood flow to that hand was restricted by a blood pressure cuff, and to rate their pain on a numeric scale at multiple time points during the test. A second set of tests measured pain inhibition by seeing how well participants could be conditioned to pain: First, participants were asked to immerse one hand four times in a cold water bath that grew increasingly colder at each immersion, and to rate the pain midway and at the end of each immersion. Then, using the temperature that evoked moderate pain for each individual, participants were again asked to immerse one hand in cold water, but then a device that delivered increasing levels of pressure was applied to the other arm, and participants were asked to report when the pressure first

became painful. Importantly, the researchers sought to account for factors known to influence pain experience by having participants complete questionnaires about depressive symptoms and sleep quality prior to undergoing the pain tests.

When they analyzed the results, the researchers found that there were significant differences between healthy women and men both in their sensitivity to pain and in pain inhibition, even after controlling for differences in sleep quality and depressive symptoms. Men were more tolerant of pain and showed more efficient pain conditioning than women. These results suggest that there are underlying biological differences between women

and men in pathways affecting pain experience that could help explain observed differences in chronic pain condition prevalence and symptom severity. Future research could help clarify this and also help determine whether targeting pathways involved in pain sensitivity and pain inhibition in different ways in women and men—e.g., by using different therapies, or by administering the same intervention in different amounts—could help better alleviate pain burden in women.

*Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, and Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. J Pain Res 8: 311-320, 2015.*



# The Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network: Yielding New Insights into Challenging Urologic Pain Syndromes

Chronic, often debilitating pain in the pelvic or genital areas, frequently accompanied by urinary symptoms such as needing “to go” urgently or many times a day: These are hallmark symptoms of urologic chronic pelvic pain syndrome (UCPPS), a term that encompasses both interstitial cystitis/bladder pain syndrome (IC/BPS, also called IC/painful bladder syndrome (PBS)), which predominantly affects women, and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), which affects men. These pain conditions reduce quality of life and productivity and incur significant health care costs for millions of Americans.

Despite many years of committed basic and clinical research efforts supported by the NIDDK and other research funding agencies, the cause(s) of UCPPS has long remained elusive, as have widely or fully effective treatments. Moreover, diagnostic tests are not currently available. Instead, because many UCPPS symptoms can be suggestive of known diseases, those diseases need to be ruled out first—making IC/BPS or CP/CPPS a “diagnosis of exclusion.” While public and clinical awareness of UCPPS is increasing due to educational efforts by the NIDDK and major health advocacy organizations, such as the Interstitial Cystitis Association and the Prostatitis Foundation, many people suffer

for years with symptoms and no diagnosis. In the face of these challenges, discoveries emerging from the NIDDK-sponsored Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network are bringing scientists and clinicians closer to understanding the cause(s) of UCPPS, improved diagnosis, better characterization of patients to help identify more effective treatments, and ways to prevent onset.

## A New Approach

Historically, much of the NIDDK-supported research on causes and treatments for IC/BPS and CP/CPPS focused on the bladder and prostate, respectively. This was because these organs and tissues were thought to be the sites of origin for the pain and other symptoms suffered by persons diagnosed with UCPPS. Despite a broad array of studies exploring many hypotheses, however, researchers were unable to find satisfactory medical explanations for symptoms or to identify definitive risk factors or viable therapies, with the exception of a single study suggesting that myofascial physical therapy might be effective in some IC/BPS patients. At the same time, a growing body of evidence was revealing that many people with UCPPS frequently have other, overlapping chronic pain conditions, such as irritable bowel syndrome

(IBS) and fibromyalgia. Taken together, these outcomes and findings suggested that, at least in some people, UCPPS might not be a localized pain condition, but instead be part of a global pain process involving the central nervous system (*i.e.*, the brain and spinal cord) and potentially other body systems, such as the immune system. These insights also suggested that there might be a greater degree of diversity among persons grouped together under a UCPPS diagnosis than was initially thought.

In 2008, armed with these new perspectives and input from the scientific and health advocacy communities, the NIDDK launched Phase I of the MAPP Research Network. Established through a Request for Applications, this phase of the Network comprised six “Discovery Sites” at research institutions across the country, complemented by a Data Coordinating Center to manage and store clinical data and a Tissue and Technology Center to centrally process, store, and disburse clinical samples. This novel, multi-site research network embraced a unique, systemic (whole-body) approach to the study of IC/BPS and CP/CPSS to address clinically relevant questions. The Network gathered together scientists with diverse research expertise—including basic science, clinical urology, behavioral science, immunology, epidemiology, neurobiology, psychology, chronic pain, neurobiology/neuroimaging and infectious diseases—all working collaboratively to better understand UCPPS. In addition to moving beyond traditional bladder- and prostate-specific research directions to an innovative, multidisciplinary strategy for studying UCPPS, Network scientists sought to investigate potential relationships between these syndromes and the other chronic pain conditions that are often seen in people with UCPPS, such as IBS, fibromyalgia, and chronic fatigue syndrome. Thus, Network leaders included

not only urologists, but investigators specializing in these overlapping pain conditions.

## Foundation Studies and Novel Findings

In Phase I, the MAPP Research Network recruited 424 men and women with UCPPS in a central, Trans-MAPP Epidemiology and Phenotyping study to better understand how these conditions progress over time (the natural history) and to learn if patients might fall into different, distinguishable subgroups based on differing symptoms that may arise from different causes—and, thus, may require different treatments. To achieve its goals, the Network also recruited “control” participants—both healthy persons without any pain syndromes (415 individuals), and those who have one or more of the overlapping chronic pain conditions (200 individuals).

