

Glomeruli are clusters of small, looping blood vessels in the kidney that perform the first step in the body's removal of waste products, salts, and excess fluid from the circulation while retaining red blood cells and blood-borne proteins. Each kidney contains approximately one million of these tiny filtering units. This image shows a group of glomeruli and the larger vessels that deliver blood to them.

*Photo credit: Susumu Nishinaga/Science Photo Library*

# Kidney, Urologic, and Hematologic Diseases

**D**iseases 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 disorders 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 to 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 23 million Americans 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 2010, nearly 600,000 patients received treatment for ESRD: over 400,000 received either hemodialysis or peritoneal dialysis and almost 180,000 were living with a kidney transplant. Minority populations, 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 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. In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in 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

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<sup>1</sup> Levey AS, et al. *Ann Intern Med* 150: 604-612, 2009.

<sup>2</sup> U.S. Renal Data System, *USRDS 2012 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, 2012.

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 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.

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, and urinary tract infections. As described in this chapter, NIDDK-supported research has identified a potential new treatment approach for urinary tract infections. Other disorders of the genitourinary tract, such as interstitial cystitis/painful bladder syndrome (IC/PBS) in women and men and chronic prostatitis/chronic pelvic pain syndrome in men, are also important components of the NIDDK's urology program. Recent research in an animal model has demonstrated that sex differences in pelvic pain exist. Additional areas of interest include research on treatments for kidney stones, such as shock-wave and laser lithotripsy to break up stones, and therapeutic approaches to inhibit their formation and growth.

IC/PBS is a debilitating, chronic, and painful urologic disorder. Based on a recent large national interview survey, it is estimated that 3.3 million (2.7 percent)

U.S. women 18 years old or older have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/PBS.<sup>3</sup> Using a community-based epidemiological survey, researchers have estimated that 1.6 million (1.3 percent) U.S. men ages 30 to 79 years old have persistent 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/PBS is focused on elucidating the causes of these conditions, identifying "biomarkers" that will aid diagnosis, and improving treatment and interventions. Ongoing epidemiologic studies will help refine prevalence estimates and demographics. These include the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research network, which supports research designed to uncover the underlying causes of IC/PBS and to characterize the disease profiles in patients. A profile of a patient who is participating in the MAPP study appears in this chapter. Other studies include the Boston Area Community Health Survey (BACH), which seeks to identify patterns and risk factors for a range of urological problems, and the Olmsted County (Minnesota) Study, which is studying lower urinary tract symptoms in men.

Urinary incontinence is conservatively estimated to affect 13 million Americans, most of them 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 the effect of different diagnostic tools and interventions on patient outcomes.

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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 07-5512, 2007*

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and chronic disease. NIDDK-supported research has recently identified a potential new approach to mitigate the severity of two serious forms of anemia in an experimental model.

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. Studies have identified a protein involved in aging of blood stem cells. An additional priority of the NIDDK's hematology research program is the development of improved iron-chelating drugs to reduce the toxic iron burden in people who receive multiple blood transfusions for the treatment of diseases.

## **CHRONIC KIDNEY DISEASE**

### **A Better Way To Estimate Kidney Function:**

Researchers have recently shown that measuring creatinine and cystatin C—two markers for chronic kidney disease (CKD)—more precisely estimates kidney function than measuring either marker alone. Creatinine is a waste product from protein in the diet and the normal breakdown of muscle tissue. Cystatin C is released by cells throughout the body. Normally, the kidneys remove both creatinine and cystatin C from the blood, and they are excreted in the urine. As kidney disease progresses, however, the kidneys do this job less well, leading to increased levels of creatinine and cystatin C in the blood.

Within the kidney, the glomerulus performs the task of filtering waste products and excess salts and fluid from the blood. The “glomerular filtration rate,” or GFR, is a measure of the kidneys' capacity to filter the blood. However, GFR is rarely measured outside of a research setting. It is most commonly

estimated using a mathematical equation that incorporates, among other factors, an appropriate biomarker, usually the level of creatinine in the blood. Physicians and scientists have long known that the method of estimating GFR by measuring creatinine alone is imprecise, because creatinine levels can vary among individuals due to factors that are not related to kidney function, such as differences in muscle mass, malnutrition, or chronic illness. This imprecision can have negative consequences, such as incorrectly classifying patients as having CKD when they may not, leading to unnecessary treatment of healthy individuals. It can also fail to detect the decreased kidney function in patients who do have CKD and who would benefit from treatment. The new study found that using a revised calculation that incorporates both creatinine and cystatin C levels produced more accurate estimates of GFR over a broader range of kidney function and body size than estimates utilizing creatinine alone, and was less likely to be altered by other medical conditions. This research was conducted as part of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study, which estimated kidney function in a diverse group of over 5,000 people from 13 studies.

Estimated GFR is routinely reported with the results of the most commonly ordered clinical blood tests. Thus, this CKD-EPI study may have important implications for routine medical care for adults. Furthermore, because there is no effective treatment to restore kidney function once it is lost, prevention and early detection of kidney disease are critically important approaches to prevent kidney failure. These results are an important step towards improving the certainty of CKD diagnosis.

*Inker LA, Schmid CH, Tighiouart H, et al. Estimating Glomerular filtration rate from serum creatinine and cystatin C. New Engl J Med 367: 20-29, 2012.*

### **Health Risks from Chronic Kidney Disease**

**Independent of Diabetes:** A meta-analysis of over 40 studies that enrolled over 1 million patients found that the presence of chronic kidney disease increases the risk of cardiovascular disease and death, and

that similar outcomes were seen in patients with and without diabetes.

There are approximately 23 million Americans with chronic kidney disease, and worldwide prevalence has been estimated to be between 10 and 16 percent of all adults. The most common cause of chronic kidney disease is diabetes, and the most common cause of death in patients with chronic kidney disease is cardiovascular disease. The current study examined patient data from 30 studies of the general population and high-risk patients and 13 studies of patients with chronic kidney disease in order to determine the risk of progression to kidney failure or cardiovascular disease and death in patients with chronic kidney disease. Specifically, the researchers were interested in learning whether the presence of diabetes in these patients had an impact on the likelihood of progression to kidney failure, cardiovascular disease, or death. When comparing patients with and without diabetes who had the same level of estimated kidney function, the risk of kidney failure, cardiovascular disease, and death were much the same in both groups.

Although patients with diabetes have an increased risk for cardiovascular disease and kidney failure, this study found that the relative risks of these outcomes were much the same irrespective of the presence or absence of diabetes when kidney function was included in the analysis. This observation highlights the association of poorer outcomes with reduction in kidney function and underscore the important role of kidney health as a predictor of outcomes independent of diabetes status.

*Fox CS, Matsushita K, Woodward M, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 380: 1662-1673, 2012.*

## **GENETIC VARIATION AND KIDNEY DISEASE**

**Genetic Variants Linked to Earlier Kidney Failure in African Americans:** Scientists have identified an association between variants in the *APOL1* gene and two measures of the severity of kidney disease in

African Americans. In two separate studies, they found that *APOL1* gene variants are associated with the rate of decline in kidney function in African Americans and also with the age at which individuals begin hemodialysis to treat kidney failure.

These studies build on information learned over the past few years about the contribution of genetic factors to the increased risk of kidney disease in this population. Previous studies have shown that African Americans with two copies of certain variants of the *APOL1* gene are at increased risk of developing kidney disease. Indeed, these two variants, termed G1 and G2, are believed to explain much of the increased risk of non-diabetic kidney disease in this population.

One study examined biosamples from participants in the African American Study of Kidney Disease and Hypertension (AASK). The AASK study enrolled African American patients with mild kidney disease due to hypertension and found that treatment with an angiotensin-converting enzyme inhibitor was better than two other drug options at slowing kidney disease progression. The investigators asked whether *APOL1* and other gene variants were associated with an increased risk of worsening kidney disease in 700 AASK participants. They analyzed archived DNA samples and found that the presence of the G1 variant of the *APOL1* gene was associated with a faster decline of kidney function compared to study participants without this variant.

Another study examined over 400 African Americans with kidney failure and asked whether the presence of 1 or 2 copies of either *APOL1* variant was associated with a younger age at which the participants began hemodialysis, a therapy used to cleanse the blood of waste products and excess fluids and salts when the kidneys no longer function. The researchers found that African Americans with two copies of the G1 variant began hemodialysis at a significantly younger age (approximately 49 years old) than those with 1 copy of the variant (about 56 years old). People with 2 normal copies of the *APOL1* gene began hemodialysis at around 62 years of age.

These findings have important implications for understanding the differences in kidney disease progression across different populations. They also suggest the *APOL1* G1 variants may be a marker of an increased rate of kidney disease progression in African Americans. Therefore, early interventions to prevent progression of chronic kidney disease in this high-risk population may be of particular benefit.

Kanji Z, Powe CE, Wenger JB, et al. Genetic variation in *APOL1* associates with younger age at hemodialysis initiation. *J Am Soc Nephrol* 22: 2091-2097, 2011.

Lipkowitz MS, Freedman BI, Langefeld CD, et al. Apolipoprotein L1 gene variants associate with hypertension-attributed nephropathy and the rate of kidney function decline in African Americans. *Kidney Int*: 83: 114-120, 2013.

**Gene Mutations Linked to Hypertension:** Scientists have identified mutations in two genes that play a role in the regulation of blood pressure and salt balance in a rare, heritable disease that causes high blood pressure (hypertension), a leading cause of chronic kidney disease and kidney failure. The researchers were studying a rare, inherited form of hypertension called pseudohypoaldosteronism type II, or PHAII. They analyzed genetic samples from 41 families with PHAII and identified mutations in either the *KLHL3* or *CUL3* genes in most. The proteins encoded by these genes, *KLHL3* and *CUL3*, come together in kidney cells as part of a complex that targets other proteins for breakdown and recycling or other processes. The scientists hypothesize that these mutations inhibit this function of *KLHL3* and *CUL3*, thereby disrupting normal cellular processes. The researchers also noted that the hypertension associated with PHAII can be successfully treated with drugs that promote increased fluid excretion by acting on a protein that regulates the absorption of sodium and chloride in the kidney—and that the *KLHL3* and *CUL3* genes are expressed in the same region of the kidney as the drug-regulated protein.

This finding suggests that *KLHL3* and *CUL3* may be involved in helping to maintain fluid and salt balance by regulating how much fluid is retained by the kidneys'

filtering system and how much is excreted in urine. A fuller understanding of the roles these proteins play in blood pressure maintenance and fluid and salt regulation in PHAII will provide further insight into the regulation of this delicate balance in these patients. It may also identify novel targets for the treatment of hypertension arising from other causes in the general population.

