Studies have shown that chronic pain conditions are often accompanied by alterations in brain structure and function. Research described in this chapter suggests that there may be brain changes unique to people with urologic chronic pelvic pain syndromes (UCPPS). Magnetic resonance imaging (MRI) is a noninvasive approach being used to try to identify unique sets of changes, or “brain signatures,” for different pain conditions, as these could lead to improved understanding, diagnosis, and therapy.

This image depicts a model of the brain constructed by combining highly detailed MRI scans obtained from people with UCPPS and from healthy people. Areas in green showed no structural differences, whereas the areas in blue, yellow, and red scattered throughout the brain differed between people with UCPPS and healthy individuals. This is one of several images that, with accompanying analyses, enabled researchers to identify a number of microstructural changes in the brains of people with UCPPS, particularly in areas related to perceiving and responding to pain, some of which may be unique to people with UCPPS as compared to people with different chronic pain syndromes.

Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new treatments for them, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and of the blood and blood-forming organs. The overall goal of the NIDDK’s research programs is to increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.

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

It has been estimated that more than 20 million American adults have impaired kidney function—also called chronic kidney disease (CKD).1 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 2014, over 678,000 patients received treatment for ESRD: over 467,000 received either hemodialysis or peritoneal dialysis, and over 196,000 were living with a kidney transplant.2 Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives, bear a disproportionate burden of CKD and ESRD. African Americans are nearly four times more likely to develop kidney failure than are non-Hispanic Whites.2 American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic Whites.2 In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

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

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diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. The Institute issued a research solicitation titled “Kidney Precision Medicine Project” that aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies. In addition, NIDDK issued a research solicitation titled “Pilot Clinical Trials in Pediatric Chronic Kidney Disease” to form a multi-center collaboration to perform pilot trials to optimize study designs for larger trials for new pediatric CKD treatment.

The NIDDK’s National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and seeks to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK’s urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urologic diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. To spur research in urinary stone disease, the NIDDK established the Urinary Stone Disease Research Network (USDRN) to: a) design and conduct a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conduct clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) provide data and collect biological samples from the studies to create a resource for future researchers. Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important research topics of the NIDDK’s urology program.

IC/BPS is a debilitating, chronic, and painful urologic disorder. Based on a recent large, national interview survey, it is estimated that among U.S. women 18 years old or older, 3.3 million (2.7 percent) have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/BPS. Using a community-based epidemiologic survey, researchers have estimated that among U.S. men ages 30 to 79 years old, 1.6 million (1.3 percent) have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with BPS.

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

Based upon national public health surveys conducted over several years, it is estimated that 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women. Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as to provide new insights into

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5 *Urological Diseases in America. NIDDK, NIH Publication Number 12-7865, 2012.*
non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes. To address this challenge, the NIDDK established the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health, established the Prevention of Lower Urinary tract Symptoms (PLUSES) Research Consortium to develop the evidence base for normal or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

The NIDDK’s hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program with current topic areas: hematopoietic stem cell determinants, non-coding RNA, macrophages, and aging.

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.

**KIDNEY FORMATION AND FUNCTION IN HEALTH AND DISEASE**

**Mining the Genome for Insights into Kidney Function and Development:** An international group of researchers, examining data from more than 230,000 people, have discovered 24 new areas of the genome (the entire set of genetic information) that are associated with kidney function or development and have confirmed 29 other genomic areas that were previously identified. Estimates suggest that more than 20 million adults in the United States have chronic kidney disease (CKD) of varying levels of seriousness. To identify genes associated with CKD, a team of scientists scanned the genomes of more than 130,000 people of European ancestry for genetic variants associated with estimated glomerular filtration rate (eGFR), a measurement of how well their kidneys are filtering wastes and extra fluid from the blood. The researchers identified 24 new regions of the genome that were associated with kidney function and confirmed the findings by further analysis in more than 42,000 additional people of European ancestry. Their analysis also confirmed 29 genomic regions already known to be associated with eGFR. Many of these genomic regions were associated with eGFR in people with type 2 diabetes or high blood pressure—populations at particularly high risk for CKD. The scientists also examined the genomes of over 16,000 people of African ancestry and more than 42,000 Asians. Several of the newly identified regions were associated with kidney function in other ethnic groups, suggesting that these results likely extend beyond people of European ancestry. Computational analyses determined that genes found to be in these genomic regions were mostly turned on in cells within the kidney or urinary tract, and were involved in processes related to kidney development and function. The specific genomic variations associated with eGFR often resided in portions of the genes that determine when, where, and to what extent the genes are turned on. Additional studies will be necessary to understand the precise role of each genomic region in kidney development and physiology, but these findings have generated numerous potential targets for therapeutic strategies to improve kidney health, including in people with type 2 diabetes or high blood pressure.