The Network also conducted a number of other collaborative studies across sites complementing the central Trans-MAPP study—for example, there is evidence that alterations in the brain and spinal cord play important roles in chronic pain, so brain-imaging studies looking at structure and function were conducted across the Network using standardized protocols. Other efforts included various studies to identify biomarkers of disease, to assess the possible role of infectious agents, and to provide a systemic view of disease. The development and assessment of clinically relevant animal models of UCPPS has also been part of the Network’s efforts. In addition, individual Network sites have conducted studies to test ideas complementary to the Trans-MAPP central clinical study. Importantly, the Network was structured so that investigators would draw upon a shared pool of participant data and samples from across the Network collected using common protocols.

To establish the central Trans-MAPP study, people with IC/BPS or CP/CPSP and control participants were initially asked to fill out a number of questionnaires covering a variety of topics, including urologic pain, emotional state, other types of pain, and other symptoms and quality of life issues. They were also asked to provide blood and urine samples for use in some of the research studies. Additionally, a subset of participants engaged in a simple pressure pain threshold procedure that is a way to directly assess pain sensitivity. For participants with UCSPS, this initial, in depth clinic-based visit was followed by two additional visits at 6 and 12 months after enrollment. During that year, these participants were also registered with an Internet-based system so that they could fill out assessments of their symptoms every 2 weeks. This regular symptom assessment, a key component of the central epidemiological study, was a particularly valuable tool, as it has enabled MAPP Research Network scientists to learn a great deal about UCSPS symptoms, symptom fluctuations, and their possible correlations with other factors. The data and samples collected for the central study have also served as a crucial resource for other collaborative studies and site-specific studies in the Network.

During Phase I, the Network made significant progress on a variety of fronts, and results of Network analyses continue to emerge in scientific meetings and in peer-reviewed publications. Recent scientific reports describing novel advances in four major areas—clinical research tools, symptom flares, brain changes, and biomarkers/potential mediators—are summarized below.

### **Clinical Research Tools**

As described previously, a large part of the central trans-MAPP study consisted of participants completing a variety of questionnaires to

describe the key symptoms of pain and bladder dysfunction, as well as issues such as depression, sleep quality, and general quality of life. While a few of these questionnaires were developed specifically for the MAPP Research Network, the majority have already been employed in health care settings and in clinical research to assess the impact of UCSPS on individuals and the outcomes of clinical trials, respectively. In the past, many of these questionnaires were used to generate a composite “score” for UCSPS that combined pain and bladder symptoms. However, a recent analysis of data from a subset of questionnaires administered in the trans-MAPP study allowed Network scientists to determine that such a composite score can “mask” independent responses in each of these areas—i.e., their analysis suggests that improvement or worsening can occur in pain independently of bladder symptoms, and vice versa, and that a composite score limits the ability of researchers and clinicians to detect such changes. In addition, they found a differential impact of these key symptoms on an important comorbidity, depression: only pain symptoms were associated with depression. These results indicate that, going forward, pain and urinary symptoms should be scored independently in UCSPS to enable more accurate research analyses and improved patient care.

### **Symptom Flares**

People with UCSPS have reported suffering from symptom “flares”—brief or extended periods of time when symptoms intensify. Understanding flares is important both for research studies—in which flares need to be taken into account when assessing the success of a therapeutic intervention for UCSPS, for example—and also in developing clinical tools to measure and improve patient quality of life. Network scientists conducted a collaborative study at four Discovery Sites to learn

more about the impact of flares on individuals' lives, as well as gain more insight into associated triggers and treatment. The study involved eight focus groups (two groups per site) of women with IC/BPS, for a total of 57 individuals.

As a result of the moderated focus group discussions and accompanying questionnaires, the researchers found that flares were common and varied widely in their nature (e.g., pain, diarrhea, nausea), intensity (moderate to severe), frequency (daily to once a year or less), and duration (minutes to years); there were also some distinctions in frequency and duration between mild or moderate flares and the more severe flares. The most disruptive flares seemed to be those that were painful in nature, were accompanied by bladder symptoms, and lasted for days. Most participants could identify at least some of their triggers, though not all. Examples included stress, diet, allergies, medications, brand of toilet paper, and emotional state. Also, many triggers were individual specific—e.g., exercise could be a trigger in one person and a management strategy in another. Participants described approaches they used for preventing and self-managing flares but, crucially, they also conveyed the immediate and longer-term impacts of flares, from having to cancel social engagements, to living in a state of constant vigilance and anxiety (which in turn affected family and other relationships), to losing jobs and educational opportunities due to the severity of flares. The very negative impact of flares suggests that future research focused on preventing and mitigating flares would have a positive impact on quality of life for these individuals. This study also revealed how a sense of control over some aspect of symptoms (through medication or other means) is an important coping mechanism for many persons experiencing flares, especially as the unpredictability of flares is part of

their negative impact; thus, empowering patients through, e.g., discussing treatment strategies for flares, could be integrated into clinical care.

### Brain Changes

Brain changes have been observed in individuals suffering from a variety of painful conditions, but identifying changes in brain structure and function in large numbers of well-characterized people with UCPPS in a standardized manner had not been attempted previously. Network scientists recently reported a variety of differences in brain structure and function between women and men with UCPPS and healthy counterparts. For example, one study focused on “white matter,” the structures that facilitate communication of information between and within brain regions. Using an imaging technique that detects a marker of white matter structural integrity, Network researchers found that, compared to healthy controls, women with IC/BPS exhibited white matter abnormalities in several different brain regions. Moreover, these alterations appear to be clinically relevant, as they correlated variously with pelvic pain severity, urologic symptoms, and quality of life as reported by participants. These results complement a prior Network study of women with IC/BPS that found that increases in pain and mood disturbance were associated with increases in the volume of “gray matter”—the brain tissues responsible for cognition, sensory perception, emotion, and muscle control, which can form connections via white matter.