Boyden LM, Choi M, Choate KA, et al. Mutations in *kelch-like 3* and *cullin 3* cause hypertension and electrolyte abnormalities. *Nature* 482: 98-102, 2012.

## GENE REGULATION AND KIDNEY DEVELOPMENT AND MAINTENANCE

**New Insights into Kidney Formation:** New research indicates that the protein called Dicer plays an important role in kidney development by regulating micro-ribonucleic acid (miRNAs). The discovery of molecules known as miRNA has challenged the prevailing scientific thinking about the role of RNA in gene expression, the process by which information stored within DNA is decoded into messenger RNA (mRNA) which in turn is translated into a protein. Different genes produce different mRNAs, which code for different proteins. Both mRNA and miRNA are made up of a string of nucleic acids; however, miRNA is much shorter than mRNA. miRNAs can interact with mRNA to block the ability of mRNA to be translated into protein, thereby adding another level where gene expression can be regulated. Mammalian genomes contain a large and diverse family of miRNAs. It is now believed that miRNAs might affect one-third of all human gene expression.

The protein Dicer plays a key role in processing miRNA from its initial long pre-miRNA form to its shortened, mature form. Scientists sought to determine the role, if any, of Dicer and miRNAs in regulating kidney development. To investigate Dicer's potential role, mice were genetically altered to remove Dicer function from cells that form the nephron and ureteric bud-derived collecting duct system—two compartments of the mammalian kidney. Kidneys were removed prior to or shortly after birth and

evaluated for developmental abnormalities. Lack of Dicer function in cells destined to become part of the nephron led to increased cell death, incomplete nephron formation, and smaller kidneys. Removal of Dicer function from the ureteric bud resulted in the development of kidney cysts (sacks of fluid that replace healthy tissue). In addition, Dicer removal disrupted normal structural features of the ureteric bud.

This study provides evidence that Dicer, and presumably the miRNAs processed by Dicer, have distinct and critical regulatory roles within different components of the developing kidney. Further investigation of the role of miRNAs might shed new light into understanding how the number of functioning nephrons in the kidney is determined and the underlying causes and development of cystic kidney diseases.

*Nagalakshmi VK, Ren Q, Pugh MM, Valerius MT, McMahon AP, and Yu J. Dicer regulates the development of nephrogenic and ureteric compartments in the mammalian kidney. Kidney Int 79:317-330, 2011.*

### **Defective DNA Repair and Chronic Kidney Disease:**

Mutations in a protein that is part of the cellular machinery that helps maintain the genome and repair DNA damage might contribute to chronic kidney disease, as well as to a rare cystic kidney disease called nephronophthisis (NPHP)-like ciliopathy.

In a recent report, scientists identified mutations in the gene that encodes the FAN1 protein as a cause of karyomegalic interstitial nephritis (KIN), a rare and slowly progressive chronic kidney disease marked by fibrosis and cells with massively enlarged cell nuclei. The FAN1 protein is part of a multi-protein complex that works to repair damage to DNA, which is contained in the cell nucleus. Specifically, this complex breaks the inappropriate chemical bonds that sometimes link one chromosome to another. These inter-chromosomal cross-links can form following exposure to toxins, or as a result of normal metabolism and aging. Such cross-links, if not repaired, can cause

enlarged nuclei, prevent gene activation and cell division, and ultimately lead to cell death.

The scientists showed that FAN1 mutants had an impaired ability to remove DNA cross-links in patients with KIN, and suggested that this diminished capacity to repair DNA damage was an important factor in progressive kidney disease. Evidence for the key role of FAN1 was also seen in animal studies. In the zebrafish, lower levels of FAN1 caused a defect similar to KIN, with diminished DNA repair, cell death, and kidney cysts.

Further support for the importance of DNA repair was observed in a well-characterized rat model of kidney disease arising from high blood pressure, in which the extent of DNA damage in the kidney was correlated with kidney failure. This observation supports the hypothesis that faulty DNA repair may be an important underlying cause of kidney disease arising from various causes.

DNA damage has been shown to be associated with exposure to toxins, and the kidney—as an organ whose primary function is to remove waste products from the blood—is likely exposed to levels of toxic agents that are higher than those in most other organs. This study identifies defective repair of DNA damage in the kidney—and the accumulation of this damage, subsequent cell dysfunction, and cell death—as an important cause of fibrosis and subsequent loss of kidney function in patients with KIN. As fibrosis similar to that seen in KIN is a feature seen in chronic kidney disease in general, the role of DNA damage and repair in other forms of kidney disease may prove to have wider applicability. Indeed, future research could explore whether these findings may be relevant to many diseases in which fibrosis is a hallmark.

*Zhou W, Otto EA, Cluckey A, et al. FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. Nat Genet 44: 910-915, 2012.*

## KIDNEY DAMAGE: NEW INSIGHTS INTO INITIATION, NEW TARGETS FOR THERAPY

*Multiple recent studies have provided important insights into the origin of scar tissue that is seen in some forms of kidney disease.*

“Fibrosis”—the term that describes 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—causing acute kidney failure—or from a slowly-progressing, chronic condition. Extensive kidney fibrosis, and the scar tissue that can sometimes arise, can impair the removal of toxins and excess fluid from the blood, cause irreversible organ damage and, in severe cases, lead to kidney failure. New reports shed more light on the origins of kidney fibrosis and identify multiple potential new targets for therapy.

One study focused on molecular regulators of gene expression (the extent to which gene functioning is on or off), and how these regulatory factors might influence the deposition of fibrous tissue following kidney injury. In this study, researchers examined two different mouse models of kidney fibrosis, and sought to identify regulators of gene expression that were elevated in the presence of scarring. The researchers focused on one molecule, microRNA 21 (miR-21) that was found to be highly elevated in two mouse models of kidney disease soon after injury but before fibrosis appeared. This molecule is also found in humans with kidney injury. Mice engineered to lack the miR-21 gene showed diminished fibrosis in response to kidney injury; similar results were observed in normal mice that had been treated with an inhibitor of miR-21. This molecule represents a potential target for antifibrotic therapies in kidney disease.

Another research group identified a cell surface protein, activin-like kinase 3 (Alk3), that is present at elevated levels following kidney injury. Deletion of this protein

in certain areas of the kidney leads to increased fibrosis, suggesting that it plays a protective role in the organ. The scientists developed a small, synthetic protein that bound to and activated Alk3. This agent suppressed inflammation and reversed established fibrosis in five different mouse models of kidney disease. Molecules such as this synthetic protein may be able to treat, and possibly reverse, kidney fibrosis.

Two other studies investigated the role of various cell types within the kidney, and tried to identify the source of the collagen-producing cells that can lead to fibrosis. One focused on pericytes, a type of stem cell that is usually associated with blood vessels, in kidney injury and fibrosis. Previous research indicated that kidney fibrosis appears to arise through a pathway involving cells derived from pericytes. The current study found that kidney pericytes increased their levels of the enzyme ADAMTS1, which plays a role in remodeling the tissue surrounding kidney cells, and downregulated an inhibitor of this enzyme, TIMP3, following kidney injury. Mice engineered to lack TIMP3 were more susceptible to kidney injury-induced fibrosis. Together, these results suggest central roles for regulators of enzymes that can modify networks of blood vessels in the kidney following injury.

In a second project focused on the role of a particular type of kidney cell, scientists used a new mouse model to study acute kidney injury and fibrosis. This study involved selective injury to the proximal convoluted tubules, which are part of the nephron, the basic structural and functional unit of the kidney. These tubules resorb about two-thirds of the fluid generated by the glomeruli, the filtering units within the kidney’s nephrons. After inducing a one-time injury in a specific region of these tubules, the scientists observed the proliferation of tubular cells and the appearance of inflammatory cells. Following this single injury, the kidney recovered completely. However, when the researchers induced three injuries at one-week intervals, they observed diminished cellular repair, with resultant

blood vessel damage and fibrotic damage to both the kidney tubules and the glomeruli. This study shows that repeated injuries, even to only a portion of the nephron, can lead to more widespread kidney damage, similar to that associated with chronic kidney disease.

Researching another form of kidney disease, a team of scientists used computational and systems biology approaches to examine signaling molecules that regulate gene expression in a mouse model of HIV-associated kidney disease. They identified the protein HIPK2 as a key regulator of kidney fibrosis. Levels of this protein were found to be elevated in both the mouse model and in patients with various forms of kidney disease. Deletion of the gene encoding HIPK2 in the mouse model improved kidney function and reduced the severity of fibrosis. HIPK2 may be a potential target for novel therapies to address kidney fibrosis.

These five studies illuminate the complex system of regulation surrounding kidney fibrosis following injury, and identify multiple potential targets for further strategies aimed at preventing and possibly reversing kidney fibrosis, thereby preserving kidney function. Understanding the cellular and molecular mediators of kidney fibrosis is a high priority for scientists studying kidney disease. The identification

of the factors that play a key role in this process might identify new targets for treatment aimed at preventing or reversing fibrosis. Furthermore, a better understanding of fibrosis in general could yield insights into how this process unfolds in other tissues and organs, potentially opening up new avenues to therapy for a range of diseases.