**A Cause of Hardening and Narrowing of Arteries in Kidney Disease Identified:** Scientists have found in mice that a certain type of stem cell contributes to artery “hardening” that can lead to cardiovascular events like heart attacks and strokes. Arterial
calcification, or “hardening” of the arteries due to the accumulation of minerals in the artery walls, is often a precursor to cardiovascular disease, especially when accompanied by atherosclerosis, or narrowing of the arteries due to formation of fatty “plaques” on the inner walls. These conditions are common among those who have chronic kidney disease (CKD) and can lead to dangerous blood clots and/or heart failure. Mesenchymal stem cells (MSCs) in the arteries have been thought to contribute to artery disease and repair, but research on these cells has been hindered by a lack of tools to follow their fate as they differentiate, or mature into more specialized cells. To study MSCs’ role in artery health, researchers identified a protein marker, called Gli, which could be used to identify arterial MSCs experimentally. These Gli+ stem cells normally reside in the outer walls of the arteries, but in a mouse model of arterial injury they differentiated and migrated to wound sites to take part in wound repair. The scientists then used a mouse model to investigate what role these Gli+ cells might play in disease. In a mouse model of CKD that includes artery hardening from calcification and fatty plaques, the Gli+ cells migrated into the arteries to cluster around arterial plaques and differentiated into osteoblasts, or bone-forming cells. When researchers genetically engineered mice to have significantly reduced numbers of Gli+ cells, these mice had significantly less artery calcification than normal mice, suggesting that the Gli+ cells were responsible for artery hardening. These results may also have important implications for people. Researchers found Gli+ cells in the arterial plaques of men with CKD, while the Gli+ cells in the arteries of men without CKD stayed in the outer arterial walls. Overall, these findings gave important new insights into stem cell biology and may offer new targets for treatment or prevention of arterial disease.


Decline in Nephron Numbers in the Kidney Even in Healthy Aging: New research shows that from young adulthood to old age, healthy adults lose about half of their nephrons—the basic functional unit of the kidney. Nephrons consist of various cells and structures that work together to filter waste products and excess fluid from the blood; the glomerulus is the fundamental filtering apparatus in the nephron. Previous research has shown that the number of nephrons correlates with the functional capacity of the kidney, and that nephron loss occurs with aging. However, most studies estimating nephron number have been done using kidney samples obtained at autopsy rather than from living people, as the only way to obtain kidney tissue from living people is through an invasive biopsy. Researchers took advantage of the fact that people donating one of their kidneys to another person—i.e., living kidney donors—undergo tests before and during the transplant procedure, including a kidney biopsy. They used data from these tests to estimate nephron numbers in 1,638 healthy male and female living kidney donors, ages 18 to 75.

The researchers found that donors who were 18 to 29 years old had about 990,000 nephrons. In contrast, 70- to 75-year-olds had about 520,000 nephrons, or roughly half the number as in the younger group. Overall, nephron number was found to decrease by about 7 percent every decade from young adulthood to old age. Men had more nephrons than women, but there was no difference in the age-related decline in nephron number between men and women. The researchers also observed a decrease in glomerular filtration rate (GFR)—a measure of kidney function—with age that correlated with the age-related decline in nephron number. In addition to older age and lower GFR, other clinical characteristics associated with lower nephron number included shorter height, family history of end-stage renal disease, and higher level of uric acid in the blood. The researchers also discovered that using other methods to estimate nephron number, such as by measuring the volume of the outer rim of the kidney (the cortex) by computed tomography scan, grossly underestimated nephron loss.