Network scientists have also used a technique called functional magnetic resonance imaging (fMRI) to explore how brain regions work together to produce pain or how they may be modified in the context of chronic pain, and to possibly identify signature alterations in this “functional connectivity” germane to UCPPS. Comparing fMRI brain scanning data from 45

women with UCPPS but no comorbid conditions and 45 healthy controls, scientists found significant alterations in functional connectivity between several brain regions and networks in symptomatic women while at rest—i.e., not actively engaged in tasks. These alterations included decoupling of two brain regions from the brain's "default mode network," a pattern of brain activity that is engaged when people are involved in undisturbed, task-free, introspective thought—suggesting that persons with UCPPS may experience dysfunction in this default network. Moreover, the two decoupled regions exhibited altered functional connectivity—both increases and decreases—with other brain regions, including ones involved in pain; sensory, motor, and emotion regulation processes; reward; and higher executive functioning. These latter alterations were associated with clinical and behavioral measures reported by the participants within 48 hours of their brain scans, including pain, anxiety, and self-esteem, and may reflect a literal shift in brain focus in persons with UCPPS from introspective thought towards aspects of pain and emotion regulation.

In another study, Network scientists used fMRI to examine the relationship between chronic pain and brain involvement in pelvic floor muscle control in men with CP/CPPS. In addition to experiencing pain in this area, men with CP/CPPS are known to have abnormalities in pelvic floor muscle activity. In the study, researchers first identified in healthy men a brain region involved when actively contracting pelvic floor muscles, as well as a distinct region involved in muscle contraction in a non-painful area in men with CP/CPPS (the right hand). They then used brain scans from multiple Discovery Sites to look at the functional connectivity of these

regions in both men with CP/CPPS and healthy men while at rest to see if there were differences that could help explain the altered pelvic floor muscle activity in men with CP/CPPS. They found that, compared to functional connectivity of the hand control region, there was a significant alteration in functional connectivity of the brain region involved in pelvic muscle control in men with CP/CPPS versus healthy controls. The alteration affected functional connectivity to a brain region involved in processing and providing an emotional response to a broad spectrum of sensory inputs from the body (e.g., it is involved in experiences such as food cravings, nausea, pain, and disgust). This altered functional connectivity was significantly associated only with pain symptoms and not with other symptoms experienced by men with CP/CPPS, and the degree of alteration tracked with the severity of pain symptoms reported by participants. This study is the first to identify brain activity changes in men with CP/CPPS compared to healthy men, points to an important role for brain control of muscle activity in this disorder, and suggests a possible signature alteration that could be explored both as a biomarker of treatment success and a predictor of response to treatment.

These findings contribute significantly to the growing body of evidence for involvement of the central nervous system (CNS) in UCPPS. Importantly, as all of these studies are "snapshots" of the brain at one point in time, it remains unclear whether the structural and functional changes are causes or consequences of UCPPS. However, these findings can now be pursued to determine the potential role(s) of these differences in symptom manifestation, maintenance, and amelioration.

### **Biomarkers/Potential Disease Pathways**

Network scientists are pursuing a variety of hypotheses and efforts to identify molecules or biological changes, or “biomarkers,” that are easily detected and consistently associated with some aspect of UCPPS. Moreover, some biomarkers for UCPPS may differ among individuals and thus could potentially distinguish subgroups of people with this condition who may benefit from different therapies. (This approach would be similar to testing for *BRCA1* gene mutations to help in selecting a specific cancer therapy.) In a recent study, scientists at a Phase I Discovery Site investigated certain inflammatory responses as potential indicators of underlying biological processes in UCPPS, and whether they are also associated with differing pain profiles in some people with UCPPS. Inflammation is a bodily process that is normally used to help defend against infection; some typical signs of inflammation are redness, heat, and pain. However, if inflammation is activated inappropriately, people can suffer needlessly from its effects, including pain. In a prior study, the research team had found that there was an association between pelvic pain symptoms in women with IC/BPS and heightened inflammatory responses mediated by two cellular proteins called toll-like receptor (TLR)-4 and TLR-2. Building on these findings, the scientists investigated whether these inflammatory responses could further differentiate between women experiencing pelvic pain and women also reporting widespread pain outside the pelvic area. The magnitude of TLR-mediated inflammatory responses can be detected in a laboratory test using blood samples. Comparing the results of blood sample tests to pain symptom data from participants at their Discovery Site, the scientists found that women with IC/BPS who had a higher than average TLR-4-mediated inflammatory response were significantly more likely to be

reporting pain symptoms outside of, as well as in, the pelvic area. As might be expected from the first finding, TLR-4-mediated inflammatory responses were also higher among women with IC/BPS who had been diagnosed with one or more overlapping pain conditions. While additional studies will be needed in a larger and more diverse sample of women, these findings suggest that the magnitude of TLR-4-mediated inflammatory responses may be a biomarker that can differentiate between subgroups of women diagnosed with IC/BPS in a way that could advance both clinical research and clinical care.

Other findings emerging from Phase I Network studies include insights into the course of UCPPS in women and men; differences between people with UCPPS and healthy controls in microbes associated with the bladder; and identification of clinical characteristics that could help differentiate potentially relevant subgroups among participants with UCPPS.