*Chau BN, Xin C, Hartner J, et al. MicroRNA-21 Promotes fibrosis of the kidney by silencing metabolic pathways. Sci Transl Med 4: 121ra18, 2012.*

*Grgic I, Campanholle G, Bijol V, et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. Kidney Int 82: 172-183, 2012.*

*Jin Y, Ratnam K, Chuang PY, et al. A systems approach identifies HIPK2 as a key regulator of kidney fibrosis. Nat Med 18: 580-588, 2012.*

*Schrimpf C, Xin C, Campanholle G, et al. Pericyte TIMP3 and ADAMTS1 modulate vascular stability after kidney injury. J Am Soc Nephrol 23: 868-883, 2012.*

*Sugimoto H, LeBleu VS, Bosukonda D, et al. Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. Nat Med 18: 396-404, 2012.*

## **INSIGHTS INTO INTERSTITIAL CYSTITIS/ PAINFUL BLADDER SYNDROME**

**Sex-specific Differences in Pelvic Pain of Interstitial Cystitis/Painful Bladder Syndrome:** Researchers studying interstitial cystitis/painful bladder syndrome (IC/PBS) in a rodent model have found evidence that females experience greater pelvic pain than males, but that this disparity does not correspond to estrogen levels or differences in bladder injury. The majority of IC/PBS patients are women, and some evidence has suggested a role for the hormone estrogen, the primary female sex hormone, in IC/PBS pain symptoms. Researchers investigated this hypothesis in a specific mouse model of IC/PBS. In this model, irritated nerves release a chemical that activates

inflammatory cells in the bladder. These inflammatory cells, called mast cells, release histamine and other chemicals that inflame the bladder lining and cause pain. This “neurogenic cystitis” model recapitulates the pelvic pain seen in human patients and is also thought to be one possible pathway for how human IC/PBS develops. Using both female and male mice, the researchers induced nerve irritation with a viral infection and then compared female and male mice for potential sex-specific differences in pelvic pain, and also examined the effects of estrogen levels and genetic background. They found that while one genetic strain of mice experienced more pain than another, female mice from either strain experienced greater pelvic pain than male mice. However, when the scientists ablated estrogen production in some of the female mice prior to

the viral infection, they found no significant differences in pelvic pain between those that had normal levels of estrogen and those that did not. Female and male mice with neurogenic cystitis also sustained similar levels of bladder inflammation and injury, making this a less likely explanation for differences in pelvic pain. These findings suggest that, in this rodent model of neurogenic cystitis, sex differences in pelvic pain exist but are not dictated by estrogen; genetic differences play a role in determining susceptibility to pelvic pain; and the two may be related. Further study of sex differences and the role of genetics in pelvic pain could have important implications for understanding IC/PBS pain in people.

*Rudick, CN, Pavlov, VI, Chen, MC, and Klumpp, DJ. Gender specific pelvic pain severity in neurogenic cystitis. JUrol 187: 715-724, 2012.*

### **Clinical Trial Shows Benefit of Specialized Physical Therapy Regimen for Women with Interstitial Cystitis/Painful Bladder Syndrome:**

**Results:** Results from a recent clinical trial suggest that a physical therapy regimen targeting muscle and connective tissue in the pelvic floor, hip, and abdominal areas could help improve symptoms in women with interstitial cystitis/painful bladder syndrome (IC/PBS). In addition to symptoms of pelvic pain, urinary frequency, and/or urinary urgency, many women diagnosed with IC/PBS exhibit tenderness and tension in the muscle and connective tissues surrounding the pelvic area. Previously, a pilot study comparing specialized pelvic floor myofascial physical therapy (MPT) to non-specific, whole-body therapeutic massage among women and men with IC/PBS (in women) or chronic prostatitis/chronic pelvic pain syndrome (in men) had indicated that pelvic MPT might be beneficial specifically for women with IC/PBS. Building on that study, researchers recruited 81 women with IC/PBS of less than 3 years duration for a clinical trial to determine the benefit of pelvic MPT as compared to whole-body therapeutic massage. Participants were randomly assigned to receive up to 10 one-hour sessions of either treatment from a trained physical therapist over the course of 12 weeks. They were then asked to assess overall symptom improvement.

Participants were also asked to rate outcomes for specific symptoms and issues related to their condition. The researchers found that, while both groups reported similar improvements in bladder pain, urinary urgency and frequency, and quality of life, 59 percent of the women in the pelvic MPT group reported that their overall symptoms had moderately or markedly improved compared to when they began treatment, versus only 26 percent in the whole-body therapeutic massage group. Neither group reported a serious adverse event during treatment. With these encouraging results in hand, researchers can now pursue questions such as the durability of treatment effects and which patients are most likely to benefit from treatment, as well as other questions that can help determine whether pelvic MPT could become a standard clinical treatment for women with IC/PBS.

*FitzGerald MP, Payne CK, Lukacz ES, et al. Randomized multicenter clinical trial of myofascial physical therapy in women with interstitial cystitis/painful bladder syndrome and pelvic floor tenderness. JUrol 187: 2113-2118, 2012.*

## **TREATMENT FOR URINARY INCONTINENCE IN WOMEN**

### **Specialized Bladder Tests Before Urinary Incontinence Surgery in Women May Be Unnecessary:**

**Results:** Results from a recent clinical trial suggest that invasive and costly tests commonly performed in women before surgery for stress urinary incontinence (SUI) may not be necessary in many cases. Millions of American women suffer from SUI, in which urine leaks from the bladder through the urethra during a physical stress, such as coughing, laughing, sneezing, or exercise. Treatment for SUI includes surgical procedures to support and compress the urethra to stop urine from leaking. Prior to surgery, many women not only receive an office evaluation to diagnose their incontinence, but also undergo specialized bladder function tests called urodynamic studies. These tests help assess how well the bladder, urethra, and muscles that support and compress the urethra work together to store and release urine. Similar to other office bladder procedures, the urodynamic tests can be uncomfortable or painful, and

can increase risk for urinary tract infections. Although the urodynamic tests were found to refine a doctor's diagnosis, the tests have not been proven to guide decisions about treatments or improve surgical outcomes.

To test whether urodynamic studies influenced the likelihood of treatment success, researchers conducted a study in 630 women who were planning to have surgery for SUI. Participants were women who had SUI that was not complicated by other health factors, such as previous incontinence surgery or pelvic irradiation. Women who also had urge incontinence (urine leakage at the time of a very strong desire to urinate, as opposed to physical stress) were not excluded as long as SUI was their predominant type of incontinence. Women with uncomplicated, predominantly SUI were randomly assigned to receive either (1) both a pre-operative evaluation in a doctor's office and urodynamic tests, or (2) the office evaluation only. One year after the surgical procedure, the researchers assessed treatment success, which was defined as a participant reporting on a questionnaire that her urinary distress had been reduced by 70 percent or more, as well as reporting that her urinary tract condition had improved "much" or "very much." The researchers found that the proportion of women in whom treatment was successful was similar in both groups—76.9 percent versus 77.2 percent in the women who had urodynamic testing and the women who received the office evaluation only, respectively—with no significant differences in quality of life, patient satisfaction, or problems voiding. While urodynamic testing did lead to changes in diagnoses for some of the women, the researchers observed that this did not lead to significant differences in either the selection of surgical treatments or the one-year outcomes between the two groups. These results indicate that, for women with uncomplicated SUI who are receiving care from urologists and gynecologists with advanced training in bladder problems, specialized bladder function tests are not necessary to achieve surgical treatment success—information that women and their physicians can consider in planning treatment.

*Nager CW, Brubaker L, Litman HJ, et al. A randomized trial of urodynamic testing before stress-incontinence surgery. N Engl J Med 366: 1987-1997, 2012.*

## **NEW WAYS TO TREAT URINARY TRACT INFECTIONS**

**Research Yields Potential New Treatment for Urinary Tract Infections:** Researchers have identified novel orally active compounds that appear to block uropathogenic *E. coli* bacteria from binding to bladder cells, thereby preventing new urinary tract infections (UTIs) and mitigating chronic infections in studies in mice. Infections of the urinary tract are common in women—about one-third of all women in the United States are diagnosed with a UTI by the time they reach 24 years of age—and many women experience repeated UTIs. Most UTIs are caused by a common type of *E. coli* bacterium. The outside surfaces of these bacteria contain hair-like projections that are tipped with a sugar-binding protein called FimH. FimH facilitates the binding of bacteria to human or mouse proteins containing mannose, a type of sugar, which are found on the surface of epithelial cells lining the bladder wall. Once attached to bladder cells, the bacteria become resistant to being flushed out by urine and can initiate infection. The bacteria form what is called a biofilm, a well-organized community that adheres to a surface. Bacteria can also invade bladder cells and establish an intracellular reservoir, thus producing a chronic infection. While antibiotic treatments are available, chronic and recurrent UTIs in women have become more challenging due to antibiotic resistance. To circumvent drug-resistant *E. coli*, there is an urgent need for new therapeutics to treat and prevent UTIs.

Because FimH is essential for successful infection by UTI-causing *E. coli*, scientists focused on finding a way to interfere with FimH binding to mannose as a possible new therapeutic strategy. First, they developed a panel of investigational compounds derived from mannose, called mannosides, and tested the ability of these mannose derivatives to block bacterial growth in a laboratory assay designed to mimic a biofilm. They then selected the most promising mannoside compound to evaluate its efficacy in treating and preventing UTI in a mouse model. Four mannoside compounds that were tested blocked biofilm growth when added at an initial stage of biofilm development or were capable of disrupting an established biofilm. The most potent

mannoside was shown not only to reduce bacterial levels in bladders of mice with chronic UTIs, but also to do so more effectively than standard antibiotic treatment. Moreover, when administered prior to exposure to bacteria, the mannoside prevented new UTIs in mice. Like standard antibiotic treatment for UTIs, the mannoside was active when administered orally. Building on these results, the researchers were also able to generate additional, further optimized mannoside compounds as a starting point for new tests.

This promising finding of an alternative approach to UTI treatment emerges from a long-term research investment to understand the virulence and life cycle of uropathogenic *E. coli*, the primary culprit in UTIs in women. Future studies will continue to develop more potent mannosides and to assess these compounds for toxicity prior to their potential testing in humans.

*Cusumano CK, Pinkner JS, Han Z, et al. Treatment and prevention of urinary tract infection with orally active FimH inhibitors. Sci Transl Med 3: 109ra115, 2011.*

## **PREVENTION OF BENIGN PROSTATIC HYPERPLASIA IN MEN**

### **Drug Therapy To Prevent Benign Prostatic**

**Hyperplasia:** The drug finasteride, which inhibits the metabolism of the male sex hormone testosterone and which has been shown to be effective in relieving the symptoms of benign prostatic hyperplasia (BPH), can also reduce the likelihood that otherwise healthy men will develop this condition. While the symptoms of BPH vary, the most common ones involve changes or problems with urination, such as a hesitant, interrupted, or weak stream; urgency and leaking or dribbling; and more frequent urination, especially at night.

Researchers examined patient data from over 9,000 men who were enrolled in the Prostate Cancer Prevention Trial, which collected information on men with BPH and related lower urinary tract symptoms, such as frequent urination, inability to urinate, and urinary tract infections. The average age of the men was 62 years. This retrospective analysis found that men

who had received finasteride, a drug that blocks the conversion of testosterone to a more potent metabolite, had a 40 percent lower rate of BPH development than men who did not. The effect of finasteride did not vary significantly by age, race, diabetes, physical activity, or smoking, suggesting that these results could be applicable to a larger population. BPH rarely causes symptoms before age 40, but more than half of men in their sixties and as many as 90 percent in their seventies and eighties have some symptoms of BPH.