These findings show that there is substantial nephron loss as a part of aging in healthy adults. The researchers note that current clinical guidelines use a single GFR threshold for diagnosing kidney disease. However, because of the observed nephron loss with healthy aging and the correlating decline in GFR, using a single cut-off value may over-diagnose kidney disease in older adults (who have a lower GFR compared to younger adults) and under-diagnose kidney disease in younger adults. The researchers also note, however, that the population included in this study was predominantly White and had only 11 participants aged 70 to 75 years, so additional research is needed to assess generalizability of the findings. Further

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research, including developing improved imagining techniques that enable nephron number to be estimated in living people without the need for a biopsy, could help shed light about nephron number in other populations and how to consider nephron loss in healthy aging when diagnosing kidney disease.


Understanding the Role of an Important Family of Genes in Human Kidney Development: New research has elucidated important differences between mouse and human kidney development in the role of a family of genes—findings that could explain why people have more nephrons than do mice, and could inform new therapeutic strategies for the prevention and treatment of kidney disease.

Nephrons are the basic functional unit of the kidney. They consist of various cells and structures that work together to filter waste products and excess fluid from the blood. The mechanisms driving the number of nephrons are particularly important to determine because low nephron number has been found to be associated with increased risk for high blood pressure and reduced kidney function in people. Humans have a vastly higher number of nephrons per kidney than do mice (1 million versus 13,000, on average). Key distinctions in kidney development could account for this difference, including the fact that nephron formation takes significantly longer to complete in humans than it does in mice.

To explore the differences in kidney development, researchers investigated two members of the “Six” gene family, Six1 and Six2, which encode regulatory proteins known to be essential for proper kidney development in the mouse, but not well explored in human developmental systems. In mouse and human kidney development, the Six2 and SIX2 genes, respectively, are turned on in a pool of progenitor cells that give rise to nephrons throughout the period of nephron generation. Previous studies have shown that the mouse Six1 gene is turned on only transiently in early stages of kidney development. By contrast, the researchers found that the human SIX1 gene is similarly turned on early, but also continues throughout the period of extensive nephron formation. They also determined that, specifically in humans, the activation of the SIX1 gene is regulated, in part, by the protein that is encoded by the SIX2 gene. These findings reveal a divergence between mice and people in the molecular regulators controlling the fates of nephron progenitor cells. The scientists hypothesize that the expanded activation of the SIX1 gene in humans could play a role in maintaining the pool of progenitor cells, which could then establish the higher number of nephrons observed in humans compared with mice. Additional research could address that possibility.

Often, translating important discoveries from mouse models to applications in humans can be challenging due to differences between species. Armed with this new knowledge on human kidney development, scientists could develop novel strategies to increase nephron number in people, and test whether this would improve kidney function.


Technological Improvements Help Transplanted Kidney Tissue Connect with a Host’s Circulatory System: Researchers have identified new experimental conditions that improve the viability of transplanted kidney tissues and their ability to interact with the host blood supply in rodent models. For people with kidney failure, the two main treatment options are dialysis or a kidney transplant. However, there is currently a significant shortage of donated kidneys that are available for transplantation. Therefore, research towards improving methods for engineering functional kidney tissues remains an urgent public health undertaking. Previous attempts in rodent models to transplant engineered kidney tissues have met with limited success, partly because the hosts’ vascular systems could not seamlessly connect with the transplanted tissue.