### **Next Steps**

In light of the progress, novel findings, important new research resources, and resulting new hypotheses developed during Phase I of the MAPP Research Network, the NIDDK decided to support continuation of its efforts. With co-funding from the NIH Office of Research on Women's Health (ORWH), the NIDDK issued a second set of Requests for Applications and in FY 2014 renewed the MAPP Research Network for a second 5 year phase. In Phase II, the Network has been enhanced by the integration of three additional Discovery Sites.

As Network studies move forward, investigators are building upon Phase I discoveries and continuing efforts to provide a foundation for

effective clinical interventions for IC/BPS and CP/CPSP. For example, Network researchers are engaged in a multi-faceted Trans-MAPP Symptom Patterns Study (SPS) designed to better understand symptom change profiles over time and associated biological changes and risk factors. The SPS includes studies to help clarify whether structure and functional changes in the brain are cause or consequence of UCPPS, by looking at participant brain images over time. Other SPS studies include evaluating promising biomarkers/potential mediators identified in Phase I, and pursuing identification of UCPPS patient subgroups defined by differences in clinical symptoms and underlying biological factors. The Network is also implementing the results of the questionnaire analysis described previously to score pain and urinary symptoms separately. To better understand findings from the clinical research, Network researchers will study animal models to examine possible biological mechanisms underlying UCPPS. They will also explore observations made initially in animal models of UCPPS to determine their relevance to humans. Finally, in Phase II the Network is expanding its collaborative efforts to include scientists outside of the Network itself—including other NIDDK-supported research networks—thereby increasing the number of and speed with which critical scientific questions can be pursued, to the ultimate benefit of persons living with or at risk of developing UCPPS.

More information can be found at the MAPP Research Network website:  
[www.mappnetwork.org](http://www.mappnetwork.org)

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## PROSTATE DISEASE RESEARCH

### Benign Prostatic Hyperplasia—Gaining Insight and Potential New Treatment Identified in

**Mice:** Two recent studies have explored the role of fibrosis in benign prostatic hyperplasia (BPH). The prostate is a male gland about the size and shape of a walnut. It surrounds the urethra just below the bladder, where it adds fluid to semen before ejaculation. The prostate gland commonly becomes enlarged as a man ages. This condition is called benign prostatic hyperplasia (BPH) and is caused by the non-cancerous (benign) growth (hyperplasia) of two different cell types—epithelial and smooth muscle—in regions of the prostate called nodules. As the prostate enlarges, it may squeeze the urethra and affect the flow of the urinary stream. The lower urinary tract symptoms (LUTS) associated with the development of BPH rarely occur before age 40, but more than half of men in their sixties, seventies, and eighties have some LUTS. The most common symptoms vary, but 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 formation of fibrosis in the prostate gland contributes to the development of BPH and LUTS. During pathological fibrosis, normal structures are replaced by scar tissue, which is characterized by excess production and deposition of proteins such as collagen—thus, collagen is often used as a marker of fibrosis.

One group of researchers sought to evaluate the contribution of fibrosis, specifically collagen, to the development of BPH in men. Normal prostate tissue was obtained from patients undergoing prostatectomy for surgical management of prostate cancer. BPH

nodule tissue was obtained from patients during prostate resection surgery (a procedure to remove part of the prostate gland in order for urine to more easily flow through the urethra). Total collagen content was determined to be similar between normal and BPH tissue samples. However, a significant increase in a type of collagen pattern called “thicker collagen bundles” was noted in BPH tissue compared to normal tissue—suggesting that these bundles may play a role in BPH. Medical management of LUTS due to BPH includes the use of prescription medications called  $\alpha$ -blockers (e.g., tamsulosin) and 5 $\alpha$ -reductase inhibitors (e.g., finasteride). Collagen levels in BPH prostate samples obtained from patients treated with either  $\alpha$ -blockers or 5 $\alpha$ -reductase inhibitors were compared to BPH prostate samples from patients not treated with medications. Collagen levels in tissue samples were found to be similar in medication-treated men compared to men not taking either of the two medications, suggesting that these medications do not lead to a decrease in fibrosis within the prostate.

Using a mouse model of bacterial-induced prostate inflammation, a second group of investigators examined the reversibility of fibrosis after eliminating the infection and inflammation with an antibiotic treatment. In this model, uropathogenic *Escherichia coli* (*E. coli*) bacteria (a leading cause of urinary tract infections) are placed in the urethra in close proximity to the ejaculatory ducts of the prostate, whereas saline is placed in the urethra in close proximity to the ejaculatory ducts of mice that serve as controls. Mice whose prostates were infiltrated with *E. coli* produced more collagen in their prostates compared to control animals. The investigators further showed that antibiotic treatment completely eliminated the bacterial infection and partly reduced the accompanying collagen buildup in the prostate.

These studies provide new information on the potential role of fibrosis in BPH. A better understanding of fibrosis, in general, could yield insights into how this process unfolds in other tissues (e.g., kidney), potentially opening new avenues to therapy for a range of conditions and diseases.

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## BLOOD CELL DEVELOPMENT AND SURVIVAL

**Elegant Study Documents How Hematopoietic (Blood) Stem Cells Find Their Niche:** A new study extends our understanding of how the hematopoietic stem cell (HSC) microenvironment, or “niche,” promotes the survival and function of these cells. HSCs, a type of stem cell, are able both to self-renew and to develop into any kind of blood cell. Immature HSCs arise from cells in a vascular structure called the dorsal aorta and then travel to the intermediate site before arriving at their final location in the body. In the model organism zebrafish, these sites are the tail and the adult kidney; in mice and other mammals, they are the fetal liver and bone marrow, respectively. The sites in these tissues in which the HSCs reside are termed niches, and the cell types and factors that interact with the immature HSCs have been of interest to investigators. Strategies that promote the interaction between the HSC and its niche may lead to greater numbers of blood stem cells that could be acquired from a blood or tissue

donor, which would be therapeutically advantageous for bone marrow and other transplant procedures.