For many years, surgery was the only viable treatment option for BPH. In 2003, the NIDDK-supported Medical Therapy of Prostatic Symptoms (MTOPS) clinical trial conclusively demonstrated that combination therapy consisting of an  $\alpha$  blocker, which relaxes smooth muscle, and finasteride was more effective than either drug alone in relieving the symptoms of BPH. The current study complements these findings, suggesting that finasteride may be an effective preventative therapy in men without overt symptoms of BPH.

*Parsons JK, Schenk JM, Arnold KB, et al. Finasteride reduces the risk of incident clinical benign prostatic hyperplasia. Eur Urol 62: 234-241, 2012.*

## **IRON: A DELICATE BALANCE**

**The Double-edged Sword of Hepcidin:** Approaches to modulate levels of hepcidin may provide benefit to people with either iron overload or iron deficiency. Hepcidin, a peptide hormone produced by the liver, is the master regulator of iron balance in humans and other mammals. Hepcidin inhibits transport of iron from cells by binding to the iron channel, ferroportin, which reduces dietary iron absorption and limits release of iron from cells that store iron, such as macrophages. Insufficient levels of hepcidin cause or contribute to iron overload in  $\beta$ -thalassemia and in hereditary hemochromatosis, while excess levels of hepcidin lead to a decline in blood iron levels, as occurs in the anemia of chronic inflammation. Thus, strategies that increase or decrease the effective level of hepcidin could help treat these diseases.

Researchers recently reported the design of smaller forms of hepcidin and tested the ability of these compounds to mimic hepcidin activity and also treat iron overload in mice. These “mini-hepcidins” contain the segment of the hepcidin protein that interacts with ferroportin, and were found to be resistant to degradation by enzymes in the blood. After injection or oral administration to mice, these “mini-hepcidins” were found to lower serum iron levels as effectively as full-length hepcidin and also to lower liver iron levels significantly in an animal model of iron overload. “Mini-hepcidins” offer certain advantages over full-length hepcidin in that they are less expensive to produce, are more stable, and can be administered orally.

In a separate study, scientists assessed the ability of two small compounds (LDN-193189 and HJV.Fc) to block the production of hepcidin in an animal model of anemia of chronic inflammation. Previous research has demonstrated that both LDN-193189 and HJV.Fc block the action of a protein that signals the hepcidin gene to be expressed. By blocking this protein, called bone morphogenetic protein (BMP), the production of hepcidin was effectively blocked. This study provided compelling evidence that both agents reduced hepcidin levels by blocking BMP, allowing iron to become more available for red blood cell production, thereby ameliorating anemia of chronic inflammation in an experimental animal model.

Past investments in basic science research provided the foundation for these two research studies targeting hepcidin levels. Ongoing studies continue to evaluate these and other promising compounds in order to develop effective treatments for iron-related blood disorders.

*Preza GC, Ruchala P, Pinon R, et al. Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload. J Clin Invest 121: 4880-4888, 2011.*

*Theurl I, Schroll A, Sonnweber T, et al. Pharmacologic inhibition of hepcidin expression reverses anemia of chronic inflammation in rats. Blood 118: 4977-4984, 2011.*

## **GENE REGULATION AND BLOOD CELL FORMATION**

**Blood Cell Formation and Regeneration:** Scientists have recently determined that two biologic pathways, the bone morphogenetic protein (BMP) and Wnt signaling pathways play a dynamic role in blood cell (hematopoietic) regeneration and maturation (differentiation). Following an insult or injury to the blood system, such as rapid blood loss due to a serious injury, the regenerative process stimulates rapid expansion of hematopoietic stem cells followed by differentiation of these cells into red blood cells and other major blood cell types. Both BMP and Wnt signaling pathways contribute to the initial formation of the hematopoietic system during development, but it was not known whether these pathways are also involved in hematopoietic regeneration and differentiation after injury during adulthood.

To evaluate whether BMP and/or Wnt signaling pathways contribute to blood cell regeneration, scientists performed experiments on zebrafish, a model organism. They subjected adult zebrafish to a sub-lethal dose of radiation to destroy their existing blood cells. They then looked for signs of blood cell regeneration by characterizing blood cell populations from zebrafish kidney marrow, which is considered the organ responsible for production of all major blood cell types, analogous to the mammalian bone marrow. The regeneration of blood cells following irradiation of the fish was shown to depend on the activation of both the BMP and Wnt signaling pathways.

The researchers next gained insight into how these signaling pathways promote regeneration of different types of blood cells. Drawing upon previous findings that the BMP and Wnt pathways act by turning genes on or off, the scientists sought to identify other factors involved in this process. Two gene-regulating factors, SMAD1, a member of the BMP signaling family, and TCF7L2, a member of the Wnt signaling family, were found to be associated with genes that play a role in the production of blood cells. Furthermore, when the researchers examined red blood cells grown in the laboratory, these factors were found to be

bound to DNA in close proximity to known “master regulators” GATA1 and GATA2, which are important for directing gene regulation in a particular progenitor cell so that it becomes a red blood cell. Interestingly and importantly, in a white blood cell line, SMAD1 and TCF7L2 were not found to be associated with red blood cell genes in the zebrafish DNA, but rather were associated with white blood cell genes. In these cells, SMAD1 and TCF7L2 were both in close proximity to a different master regulator, C/EBP $\alpha$ —important for directing a particular progenitor cell to become a white blood cell.

This study identifies signaling pathways and factors associated with different master regulators of genes, which direct the regeneration and differentiation of distinct blood cell types in adults following injury.

*Trompouki E, Bowman TV, Lawton LN, et al. Lineage regulators direct BMP and Wnt pathways to cell-specific programs during differentiation and regeneration. Cell 147: 577-589, 2011.*

## **NEW APPROACHES TO TREATING ANEMIA**

### **Nutritional Supplements Can Mitigate the Severity of Two Serious Forms of Anemia in an Experimental Model:**

**Diamond-Blackfan anemia (DBA), an inherited form of bone marrow failure, and myelodysplastic syndrome arising from a deletion of a portion of chromosome 5 [del(5q) MDS], an acquired disease, are both characterized by anemia, or insufficient levels of red blood cells. As these cells carry oxygen from the lungs to all of the body’s organs and tissues, deficient numbers of red blood cells can cause a wide range of serious medical issues. A new study, based on experiments in an animal model and with cultured human cells, suggests that the anemia associated with these disorders can be alleviated with an amino acid supplement.**

Previous studies have indicated that DBA and del(5q) MDS are caused by an insufficient level of one or more of the protein components of ribosomes.

Ribosomes are complex structures of proteins and ribonucleic acids that perform the final step of translating the DNA “blueprint” into proteins that carry out the functions of cells and tissues. Mutations in nine genes that encode ribosomal proteins have been identified in patients with DBA, accounting for about half of the diagnosed cases.

In the current study, researchers modeled DBA and del(5q) MDS in zebrafish using a targeted approach to reduce the levels of two specific ribosomal proteins that have been implicated in these diseases. They observed that, as in humans, diminished levels of these proteins in zebrafish resulted in severe anemia. When the animals were treated with leucine, there was a significant improvement in the animals’ anemia. Similar results were seen in experiments with cultured human blood cells. Leucine is an “essential” amino acid that must be part of the diet because it cannot be synthesized in animals and humans. Leucine has previously been shown to enhance ribosome function in cells, potentially by activating a biologic pathway (known as the mTOR pathway) that integrates multiple signals inside the cell and plays a role in protein synthesis. In this study, the researchers showed that leucine activates this pathway in blood cells, a result that may explain how leucine improved anemia in the zebrafish.

The only true cure for DBA is a bone marrow transplant, which is a very limited treatment approach because suitable bone marrow donors are rarely available and serious risks are associated with the procedure. The novel findings described in this study show that simple nutritional supplementation may reverse many of the manifestations of DBA and del(5q) MDS and provide a rationale for future studies that will explore the potential effectiveness and safety of nutritional leucine supplements to treat patients with these rare disorders.

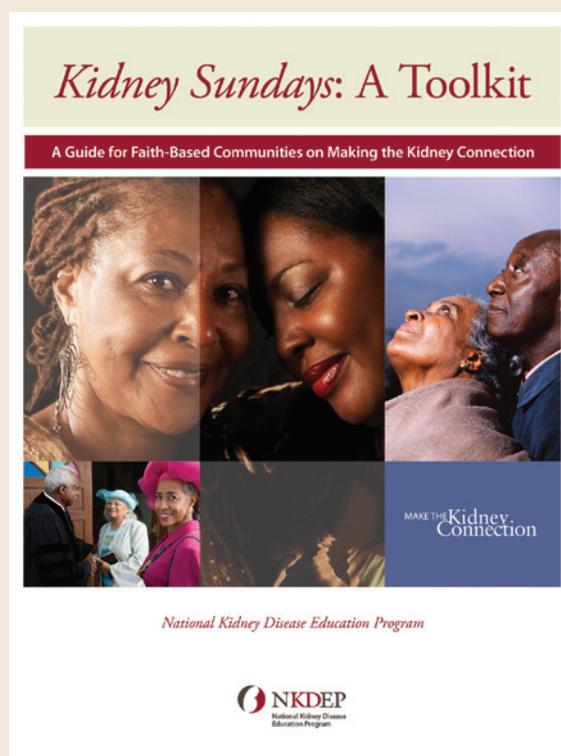
*Payne EM, Virgilio M, Narla A, et al. L-leucine improves the anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q) MDS by activating the mTOR pathway. Blood 120: 2214-2224, 2012.*

# National Kidney Disease Education Program's *Kidney Sundays Initiative* *Raising Awareness of Kidney Disease Risk Factors* *Among African Americans*

According to data from the National Health and Nutrition Examination Survey (NHANES), 16 percent of African Americans may have chronic kidney disease (CKD). African Americans between the ages of 30 and 39 are 11 times more likely than Caucasians to develop hypertension-related kidney failure. Additionally, the same age range of African Americans is almost four times more likely than Caucasians to develop diabetes-related kidney failure.<sup>1</sup> While African Americans made up just 13 percent of the United States population in 2009, they accounted for 32 percent of new cases of kidney failure.<sup>2</sup> In addition to diabetes and hypertension, heart disease and a family history of kidney failure also increase kidney disease risk.

To help African Americans learn about kidney disease and what steps they can take if they have risk factors, the National Kidney Disease Education Program (NKDEP) conducts community outreach and education programs. In recognition of National Kidney Month in March 2012, NKDEP collaborated with the American Diabetes Association's *Live Empowered* program; the National Coalition of Pastors' Spouses (NCPS); and Chi Eta Phi Sorority, Incorporated to kick off the first nationwide *Kidney Sundays* event.