To further understand how HSCs occupy the niche, researchers studied the intermediate maturation sites of HSCs. Because zebrafish embryos are transparent, the investigators “tagged” HSCs by genetic manipulation to allow live imaging of individual cells taking up residence. The researchers discovered a multi-step, dynamic remodeling process between the HSC and its niche in the zebrafish tail. On arrival, the HSC adheres to the inside of the blood vessel wall. Next, the HSC moves from the inside of the blood vessel wall to the outside of the blood vessel wall. A small group of endothelial cells from the blood vessel wall then surround and form a pocket around the HSC, creating a space for it. A similar process of HSC binding and pocket formation was observed in mouse fetal liver. This suggests that this mechanism may be a common feature of HSC-niche interactions across multiple species.

Further studies in zebrafish showed that the HSC is also either in direct contact or very near to a different type of cell, a mesenchymal stromal cell, in this pocket. The stromal cell seems to help the HSC to divide to form two daughter HSCs. These observations were confirmed using electron microscopy, which offers much higher resolution images than light microscopy.

Current and future research efforts may include the study of the cellular dynamics in the mammalian adult marrow and the identification of small molecules that have the ability to promote critical interactions within the niche and foster an expansion of the HSC population.

Tamplin OJ, Durand EM, Carr LA, ...Zon LI. Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. *Cell* 160: 241-252, 2015.

## HOW CELLS WITH A CHOICE CHOOSE THEIR FATE

**Learning To Leverage the Potential of Pluripotent Stem Cells:** Results from a new study of mouse cells provide insight into the factors that contribute to the behavior of pluripotent stem cells (PSCs). Scientists have developed ways of reprogramming cells, such as those derived from blood or skin, to revert back to an embryonic stem cell-like state. PSCs—including embryonic stem cells and cells experimentally induced to be pluripotent—have the potential to give rise to more stem cells (self-renewal) and to cells of many different types of tissues (pluripotency). Scientists have been grappling with how to determine what controls or drives PSCs to either self-renew or undergo a state of pluripotency, which could lead ultimately to becoming more specialized cells. The ability or knowledge to control the path a PSC takes will allow for more efficient use of this cell in disease treatments or regenerative medicine.

Using an approach called single-cell expression profiling, researchers have begun to understand what cell and tissue types will arise from an individual PSC. In single-cell expression profiling, the “transcriptome” is characterized to provide a measure of a single cell’s gene activity. Active genes produce transcripts, which may have independent functions or may serve as instructions for making proteins. A transcriptome is a collection of all the transcripts present in a given cell. Because of technical limitations, most gene activity studies are performed on cell populations rather than an

individual cell. As the individual cells within a cell population may be quite variable in terms of their respective gene activity, this could have profound implications for understanding biological responses under differing experimental conditions. Using the single-cell expression profiling, the researchers were able to learn that different classes of genes have differing variability in PSCs. For example, “housekeeping” genes, which are required for basic cellular function, showed consistent activity across individual cells. Genes involved in signaling pathways, however, showed more variability. The variability of signaling pathways may be characteristic of the different types of cells that a PSC could become. From the extensive data obtained, the researchers constructed a model that reflected why a PSC would toggle from self-renewal to pluripotency. They identified several regulatory molecules called miRNA-294, miRNA-148, and Let-7—some of which drive a cell toward pluripotency and potentially specialization, and others toward self-renewal as stem cells.

The ability to analyze the transcriptome at the single cell level has allowed investigators to begin to identify regulatory circuits governing transitions between pluripotent and self-renewal cell states. This foundational information is critical to developing strategies to “program” PSCs to address needs associated with disease and injury.

*Kumar RM, Cahan P, Shalek AK,...Collins JJ. Deconstructing transcriptional heterogeneity in pluripotent stem cells. Nature 516: 56-61, 2014.*

# Erythroferrone – New Regulator of Iron Balance

The proper maintenance of blood iron levels is complex, and multiple diseases can result when iron balance goes awry. Hepcidin, a small protein made in the liver, has been previously shown to play a key role in preserving proper iron balance. Critically important to the overall understanding of iron balance is the recent discovery of erythroferrone—with potential implications for treating multiple blood disorders and diseases.

Iron is essential to the body's oxygen-delivery system. Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. Most of the 3 to 4 grams (0.1 to 0.14 ounces) of iron in adults is in hemoglobin. Much of the remaining iron is stored in the liver, spleen, and bone marrow. Because excess iron damages tissues, total body iron is carefully regulated, with most of it being constantly recycled. While small amounts are absorbed daily via the digestive tract, about 10 times more iron is simply retrieved from aged red blood cells and reused. A protein called transferrin picks up this iron, along with dietary iron that has been absorbed via the digestive tract. Transferrin then carries the iron to the bone marrow, where it is used to produce new red blood cells. Unfortunately, the human body does not seem to have an efficient or regulated way to rid itself of excess iron.

If insufficient iron flows to the bone marrow, normal red blood cell production drops and anemia can result. Thus, an important, and the most common, cause of anemia is iron deficiency, which can be corrected through administration of iron supplements. Another form of anemia, however, is associated with inflammation. Called the anemia of inflammation and chronic diseases, this condition affects people who have infections, chronic inflammatory disorders—such as rheumatoid arthritis—and many other chronic disorders, including cancers. Patients with this form of anemia typically have inadequate red blood cell production, low levels of iron in the blood, and low levels of transferrin; they may also be resistant to the effects of erythropoietin, the hormone that normally stimulates and regulates red blood cell production.