"We want people to take a more active role in protecting their kidneys—and that means if they have diabetes or high blood pressure, making lifestyle changes to manage these diseases," said Griffin P. Rodgers, M.D., M.A.C.P., Director, National Institute of Diabetes and Digestive and Kidney Diseases. "*Kidney Sundays* provided us an opportunity to make sure people know how important staying healthy is and what they can do if they are at risk for kidney disease."



On March 25, 2012, more than 350 African American faith communities from Baltimore to St. Louis, and from Dallas to Los Angeles, used NKDEP's newly revised *Kidney Sundays* Toolkit to help their members learn about the connection between diabetes, high blood pressure, and kidney disease. Thirty-nine of the participating congregations also conducted more than 1,500 blood pressure screenings with Chi Eta Phi nurses on site. The nurses shared kidney health information and encouraged those at risk to have their kidneys tested. In addition, more than 300 NCPS members also shared kidney health information in their churches.

“We still have lots of work ahead of us in terms of educating people on the serious effects of diabetes, obesity, high blood pressure, and eating habits, and how each relates to kidney health,” said NCPS President Vivian Berryhill, of New Philadelphia Baptist Church in Memphis, Tennessee. “Efforts such as *Kidney Sundays* are a great start to help... get the conversations started.”

The *Kidney Sundays* partnership with participating faith organizations helped NKDEP reach an estimated 280,000 individuals through distribution of 50,000 educational pieces, including *Kidney Sundays* Toolkits, brochures, and information cards.

In addition to the faith community engagement, NKDEP also conducted a national media campaign featuring Dr. Rodgers. The campaign included a radio tour with segments on African American stations and shows including the *Tom Joyner Morning Show* and a partnership with *BlackDoctor.org* to host a kidney health chat with Dr. Rodgers on its Facebook page. These efforts, along with a robust social media conversation on the NKDEP *Make the Kidney*

*Connection* Facebook page, garnered 3,667 visitors and 458 material downloads on the NKDEP web-site, and reached more than 60,000 individuals through eNewsletters distributed by NKDEP, the NIDDK and its programs, and partner organizations from January through March 2012.

More information about the NIDDK’s National Kidney Disease Education Program can be found at <http://nkdep.nih.gov>. To learn more about *Kidney Sundays*, visit <http://nkdep.nih.gov/kidneysundays>

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<sup>1</sup> U.S. Renal Data System, *USRDS 2012 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, 2012.

<sup>2</sup> U.S. Renal Data System, *USRDS 2010 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, 2010.

## STORY OF DISCOVERY

### *Small Molecule Stabilizers as a Potential Treatment for Transthyretin Familial Amyloid Polyneuropathy*

Proteins are important building blocks for all body parts, including muscles, bones, hair, and nails, and perform vital biologic functions. Proteins circulate throughout the body in the blood and, when functioning properly, are beneficial. Occasionally, cells produce abnormal proteins that can misfold, form fibrillar deposits or aggregates and cause disease; the exact cause of fibril formation is unknown. When these deposits of abnormal proteins were first discovered, they were called amyloid, and the disease process was called amyloidosis. A common feature is that the deposits share a “ $\beta$ -pleated sheet” structural conformation with characteristic properties detectable by certain color dyes. Diagnosis of the various forms of amyloid disease is confirmed by tissue biopsy. In systemic amyloidosis, proteins produced in one part of the body travel to a different location where they become insoluble and form fibrillar deposits that impair organ function. One such form of systemic amyloid disease is transthyretin (TTR) amyloidosis. Past investments in basic science research have provided the foundation for an exciting small molecule approach to inhibit TTR amyloidosis.

#### **The Blood Transport Protein Transthyretin in Health and Disease**

TTR is a blood transport protein for the hormone thyroxine and the vitamin A-retinol binding protein complex. TTR has two binding sites for thyroxine but only a small percentage, perhaps less than 1 percent, of TTR in blood has thyroxine bound to it. The liver is the main site of TTR synthesis in the body, but

TTR is also made in the retina and pancreas. Both mutant and normal forms of TTR can give rise to amyloid deposits. TTR consists of four identical subunits; scientists refer to the assembled subunits as a tetramer. Dissociation of the tetramer is the rate-limiting step for amyloid fibril formation. Any one of nearly 100 different mutations in the gene encoding TTR can cause amyloidosis. Disease-associated mutations destabilize the tetrameric structure and some increase the rate of tetramer dissociation. Specific clinical syndromes associated with mutated TTR are familial amyloid polyneuropathy (FAP) and familial amyloid cardiomyopathy. Senile systemic amyloidosis is a disorder that occurs in very elderly individuals (mostly men) in which amyloid fibrils formed from the normal TTR protein are deposited primarily in the heart, but also in the gut and in the carpal tunnel space of the wrist.

#### **What is Transthyretin Familial Amyloid Polyneuropathy?**

TTR FAP is a rare, progressive, and ultimately fatal hereditary neurodegenerative disease that affects the nerves and often the heart and kidneys as well. Symptoms include sensory loss, erectile dysfunction, alternating diarrhea and constipation, urinary incontinence, urinary retention, and delayed gastric emptying. TTR FAP is inherited in an autosomal dominant manner. The phrase “autosomal dominant” means that if one parent has the disease, there is a 50 percent chance that the disease gene will pass to a child. A mutation in TTR called “V30M” is the most common cause of FAP. Currently, there are no

# STORY OF DISCOVERY

FDA-approved drugs to treat this rare but serious disease. Liver transplantation is a treatment option, as replacing a liver producing mutant TTR with a liver synthesizing normal TTR can slow, if not halt, disease progression. However, this approach has its limitations, which include matched donor availability, surgery, and a need for long-term immunosuppressive steroid treatment after the transplant.

## **Development and Characterization of TTR Small Molecule Stabilizers**

*NIDDK-supported scientist Dr. Jeffery W. Kelly of the Scripps Research Institute and his collaborators have conducted seminal research studies to design and characterize small molecules that stabilize the native TTR tetrameric structure. Highlights of some of these pre-clinical and clinical studies are presented here.*

In the laboratory, the stability of TTR's tetrameric structure can be assessed by placing it in a chemical known to disrupt or "denature" protein structures. In 1996, a strategy was developed to design an orally bioavailable small molecule stabilizer that binds to TTR in blood with both high affinity and selectivity, and prevents or significantly slows dissociation of the TTR tetramer. In a proof of principal experiment, thyroxine concentrations slightly higher than necessary to occupy all TTR binding sites stabilized both normal and mutant-containing (e.g., V30M) tetramers from dissociation under conditions of denaturation. Unfortunately, thyroxine cannot be used as a stabilizer of tetramer structure—owing to its hormone activity. However, results of this study did provide evidence that a small molecule having a similar structure to thyroxine without hormonal activity but with specific and selective binding affinity might effectively stabilize normal and mutant TTR. A related research study subsequently showed that

occupancy of only one of the two thyroxine binding sites is sufficient to stabilize most TTR tetramers from dissociation under denaturing conditions.

Based on structural similarity to thyroxine, a small molecule called diclofenac was tested for its ability to stabilize TTR under denaturing conditions, and researchers also evaluated a set of 12 similar molecules. These were nonsteroidal anti-inflammatory small molecules (NSAISMs), and in 2002, several, including diclofenac, were shown to stabilize the normal TTR tetramer effectively, but they were less effective at stabilizing mutant TTR tetramers (e.g., V30M). In 2004, diclofenac and several additional NSAISMs were evaluated for their ability to stabilize tetramer structure of the most common disease-associated TTR variants, including V30M. This study demonstrated that the NSAISM diflunisal provided effective stabilization for the majority of the mutant variants and was more effective in this regard than diclofenac. Used as an FDA-approved non-steroidal anti-inflammatory drug for more than 2 decades, diflunisal has been commercially available in over 40 countries, including the United States.

Diflunisal was evaluated further for its ability to selectively bind and stabilize 1) FAP variant TTR in blood samples, and 2) TTR when orally administered to healthy volunteers or patients with FAP. When diflunisal was added to the blood of FAP patients at potentially therapeutic concentrations, mutant V30M TTR tetramers in the blood were significantly stabilized under denaturing conditions, more so than the TTR of healthy volunteers. Already FDA-approved for mild to moderate pain, fever, and inflammation, orally administered diflunisal at 250 mg twice daily for 7 days was shown to stabilize TTR in blood samples obtained from a small pilot study of patients with FAP. Moreover, a second pilot study of orally administered diflunisal at

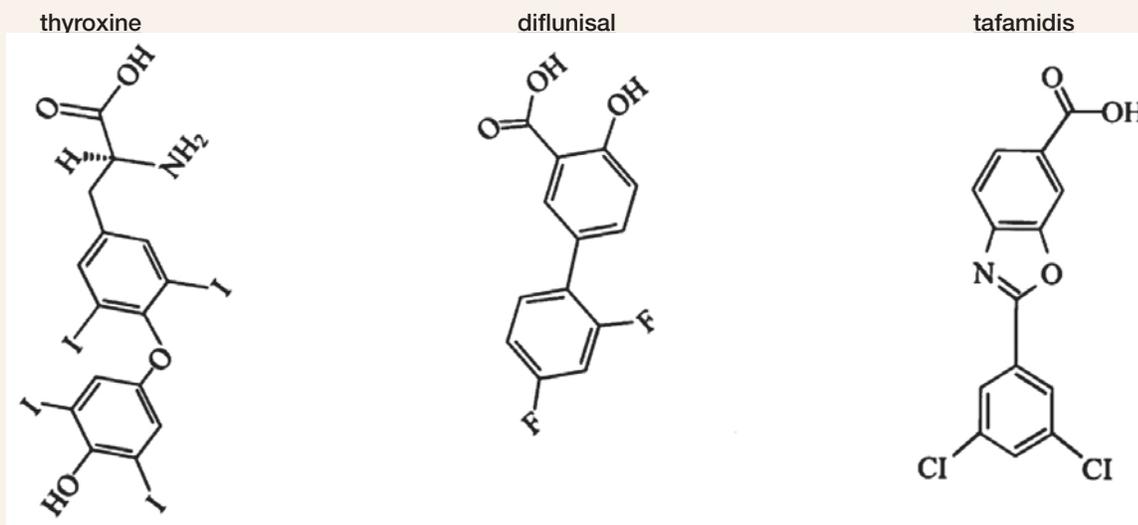
# STORY OF DISCOVERY

250 mg or 500 mg twice daily for 7 days showed that the drug occupied at least 1 of the 2 thyroxine binding sites and stabilized TTR tetramer structures from healthy volunteers. Published in 2006, the results of these two pilot studies suggested that diflunisal might be an effective small molecule therapeutic for treating TTR amyloidosis.

In 2003, a series of compounds called benzoxazoles, which also have a structure similar to thyroxine, were synthesized, and 11 of 28 were shown to effectively stabilize normal TTR tetramers and to prevent amyloid fibril formation under denaturing conditions. One of these analogs, tafamidis, exhibited particularly favorable binding selectivity and stabilization of the normal TTR tetramer. Because of these findings, tafamidis was selected for additional pre-clinical and clinical research studies. As reported in 2012, tafamidis was found to effectively stabilize tetramers of the

most clinically significant mutant form of TTR (V30M) associated with FAP, such that it behaves like normal TTR under denaturing conditions. Occupancy of one of the thyroxine binding sites by tafamidis stabilized 67 percent of normal TTR tetramers from dissociation under denaturing conditions. Increasing tafamidis concentrations such that both thyroxine binding sites were occupied stabilized 97 percent of the TTR tetramers. Consistent with previous results, tafamidis was also shown to selectively bind and stabilize TTR in human blood. When added to blood from patients with FAP at concentrations sufficient to occupy one or both of the thyroxine binding sites, tafamidis was found to significantly stabilize the V30M TTR under denaturing conditions. Furthermore, tafamidis was shown to stabilize a broad range of other pathogenic TTR variants in blood; these TTR variants contained mutations called Y69H, F64S, I84S, L111M, or V122I.

## Thyroxine and Candidate Small Molecule Stabilizers



# STORY OF DISCOVERY

## **Clinical Trial Assessments of Diflunisal and Tafamidis**

These two small molecule stabilizers have now been studied in clinical trials to test their safety and efficacy. Boston University has sponsored a placebo-controlled multicenter phase III clinical trial testing the efficacy of diflunisal for the treatment of FAP, and results are expected to be reported in 2013. This trial, with a target enrollment of 140 participants, has compared 250 mg diflunisal taken orally twice daily with a placebo. Results of a separate clinical trial evaluating tafamidis were reported by the Scripps Research Institute in July 2009. This 18-month phase II/III clinical trial, conducted by a pharmaceutical company co-founded by Dr. Kelly, showed that tafamidis slowed the progression of FAP in patients with the V30M TTR mutation. Further testing is now ongoing to achieve U.S. Food and Drug Administration approval of tafamidis for the treatment of TTR FAP.

## **NIDDK-supported Translational Research**

The translation of scientific knowledge and technology into improvements in the practice of medicine is central to the missions of the NIH and the NIDDK. As this story illustrates, the clinical understanding of the pathological underpinnings of TTR amyloidosis spurred NIDDK support of basic science research that led to the development of small molecule stabilizers that effectively decrease TTR tetrameric dissociation and amyloid fibril formation. Once identified and characterized, these small molecules were well positioned to attract academic and industry interest in conducting clinical trials of these potential therapeutics. The NIDDK continues to be committed to supporting innovative strategies for improving the health of patients with FAP and many other diseases.

## SCIENTIFIC PRESENTATION

### From Genes to Therapies: Platelets at the Center of the Universe

*Dr. Kenneth Kaushansky*

*Dr. Kenneth Kaushansky is Senior Vice President, Health Sciences and Dean, School of Medicine at Stony Brook University. A physician-scientist and leading hematologist, Dr. Kaushansky has conducted groundbreaking research on the molecular biology of blood cell production. His team has discovered several of the genes important in the growth and development of blood cells, including thrombopoietin, a key regulator of stem cell and platelet production. Dr. Kaushansky's laboratory work has led to several significant discoveries, for which he has received the Dameshek Award from the American Society of Hematology, awarded annually to the scientist who has made seminal contributions into the field of hematological disorders, and the Outstanding Investigator Award from the American Society for Medical Research, the most prestigious award of the Society.*

*Dr. Kaushansky earned his B.S. and M.D. degrees from the University of California, Los Angeles, and completed his Internal Medicine Internship, Residency and Chief Medical Residency, and Fellowship in Hematology at the University of Washington. He joined the faculty at the University of Washington as an Assistant Professor in 1987, was promoted to Associate Professor in 1991 and to Professor in 1995. Following his service as Hematology Section Chief at the University of Washington Medical Center, Dr. Kaushansky was named Helen M. Ranney Professor and Chair of the Department of Medicine at University of California, San Diego, in February 2002. In July 2010, Dr. Kaushansky moved to Stony Brook University.*

*Dr. Kaushansky has been an NIH-supported researcher for the past 30 years, including research support from the NIDDK for 27 years. At the September 2012 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Kaushansky gave a presentation on the role of the platelets in bleeding and clot formation.*

Platelets are cell fragments that help blood clot. Too few platelets can lead to spontaneous or uncontrolled bleeding. Too many platelets can trigger a heart attack, stroke, or other arterial thromboses. As such, regulation of the numbers and function of platelets is vital for health—the yin and yang of platelets.

#### **How is Platelet Production Regulated?**

Dr. Kaushansky recounted that, for many years, the primary regulator of blood platelet production remained elusive. Then, in the late 1980s and early 1990s, a French research group reported that a gene, *v-mpl*, from the murine (mouse) myeloproliferative leukemia virus (MPLV) had the ability to transform, or “immortalize,” a variety of early stage blood cells. The protein encoded by *v-mpl* was shown to be very similar (homologous) to members of a family of cell-surface receptors, including those for growth hormone and erythropoietin, the regulator of red blood cell production. This finding prompted speculation that a non-viral, mammalian form of this receptor may exist and transmit signals for a critical hormone. In 1992 and 1993, two research groups described the identification of human and mouse

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*c-mpl*—the homolog of *v-mpl*—and confirmed that the *c-mpl* gene encoded for a new protein belonging to the blood cell (hematopoietic) growth factor family of receptors. Knowledge of the c-Mpl protein quickly led to the discovery of the protein that binds to this receptor. Dr. Kaushansky's research group reported the isolation of the protein—thrombopoietin—that specifically interacts with the c-Mpl receptor. To confirm its function in blood cell production, Dr. Kaushansky and his colleagues treated mice for several days with thrombopoietin that had been synthesized in the laboratory and showed that platelet numbers increased dramatically compared to untreated (control) animals. These reports, and subsequent in-depth molecular and cellular studies, helped to delineate the physiological control of platelet production.

All blood cell types are derived from a population of self-renewing hematopoietic stem cells (HSCs) that first appear during embryonic development. In the cell lineage leading to platelet production, the HSC gives rise to progressively committed progenitor cells that each ultimately yields thousands of platelets. Specifically, HSCs become multipotent progenitor cells, which then become progenitors for specific types of blood cells, such as platelets. In the lineage of platelet development, progenitor cells give rise to large cells called megakaryocytes, first in immature and then mature forms. In a step that is unique in biology, individual megakaryocytes fragment into 1,000 to 3,000 platelets. Prior to fragmentation, the megakaryocyte undergoes a process that results in a many-fold geometric increase of chromosome numbers in a single “polyploid” nucleus. In humans, a cell normally contains 23 chromosome pairs, or a total of 46 chromosomes, in its nucleus. Mature megakaryocytes usually contain 8, 16, 32, or 64 times this normal number of chromosome pairs.

So how does the megakaryocyte become polyploid? Dr. Kaushansky explained that the megakaryocyte undergoes a process called endomitosis—the DNA is repeatedly replicated in the absence of cell division (mitosis); the cell does not divide into two daughter cells as would normally occur during chromosome duplication. This process is not simply the absence of mitosis but rather a culmination of several cycles of aborted mitoses. Dr. Kaushansky and his team systematically evaluated a handful of proteins which could be responsible for endomitosis. By comparing how these proteins behaved in normal cells undergoing cell division compared with the unique process taking place within the megakaryocyte, the evidence pointed to a protein called RhoA. The exact role that RhoA is playing in this process is now under investigation.

Although many researchers in the field predicted that thrombopoietin had physiological effects limited to megakaryocytes and platelets, Dr. Kaushansky and his colleagues studied its potential effect on HSCs. HSCs were placed in a less than optimum cell culture broth in the laboratory, and the ability of thrombopoietin and other factors to support cell survival was then evaluated. In the absence of thrombopoietin or other factors, all cells died within a few days. When stem cell factor or IL-3 were added to the cells, 80 to 90 percent of the cells survived up to 7 days. Somewhat surprisingly, thrombopoietin was also found to support the survival of HSCs similarly.

In medical situations where patients have blood cell cancers or are undergoing high-dose chemotherapy, HSCs harvested from related family members or from the patients themselves are commonly used by physicians to infuse into the patients' blood stream in order to repopulate their hematopoietic system. Although these “transplantations” have been used for several decades, the exact mechanisms governing the re-establishment of the hematopoietic system

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and blood cell production are not fully understood. Paramount to successful repopulation of the hematopoietic system is the “homing” of HSCs from the blood stream to the bone marrow. It is in the bone marrow that HSCs undergo multiple cell divisions to produce an expanded number of HSCs and to begin to mature into committed progenitor cells.

Dr. Kaushansky’s team next sought to determine whether thrombopoietin plays a biologically relevant role in HSC expansion. Bone marrow cells were obtained from either mice genetically altered to no longer produce thrombopoietin or normal mice, and normal HSCs were transplanted into the two types of mice. Five to 8 weeks post-transplantation, the number of HSCs in the transplanted mice was determined; Dr. Kaushansky found approximately 17-fold greater numbers of HSCs in the normal mice than in animals in which thrombopoietin was missing. Clearly, in mice, thrombopoietin and the thrombopoietin receptor are critical for stem cell expansion.

Dr. Kaushansky and his collaborators have also begun mapping how thrombopoietin exerts its influence on cells and tissues. After thrombopoietin binds to its cell surface receptor, c-Mpl, the receptor initiates a cascade of events that results in a cellular response. This process is termed signal transduction; the key intracellular protein involved in thrombopoietin signaling is the protein phosphorylation enzyme, JAK2.

## **Thrombopoietin: Clinical Implications**

Approximately 30 to 50 children each year are born with congenital amegakaryocytic thrombocytopenia—born without megakaryocytes and hence very low platelet counts, approximately 5 to 10 percent of normal. By age one, most of these children have developed bone marrow failure and pancytopenia (or low numbers of all blood cells) due to the lack of HSC

in their bone marrow. The molecular defect in this rare disease has been determined to be mutations in c-Mpl. Thus, this “accident of nature” proves that human HSCs, and hence all blood cell types, like those of mice, are very much dependent on the presence of thrombopoietin and c-Mpl.