Patients with anemia of inflammation and chronic diseases are usually not iron-deficient. Instead, the iron balance in their bodies has been altered, such that more iron is sequestered in the cells involved in iron recycling and absorption, as well as in liver cells that store iron. The cellular sequestration of iron leaves less available for transport to the bone marrow. Attempts to treat this condition with oral iron supplements typically do not work (a condition referred to as iron-refractory anemia), even though this form of anemia mimics iron deficiency.

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Anemia of inflammation generally improves if the underlying condition resolves.

An additional form of anemia can arise in people with a condition called thalassemia. Characterized by the under-production of normal hemoglobin, some people with thalassemia are treated with blood transfusions to provide much-needed red blood cells. While improving anemia, these transfusions can also lead to iron overload.

## Regulation of Systemic Iron Levels by Hepcidin

NIH-supported research showed that hepcidin, a small protein produced in the liver, is the master regulator of iron absorption and tissue distribution. Hepcidin was identified in 1998 in a search for small molecules active in “innate immunity,” the body’s first line of defense against invading bacteria, fungi, and other microorganisms.

In 2001, a research team in France found that when the levels of hepcidin were disrupted in mice, the animals developed iron overload, while mice that were genetically altered to “turn on” the *Hepcidin* gene to a higher level than normal were severely anemic and died within hours of birth. In 2002, while studying abnormally high iron storage levels in the liver, using a mouse model of the most common form of inherited iron overload (hereditary hemochromatosis), researchers found that the *Hepcidin* gene was turned off to

a greater extent in the mice with the excess liver iron compared to normal mice. When rats were fed an iron-abundant diet and then switched to an iron-deficient diet, investigators reported that the *Hepcidin* gene was significantly turned off in the liver while genes encoding iron transporters were significantly turned on in the digestive tract.

From this research, hepcidin emerged as a fundamental regulator of iron balance that inhibits iron absorption and iron release from tissue stores when iron levels in the blood are high, and eases off when blood iron levels decline. When the *Hepcidin* gene was always turned on, the iron accumulation normally seen in two different mouse models of iron overload was prevented. Mechanistically, hepcidin was shown to bind to the iron transport protein ferroportin and induce its destruction, thereby leading to both decreased iron absorption and release of iron into the blood. In a proof of principle of its systemic action, investigators observed a significant decrease in blood iron levels within an hour when mice were injected with hepcidin. Thus far shown to detect elevated or diminished human hepcidin protein levels in a spectrum of human diseases and conditions, a clinical assay to standardize measurement of human hepcidin is under development for commercial release to the clinical community. NIH investments in discovery research have provided a clear understanding of the role of hepcidin in normal physiology and its role in certain disease conditions.

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## Discovery of Erythroferrone as New Regulator of Iron Balance

During times of acute blood loss, there is an immediate need for the bone marrow to produce new blood cells, including red blood cells (RBCs), to replenish lost cells. Newly made RBCs demand an ample supply of iron—a component of hemoglobin. Erythropoietin drives RBC production within a few hours following blood loss, and this process continues for several days. Just how the body suppresses the action of hepcidin to allow increased iron absorption and mobilization from stores has been unclear. Recent NIH-supported research has been instrumental in identifying a key factor responsible for controlling the supply of iron needed for RBC production.

Research conducted in the mid-2000s strongly suggested that the factor responsible for hepcidin suppression during RBC production arose from the bone marrow. This critical piece of the puzzle came from a study in which drugs were used to interfere with RBC production in mice, and then the mice were subjected to blood loss. These animals lost their ability to turn off the *Hepcidin* gene, in contrast to animals not treated with these drugs.

NIDDK-supported scientists have conducted seminal studies designed to identify and characterize the factor emanating from the bone marrow that regulates hepcidin. For these studies, they used an animal model (male mice). They found that the time needed for erythropoietin administration or blood loss to significantly turn off the *Hepcidin* gene in liver or decrease hepcidin

protein blood levels was between 4 and 9 hours. Thus, the unknown hepcidin suppressor must be produced in the bone marrow within the first 4 hours following erythropoietin administration or blood loss.

While evaluating the set of bone marrow genes turned on in mice subjected to blood loss, a previously uncharacterized gene was identified that was turned on within 4 hours of blood loss and was predicted to encode for a secreted protein. In this case, the protein must be able to exit the bone marrow cell and travel to the liver to exert its anti-hepcidin activity. This protein was named “erythroferrone” (*Erfe*), as it functions as a link between production of red blood cells (erythrocytes) and the regulation of iron (which has a Latin name of ferrum). Erythropoietin administration in mice was also shown to significantly turn on the *Erfe* gene in bone marrow. The bone marrow cell types responsible for turning on *Erfe* were shown to be the developing RBCs called erythroblasts. Mice genetically engineered to lack a functional *Erfe* gene were incapable of turning off the *Hepcidin* gene. To further confirm that *Erfe* suppressed hepcidin activity, they administered laboratory-made *Erfe* protein to the mice, and found that it turned off the *Hepcidin* gene in liver and reduced hepcidin protein levels in blood. In a mouse model of  $\beta$ -thalassemia that recapitulates features of the human disease, including low levels of hepcidin in blood and iron overload in the liver, the *Erfe* gene was turned on to a significantly higher level in bone marrow compared to normal mice. When the *Erfe* gene was inactivated in the mouse model, hepcidin expression was restored and iron overload in liver

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reduced—providing an initial proof of concept that this pathway may be useful to prevent iron overload in patients with thalassemia.