Dr. Kaushansky noted several potential clinical uses of thrombopoietin therapy to increase stem cell and platelet population: hematopoietic recovery following chemotherapy for cancer; thrombocytopenia (inadequate platelet count) associated with HIV; aplastic anemia; myelodysplastic syndrome, a condition in which stem cells are defective and do not mature normally; and immune (antibody-mediated) thrombocytopenia. Another potential use was to improve platelet donor yields. Until now, the only therapy available for patients with thrombocytopenia has been the transfusion of platelets obtained from healthy donors. In one trial, administration of a normal form of thrombopoietin was found to ameliorate chemotherapy-induced severe thrombocytopenia in patients with ovarian cancer and reduced the need for platelet transfusions. In a second trial, a single dose of a modified form of thrombopoietin increased platelet counts from 225,000 to 600,000 in healthy platelet donors, allowing recovery of three times more platelets for transfusion. Unfortunately, multi-dose treatment of the healthy donors with this modified form of thrombopoietin led to the development of neutralizing antibodies to the modified protein that also recognized the donors’ own thrombopoietin and caused thrombocytopenia in the donors. Because of this serious side effect, the modified form of thrombopoietin was withdrawn from clinical trials in the United States in 1998.

Hampered by the appearance of neutralizing antibodies to the modified form of thrombopoietin,

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researchers have sought to develop small molecules that would mimic the effect of thrombopoietin but without stimulating the production of neutralizing antibodies. Two small molecules—Romiplostim and Eltrombopag—have been developed that bind to different sites on c-Mpl, but nonetheless mimic thrombopoietin signal transduction. In 2008, both Romiplostim and Eltrombopag were shown to be effective and well tolerated in patients with chronic immune thrombocytopenia (ITP) who had failed several therapies for the disease; platelet counts increased within 1 to 2 weeks and were sustained throughout 24 weeks of treatment. Both drugs were then approved for use in patients with refractory ITP.

## Blood Cell Cancers

Dr. Kaushansky then informed Council members that mutations have been identified in both *thrombopoietin* and *c-mpl* genes in humans. Congenital *thrombopoietin* mutations cause an overproduction of thrombopoietin, leading to familial thrombocythemia (high platelet counts) and are associated with blood clots. Congenital *c-mpl* mutations cause both amegakaryocytic thrombocytopenia (see above) and familial thrombocythemia, due to activating mutations, while acquired activating mutations of *c-mpl* have been found to cause essential thrombocythemia and primary myelofibrosis—two blood cell cancers (myeloproliferative neoplasms) that lead to morbidity and mortality from either bleeding or excessive blood clotting.

The most common mutation that causes myeloproliferative neoplasms is found in the thrombopoietin signaling protein JAK2. In fact, a single acquired mutation in JAK2 is found in up to 97 percent of patients with polycythemia vera (increase in all blood cell types, particularly red blood cells), approximately half of patients with essential thrombocythemia and

patients with idiopathic myelofibrosis (increased collagen deposition in bone marrow), but is absent in healthy individuals and patients with other hematological cancers. How the same JAK2 mutation can cause three distinct clinical conditions remains unclear.

To better understand the consequences of mutated JAK2, a colleague of Dr. Kaushansky's, Dr. Radek Skoda, designed a genetically engineered mouse model which allowed the researchers to control mutant JAK2 production in the mice, compared to normal JAK2. This study showed that mice producing lower levels of mutant JAK2 compared to normal protein displayed characteristics of essential thrombocythemia, whereas those with high levels of the mutant protein exhibited characteristics resembling polycythemia vera. Thus, the ratio of mutant JAK2 to normal JAK2 determines the myeloproliferative neoplasm phenotype in this mouse model.

Dr. Kaushansky's research group has recently established additional mouse models to further explore the role of mutant JAK2 in myeloproliferative neoplasms. They engineered different mice to produce mutant JAK2 in certain, but not all, of their cells. One group of mice produced mutant JAK2 in their hematopoietic and endothelial cells (the cells lining the inside of blood vessels); another group produced the mutant protein in their megakaryocytes and platelets; and other groups of mice produced this protein only in their white blood cells, endothelial cells, or bone marrow cells. The mouse line producing mutant JAK2 in hematopoietic and endothelial cells was found to have increased platelets and white cells but not red blood cells. These five mouse lines are currently under investigation to determine whether they have abnormal clotting or bleeding characteristics.

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## **Future Directions**

Dr. Kaushansky noted that there are many future research challenges for studies of thrombopoietin. These include 1) understanding the molecular mechanisms underlying thrombopoietin's effects on hematopoietic stem cell cycle regulation and cell survival; 2) determining the exact role that RhoA plays in endomitosis; and 3) delineating the

molecular mechanisms of thrombopoietin on platelet formation. In addition, research is needed to better understand myeloproliferative neoplasms, including the role played by thrombopoietin and its receptor c-Mpl. Once researchers gain a better understanding of these disorders, they can use this knowledge to design and develop new, more effective therapies.

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### Veronica Garcia

#### *Balancing Life with Interstitial Cystitis/Painful Bladder Syndrome Includes Participating in Research*



Veronica Garcia

Thirty-four-year old Veronica Garcia has been dealing with urinary pain symptoms for most of her life. “Even as a 3- or 4-year old child, I remember it was painful to go,” she recalls. Although her doctors suspected chronic urinary tract infections and over the years treated her with antibiotics and palliative measures for pain, no one could give her a specific diagnosis. However, when her symptoms changed about a year or so ago, Veronica and her doctor became concerned, and she received a new workup from a urologist specializing in pain issues. Now diagnosed with interstitial cystitis/painful bladder syndrome (IC/PBS), a chronic urologic pain syndrome for which there

is no widely effective treatment or cure, Veronica has enrolled in the NIDDK-supported Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. This multi-site study is seeking answers about the fundamental causes, progression, and nuances of urologic chronic pelvic pain syndromes that may help people with IC/PBS.

#### “Bad Plumbing”

Veronica, who works full-time as a computer programmer/analyst, is a problem solver who is determined not to let symptoms of IC/PBS get in the way of her life. A wife and mother of two, Veronica bubbles with enthusiasm as she describes how much she enjoys her family and volunteering, especially for activities with her son, Jesse (age 13), and daughter, Isabella (age 10). “Jesse is in football, and Isabella, who is very athletic, is doing cheer(leading),” she says. Both children are also in Scouts, which Veronica thinks is great for skill building and being part of the community. Additionally, Veronica says she is “very crafty” and loves to do holiday decorations and creative projects with her children any chance she gets. According to Veronica, activities like these are “very important,” as is her involvement. She also loves to read and share book recommendations with friends—“a great way to keep in touch,” she says.

However, Veronica has been dealing with pain and pelvic and abdominal discomfort issues most of her life. “The running joke is that I have ‘bad plumbing,’” she says with a laugh. After first noticing pain in

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childhood, Veronica’s urinary pain worsened as she grew older, peaking in her teens and twenties. She also sometimes had blood in her urine. Sometimes the pain would be so bad that she couldn’t sit still, and she would be afraid to go to the bathroom. Tightly fitted clothing wasn’t an option. “There was a period of time when I wore overalls all the time,” says Veronica. During the worst times, she says, “I sometimes felt like life wasn’t fair. Other people could run around and do things without a care, and I was having to make all kinds of life adjustments.”

About 6 years ago, Veronica had her first workup with a urologist. While she was glad to find out that she did not have cancer or another life-threatening condition, she still did not have a diagnosis. She says that she was told that, regarding her urinary pain and sensitivity, “some people are just like that.” She continued to be prescribed antibiotics, which, Veronica says, sometimes helped, but sometimes didn’t—most likely, she thinks, because she occasionally did have an actual urinary tract infection that was causing pain.

Five years later, Veronica says, her symptoms changed. She started experiencing pain in her lower back, near her kidneys, which, she says, was “very scary”; she also felt increased pressure on her bladder. At about the same time, a relative was diagnosed with IC/PBS. As she discussed the symptoms with her relative, Veronica began to think that she might also have IC/PBS. After discussing her new symptoms and her relative’s diagnosis with her primary care doctor, she was sent to a different urologist specializing in these issues, who did another workup—and diagnosed Veronica with IC/PBS.

## What Is IC/PBS?

Interstitial cystitis (IC) is a condition that results in recurring discomfort or pain in the bladder and the

surrounding pelvic region. Symptoms vary from person to person and even in the same individual at different times. People may experience mild discomfort, pressure, tenderness, or intense pain in the bladder and pelvic area. Symptoms may also include an urgent need to urinate, a frequent need to urinate, or a combination of these symptoms. Pain may change in intensity as the bladder fills with urine or as it empties. IC/PBS is more common in women than in men. In women, symptoms often get worse during menstruation.

However, because IC varies so much in symptoms and severity, most researchers believe it is not one, but several diseases. In recent years, scientists have started to use the terms bladder pain syndrome (BPS) or painful bladder syndrome (PBS) to describe a set of painful urinary symptoms that may not meet the strictest definition of IC. The term IC/PBS includes all cases of urinary pain that cannot be attributed to other causes, such as infection or urinary stones.

Currently, the cause(s) of IC/PBS remain unknown, and no widely effective treatments are available to treat IC/PBS. Therapeutic approaches are aimed at symptom relief, and include identifying and avoiding factors, such as certain behaviors, foods, or additives, that may trigger symptoms.

## Overlapping Conditions, Multiple Strategies

At the same time that she was dealing with urinary pain symptoms, Veronica also grew up with another set of symptoms that was diagnosed about 10 years ago as irritable bowel syndrome (IBS). IBS is a functional gastrointestinal (GI) disorder, meaning it is a problem caused by changes in how the GI tract works, but it is not a disease, nor does it cause damage to the GI tract. People with IBS have abdominal pain or discomfort, often reported as cramping, along with diarrhea,

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constipation, or both. IBS, like IC/PBS, is much more common in women. Veronica says that, for her, the IBS symptoms are worse in some ways, especially at work, because they are more obvious. “I get gas and bloating and have to use the bathroom. I can hide the IC/PBS symptoms, but not these.”

Asked how she has coped with all of these symptoms, especially before she had any diagnoses, Veronica notes that she developed a number of strategies over the years. For the urologic symptoms from IC/PBS, she says, “I’m very careful about what clothes I wear, and wash my clothes and underwear separately from all the other laundry, using fragrance-free detergents,” which, she explains, helps with the urinary pain symptoms. She also learned not to hold urine for long periods of time, which she found exacerbated her symptoms. Following her diagnosis, she figured out that other known triggers for IC/PBS symptoms, such as caffeine, were also triggers for her. For urinary pain, she uses over-the-counter medications, such as ibuprofen or acetaminophen.

She also has strategies to deal with the digestive symptoms from her IBS. “I know what foods are triggers,” such as fried foods, she says, and is especially careful during the work week. With her doctor’s knowledge, she has also recently been following a nearly gluten-free diet. While she has not been diagnosed with celiac disease, an autoimmune disease that damages the digestive tract of affected people when they eat gluten, gluten-containing foods seem to be a trigger for her IBS symptoms. After several weeks on a gluten-free diet, she says that her “stomach is flat (not bloated) now,” and she feels much better. Stress is another trigger, and Veronica says she may also look into mindfulness/cognitive behavioral therapy approaches to help with symptom management. However, another part of her personal strategy is to

keep things in perspective and try not to get “too crazy” with management—quoting her husband, she says, “everything in moderation, even moderation.”

### **IC/PBS, Urologic Chronic Pelvic Pain, and the MAPP Research Network**

Veronica’s experience is not unique. Researchers, clinicians, and patients alike have been baffled and challenged by IC/PBS. Despite years of committed basic and clinical research efforts, the cause(s) of IC/PBS remain elusive, as do effective treatments. Moreover, a diagnostic test for IC/PBS is not currently available. Instead, because many IC/PBS symptoms can be symptoms of other diseases, those diseases need to be ruled out first—making IC/PBS a “diagnosis of exclusion.” While public and clinical awareness of this condition is increasing due to educational efforts by the NIDDK and major health advocacy organizations, such as the Interstitial Cystitis Association, many people still suffer for years with pain symptoms and no diagnosis. Recent data from a major NIDDK-supported epidemiological study suggest that, among adult women in the United States, as many as 2.7 percent have symptoms consistent with IC/PBS. Further, a growing body of evidence suggests that, like Veronica, many people with IC/PBS and other urologic chronic pelvic pain syndromes frequently have other chronic pain conditions, further affecting quality of life.

In 2008, to help better understand the underlying causes of the two most prominent chronic urological pain syndromes, IC/PBS and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), the NIDDK launched the MAPP Research Network. This novel, multi-site research network embraces a unique, systemic (whole-body) approach to the study of IC/PBS and CP/CPPS. In addition to moving beyond traditional bladder- and prostate-specific research

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directions, MAPP Research Network scientists are investigating potential relationships between these two urological syndromes and other chronic pain conditions that are sometimes seen in people with IC/PBS and CP/CPPS, such as IBS, fibromyalgia, and chronic fatigue syndrome.

The MAPP Research Network includes scientists with diverse research expertise, all working collaboratively. Clinical researchers bring experience treating patients. Epidemiological investigators study the occurrence of, and identify risk factors for, IC/PBS and CP/CPPS. Basic research scientists examine what is happening on a cellular level. Network leaders include not only urologists, but investigators specializing in the overlapping pain conditions. The MAPP Research Network researchers and staff are assembled at six Discovery Sites that conduct the research studies and two Core Sites that coordinate data collection, analyze tissue samples, and provide technical support.

Currently, the MAPP Research Network is recruiting and characterizing people with IC/PBS and CP/CPPS in a central study to better understand the natural history of these conditions and to see if people with these conditions fall into different, distinguishable subgroups that may suffer from different causes and require different treatments. The Network is also conducting key brain-imaging studies, studies to identify biomarkers of disease, efforts to assess the possible role of infectious agents, and other studies designed to provide a systemic view of disease. Additionally, the Network includes “control” participants—both healthy persons without any pain syndromes, and those who have one or more of the overlapping pain conditions. Through its multi-pronged approach, the MAPP Network aims to discover new and clinically relevant insights that may lead to improved treatment options and better patient care.

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*For Veronica, an unexpected but large part of the benefit of being part of the MAPP Research Network has also come from knowing she isn't alone. She says that this is the first time she's been around many people with similar health issues.*

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## **Participation in the MAPP Research Network**

Following her diagnosis with IC/PBS in January 2012, Veronica said she felt relieved. After so many years, “It was great just to have a diagnosis...to know that there wasn't something ‘wrong’ with me,” she says. Her urologist, who is also a principal investigator for the University of California, Los Angeles (UCLA) site of the MAPP Research Network, then told her about the study and encouraged her to consider joining. “I thought that was great,” says Veronica, “anything that can help advance what we know about IC/PBS. Not only that, but at the very least, I thought I could learn a little bit about it from people who've been working on it.”

People with IC/PBS or CP/CPPS who enroll in the MAPP Research Network are 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 also participate in a pressure pain threshold procedure that is another way to assess pain sensitivity, and are asked to provide blood and urine samples for use in some of the research studies. This initial, in-depth clinic-based visit is followed by 2 additional visits at 6 and 12 months after enrollment. During that year, participants are also registered with an internet-based system so that they can fill out assessments of their symptoms every 2 weeks. This ongoing symptom assessment, a key component of the central epidemiological study, is a particularly valuable tool, as it is allowing MAPP Research Network

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scientists to learn a great deal about symptoms, long-term symptom fluctuations, and their possible correlations with other factors.

Veronica enrolled in the MAPP study in spring 2012. She says she was intrigued by the questionnaires. She says, “The questions have been wildly interesting... they bring out the detective in me”—especially questions she wasn’t initially expecting, such as psychological and emotional questions, or questions about things like sinus pain. Answering the questions “makes (her) think and wonder” about the possible connections. She says that keeping up with the biweekly self-assessments can sometimes be a bit of a challenge with her busy schedule, but she has managed to pretty much stay on top of it.

Many MAPP Research Network participants, including Veronica, also have participated in a brain imaging study that could help scientists better “map” changes associated with chronic pain. Veronica also participated in a special brain imaging study being conducted at the UCLA MAPP Research Network site, focused on using common “stressors,” such as cold water, to understand if the brain processes pain differently in people with IC/PBS versus those without the condition.

Veronica also enjoyed being part of one of several special focus groups in which participants have talked about symptom “flares”—*i.e.*, how their symptoms seem to wax and wane. Through these focus groups, the MAPP Research Network scientists are learning a great deal more about flares, their specific nature, why and when they occur, and their short- and long-term impact on individual persons—for example, participants have drawn a distinction between “flares” and “mini-flares.” Veronica says she has had flares all her life, but the intensity of flares has changed for the

better in recent years—now, she says, she “only gets a bad one once or twice a year,” while she may get “mini-flares” five or six times a year. Knowing more about flares will also be helpful to researchers as they consider research studies about IC/PBS and other pain syndromes, as the “flare status” of a participant could potentially affect research results.

## A Family Affair

When asked what she hopes to see from the MAPP Research Network studies, Veronica says that, while she doesn’t expect an immediate result, she does look forward to seeing something down the road, such as an article in the paper saying that researchers from the MAPP Research Network study have made a discovery about IC/PBS. She also wonders generally whether there might be some discoveries about family associations for IC/PBS and overlapping pain conditions. Several members of Veronica’s family also have pain syndromes, including IBS, IC/PBS, and fibromyalgia. “If they find that there is a genetic link here, I won’t be surprised,” she says.

Veronica also can’t say enough about how important her family support has been through the years. She and her husband, David, who is also a computer specialist, have been together for 20 years—“We were high school sweethearts,” she says happily—and have struggled together through many life challenges, including Veronica’s pain and digestive symptoms. “He helps me so much. When he sees me wallowing—which is rare now—he knows it’s bad, and does special little things for me.” To Veronica, family communications and everyone putting each other first helps immensely in dealing with the challenges of her pain conditions. In her eyes, “You get out what you put in.”

Her children are also very supportive. Veronica has explained as much as she can at this point about

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what she is going through, trying to be honest without getting into too much detail. They also like that she is participating in a research study. Veronica says, “I try to make it fun,” such as by sending them funny photos of herself during a test or when she goes for the acupuncture sessions that help her symptoms. That helps them to ask and talk about it, she says. Veronica says they have also been supportive of new strategies she is trying—for example, they are sharing the gluten-free food she is eating while not totally excluding wheat from their own diets.

Veronica says that her biggest fear is that her daughter may develop IC/PBS symptoms someday. Thus, based on her own experience, she is already introducing routines and strategies that may reduce symptom risk as the “normal” for her daughter, such as separate laundry and no bubble baths. At this point, she says, there have only been a couple of possible episodes, nothing like Veronica’s experience as a child.

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*Veronica says she would definitely recommend participating in the MAPP Research Network. As Veronica puts it, it is “Reassuring to know that people are working on chronic pain, that something is being done.”*

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## Perspectives

Veronica admits that dealing with all of her symptoms has been and remains challenging. Still, her perspective is that, while things are not perfect, her symptoms are better than they were several years ago, she has a diagnosis—which has brought peace of mind—and that, while she would “jump at the chance” not to have to do all the extra work they entail, she has strategies that help her to manage

her symptoms. She is also grateful that IC/PBS isn’t intrinsically life-threatening, but still happy that scientists are researching it to help people like her who are dealing with chronic pain issues. Most of all, she is determined, especially since having children, not to let her symptoms keep her from life activities. “When you have kids, they have to come first,” she says. As for participating in a research study, for Veronica, an unexpected but large part of the benefit of being part of the MAPP Research Network has also come from knowing she isn’t alone. She says that this is the first time she’s been around many people with similar health issues, and it has opened up a wealth of opportunities. She has enjoyed getting the opportunity to relate with others and hear their stories, share home remedies and information about food issues and health resources, and even to network. She likes knowing that she is “helping people just like [me].”

Veronica is also very positive about her experience with the MAPP Research Network staff. “The staff have been great—they are very compassionate, show genuine concern, and want to know what is going on with me. I feel safe, and that my confidentiality is assured. I also have access to resources—especially people—I never knew I could. I’ve had a great experience.” Veronica says she would definitely recommend participating in the MAPP Research Network, both for the opportunity to meet other people with similar issues and the opportunity to get peace of mind and know the condition better. As Veronica puts it, it is “reassuring to know that people are working on chronic pain, that something is being done.”

*For more information about the MAPP Research Network, see: <http://www.mappnetwork.org/>*