This groundbreaking study, published in 2014, was the first to demonstrate that *Erfe* is a biologically important regulator of hepcidin, with implications for supplying iron on demand for RBC production during blood loss. In addition, further experiments conducted in this study suggest that *Erfe* may serve as a target for treatment or prevention of iron overload in patients with  $\beta$ -thalassemia.

Building upon the critical finding that *Erfe* plays an important role in hepcidin suppression, NIDDK-supported researchers designed experiments to evaluate whether *Erfe* contributes to recovery from the anemia of inflammation and chronic diseases. Examining male mice with bacterial-induced anemia of inflammation, the researchers found that the *Erfe* gene was significantly turned on in the bone marrow. To gain insight into *Erfe*'s role in responding to bacterial-induced anemia of inflammation, the researchers compared normal mice to genetically engineered mice that could not produce *Erfe* (*Erfe*-deficient). Compared to normal mice, the *Erfe*-deficient mice: 1) exhibited a more severe form of anemia of inflammation (greater loss of hemoglobin), 2) turned on the *Hepcidin* gene to a significantly higher level, 3) were less capable of properly regulating serum hepcidin levels, 4) had significantly lower blood iron levels, and 5) had prolonged production of serum erythropoietin, reflecting the body's attempt to compensate for low hemoglobin levels. In addition, the

*Erfe*-deficient mice produced greater numbers of immature RBCs, as a step toward replenishing mature RBCs. However, these immature RBCs were smaller than those produced by the normal mice, because less iron was available for blood cell use in the *Erfe*-deficient mice. Taken together, this set of experiments highlights the important contribution *Erfe* makes to the recovery of anemia of inflammation by turning down hepcidin and increasing blood iron levels.

## Looking to the Future

As described in this story, knowledge gained from studying hepcidin has led to the identification of *Erfe* as a new regulator of iron balance in mammals. Future studies will determine how *Erfe* exerts its mechanism of action in liver to suppress hepcidin. If *Erfe* functions similarly in humans, then future studies may lead to potential therapies targeting *Erfe* or hepcidin for disorders of iron balance—absorption, storage, and mobilization.

The NIDDK continues to support a robust portfolio of research projects designed to shed new light on iron homeostasis—the body's establishment and maintenance of iron balance. For example, investigators are exploring various strategies for increasing hepcidin blood levels and bolstering the effectiveness of chelation therapy for iron overload. Other investigators, funded via the Stimulating Hematology Investigation: New Endeavors (SHINE) program, are examining the role of transferrin in red blood cell production and iron balance, and mouse models of iron recycling.

# A Trip to the Emergency Room Prompts One Man To Join a Clinical Trial for the Treatment of Kidney Stones



**Bob Schwarz**

The weather in Washington, DC, was crisp the December afternoon in 2014 when Bob Schwarz walked back to his office after having lunch with a friend. Bob had a long career working with the Peter Pan bus lines, overseeing the company's legislative interests, customer service, marketing, and real estate for 26 years. In his time with the family-owned business, he'd met five U.S. Presidents and been invited to the White House.

Now 67, Bob continues his work in the area of transportation as a government affairs representative for Greyhound Bus Lines and the American Highway Users Alliance. Although he works in Washington, Bob considers

Wilbraham, MA, "home," as does his wife of 27 years, who lives there year round.

As Bob settled back into his office and surveyed the stacks of papers on his desk, he was struck with a sharp, shooting pain in his lower back. "It was unlike anything I had ever experienced before. It felt like someone was putting a hot poker right into my back," he says. Although intense, the pain was also fleeting, leaving just 5 minutes after it arrived.

Bob was working late that evening, and around 8 p.m. his back pain returned, though this time it was worse and accompanied by abdominal pain. "It felt like someone had kicked me and totally knocked the wind out of me," he says. The pain was so intense he walked over to the sofa in his office to lie down, hoping that this second round of pain would pass as quickly as the first. A colleague, concerned about Bob's obvious discomfort, suggested that he go to the emergency room, but Bob demurred, describing himself as "stubborn." Instead, he placed a call to his primary care physician's office in Massachusetts. After hearing his symptoms, Bob's doctor said, "I think what you're describing is a kidney stone." Bob decided that he would wait until the next morning to go to the emergency room. After all, he describes himself as having been "blessed with very good

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health" throughout his life, only missing a single day of work in his 40-year career due to illness. Certainly, whatever he was experiencing could wait until the next day. How bad could a kidney stone possibly be?

Reflecting on whether it was a good idea to wait until the next morning before seeking medical treatment for his suspected kidney stone, Bob notes dryly, "Knowing now what I didn't know then, I certainly would not have done that."

## The Urinary Tract and Kidney Stones

If the circulatory system can be thought of as the body's plumbing, the urinary tract represents the body's waste management system. It consists of two kidneys, which filter the blood to remove waste, salts, and excess fluid; the ureters, the two tubes that connect the kidneys to the bladder that stores urine; and a urethra, through which the urine in the bladder is excreted from the body. A kidney stone is a crystal that forms when substances in the urine become highly concentrated and can no longer stay dissolved. Once formed, a stone may remain in the kidney or travel through the urinary tract and be passed out of the body in the urine.

Kidney stones vary in size, but the severity of symptoms is influenced by factors other than simply the size of the stone. Some people who

have small kidney stones may pass them relatively easily and have mild or no symptoms at all. Larger stones can sometimes also pass with little difficulty, if they are smooth and rounded. However, if stones are irregularly shaped or have sharp edges, even small ones can cause great pain, because they can irritate or lodge in the ureter. In severe cases, where the stone cannot be passed, approaches including shockwave therapy to break up the stone into smaller pieces or surgery to remove it may be required.

## The Next Morning

A neighbor accompanied Bob to the emergency room at George Washington University Hospital (GW) the next morning. Once there, the staff took his medical history and questioned him about his symptoms. They determined that he was dehydrated, and started an intravenous drip that included fluids and pain-relieving medications. An abdominal scan revealed a relatively small stone, approximately 3 millimeters, or about one-tenth of an inch, in diameter. As Bob confirms, even a stone of this size can cause "excruciating" pain.

At this point, he was approached by the patient coordinator for a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-supported clinical study of treatment for kidney stones that was underway at GW. This trial was testing whether

***Reflecting on whether it was a good idea to wait until the next morning before seeking medical treatment for his suspected kidney stone, Bob notes dryly, "Knowing now what I didn't know then, I certainly would not have done that."***

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a drug could help stones pass more quickly and/or with less pain; would he be willing to participate? Bob didn't need to think long about his reply. "If I can do something to help future people with kidney stones not go through what I went through, let's do it," he said. With that decision, Bob became a volunteer in the STONE clinical trial.

## The STONE Trial

Study Tamsulosin for urolithiasis in the Emergency department (STONE) is an ongoing, multi-center clinical trial of the drug tamsulosin to treat kidney stones. STONE expects to enroll 500 people through four participating hospitals—the George Washington University Hospital (in Washington, DC), the University of Pittsburgh, the Thomas Jefferson Hospital (in Philadelphia, PA) and the University of Alabama at Birmingham—who come to the emergency room with symptoms of a kidney stone. If the presence of a stone is confirmed via an abdominal scan, patients are asked whether they would like to enroll in the study.

Tamsulosin (which is marketed under the brand name Flomax® and also is available in generic form) is already approved for the treatment of difficult urination caused by an enlarged prostate in men. It is a member of a class of drugs known as "alpha blockers." This medication relaxes the muscles in the bladder neck, the prostate gland, and the ureter, making it easier for urine to pass. Given these effects, many researchers and physicians think that this drug might also increase the rate at which people with kidney stones might successfully pass their stones.

Over the past 10 years or so, several trials have examined the usefulness of this drug in the treatment of kidney stones, with varying results. Many of these studies, however, were small or had other limitations. The STONE clinical trial is larger and more robust than these earlier studies. It is a randomized and placebo-controlled study, meaning that patients are randomly assigned to receive either a placebo (sugar pill) or tamsulosin. Additionally, it is a double-blind trial, meaning that neither the researchers who are examining the patients nor the study participants themselves will know into which arm of the study the patients have been enrolled until the study ends. Furthermore, STONE is testing the effectiveness of tamsulosin in both men and women who have kidney stones. This is important, because tamsulosin has historically been used to treat difficult urination in men with enlarged prostates, and has not been widely studied in women.

## Enrollment, Follow-up, and Resolution

After Bob agreed to enroll in the STONE study, he was given medications and sent home. As with all volunteers, follow-up consisted of contact via telephone, email, or text message several times over the next 29 days, when the participants are asked whether or not they have passed their stone and whether they have experienced any complications or side effects, such as additional medical visits related to their stone, urinary tract infections, dizziness, or headaches. A subsequent abdominal scan is scheduled on or around day 29 to determine definitively whether the stone has passed. A final follow-up conversation takes place on day 90.

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Bob was conscientious about his participation in the study. The day-29 scan at GW revealed no sign of a stone in his urinary tract, and he was declared stone-free. Bob reports that he has felt fine ever since his participation in the trial ended. Moving forward, he has been advised to drink more water and cut back on coffee, in order to stay more hydrated and decrease the risk of a subsequent stone.

Because STONE is not yet complete, as of November 2015, neither Bob nor his doctor know whether he was given the placebo or tamsulosin. If, at the end of the trial, tamsulosin is shown to be effective, more widespread use of the drug in treating kidney stones could result in a significant improvement in the quality of life for patients with stones by reducing pain, shortening the time taken to expel the stone, and potentially decreasing the number of patients who require surgery to remove their stones. It could also reduce the so-called "indirect" (i.e., non-medical) costs related to kidney stones, such as days lost from work waiting for the stone to pass. If tamsulosin is shown to be not effective, then patients could be saved the unwanted side effects of the medication, and health care dollars would be saved.

Because Bob splits his time between Washington and Massachusetts, he admits to feeling a bit like

"a stranger in a community" when his kidney stone developed. He credits the staff at GW for helping him navigate his diagnosis and treatment, saying that they were welcoming and efficient, even working around his schedule when he had an early morning meeting on Capitol Hill the same day as his day-29 scan. "I

***"If I can do something to help future people with kidney stones not go through what I went through, let's do it," he said. With that decision, Bob became a volunteer in the STONE clinical trial.***

can't say enough about the follow-up of staff at the hospital," Bob says, noting that he especially appreciated the team "just being there when I needed them. They treated me like a platinum card carrier." Six months after his kidney stone, Bob happily reported that he had not suffered a recurrence of a stone...and that he had still not taken a sick day off from work.

*In addition to this trial, the NIDDK supports a broad range of studies in urology, including basic research urinary tract biology and clinical trials of novel therapies. The NIDDK supports a George M. O'Brien Urology Cooperative Research Center Program to improve stone disease treatment and a Rare Kidney Stone Consortium. To discuss and prioritize research needs and identify strategies to address those needs, the NIDDK held a workshop titled "Urinary Stone Disease Research Challenges and Opportunities" on April 1-2, 2015, and intends to fund a Urinary Stone Disease Research Network in 2016.*