In a recent study, scientists addressed this problem by isolating rat glomeruli (balls of capillaries through which the blood is filtered in the kidney) that were suspended in an engineered matrix and transplanted into host female mice. They compared the viability of transplanted glomeruli with and without the addition of human endothelial cells (ECs) that were genetically modified to produce the cell survival protein Bcl-2. ECs
form the inner lining of blood vessels. These genetically modified cells, which spontaneously form small blood vessels in a matrix suspension, were termed Bcl-2-ECs. After 2 weeks post-transplantation, overall survival of rat glomerular cells in the presence of Bcl-2-ECs was about 10 percent, whereas almost no glomeruli survived without Bcl-2-ECs. Using injected dyes, the researchers then found that the glomerular capillaries were capable of connecting to the newly formed blood vessels derived from the Bcl-2-ECs. Furthermore, 15 days after transplantation, all viable glomeruli suspended in the presence of Bcl-2-ECs were acquiring blood from the host mouse's circulatory system. However, a closer look at the transplanted glomeruli by electron microscopy revealed a few structural problems in some of the cells. Together, these data uncover an important technological advance in generating viable kidney tissue that can connect with a recipient’s blood vessels after transplantation. Although this new knowledge represents an important step forward, additional research will be needed to improve the efficiency, integrity, and function of transplanted engineered kidney tissues.


KIDNEY STONE TREATMENT

Moving Stones with Sound—New Ultrasound Technology Repositions Kidney Stones in People:
Researchers have developed new ultrasonic propulsion technology that can reposition kidney stones and facilitate stone fragment passage in people. Kidney stones are one of the most common disorders of the urinary tract. Smaller stones may pass with little or no pain, while larger stones may get stuck along the lower urinary tract and block the flow of urine, causing severe pain and/or bleeding. Current treatments for kidney stones, such as lithotripsy, may leave behind residual stone fragments. Most fragments will pass on their own, but others may grow larger, cause pain, and lead to the need for additional treatment.

Toward the goals of finding safe ways to reposition kidney stones and encouraging the passage of stone fragments, scientists developed ultrasonic propulsion technology. The technology uses a handheld device to generate a real-time ultrasound image to visualize the kidney stone, and directs controlled, short bursts of ultrasound waves toward the stone to try to make it move. In the first human clinical trial testing this technology, scientists found that it could reposition kidney stones in 14 of 15 men and women studied, and cause some degree of movement of both large and small stones. In fact, one person experienced pain relief after a large, obstructing stone was moved. These findings suggest that the procedure could successfully reposition kidney stones in some people. The scientists then examined six study participants who had residual stone fragments after previously undergoing a lithotripsy procedure to treat their kidney stones. Four of them passed more than 30 fragments within days after undergoing the ultrasonic propulsion procedure, demonstrating that the technology could facilitate the passage of stone fragments. An unexpected finding was that the technology may also be useful for diagnosis—in four people, what was thought to be one large stone was actually found to be a cluster of small, passable stones after they were moved. Stone size is an important factor that doctors consider when making treatment decisions, so having this diagnostic information could aid them in making those decisions. Importantly, the technology was found to be safe and did not cause pain. It is also noninvasive and could be performed in a clinic setting while people are awake without the need for sedation. Ultrasound propulsion technology is still being refined and tested in people, but with further research, it may eventually be possible to use this new technology after procedures that leave residual stone fragments to facilitate their passage and potentially reduce the need for future intervention. The technology may also be useful for moving large, obstructing stones; repositioning stones before surgery; and serving as a diagnostic tool.


New Approach May Reduce Risk of Kidney Stone Formation: A molecular tool called RNA interference (RNAi) has been shown to be effective in reducing oxalate production in animal models of primary hyperoxaluria (PH), a rare, inherited condition characterized by recurrent kidney and bladder stones. PH type 1 often results in end-stage renal disease (ESRD), a life-threatening condition in which the kidneys
can no longer filter fluids and eliminate waste products from the body effectively. People with PH type 1 have excess oxalate in their bodies because they lack a liver protein that would normally prevent oxalate overproduction. Most kidney stones consist mainly of crystallized calcium oxalate and small amounts of other compounds. Both calcium and oxalate are components of a normal diet, and a high level of oxalate in the urine correlates with increased risk of stone formation. However, decreasing dietary intake of oxalate has not been demonstrated to be effective in preventing kidney stone formation. Although surgical interventions, such as combined liver-kidney transplantation, represent an option for a small number of patients, there are no FDA-approved medically based therapeutic approaches for the treatment of PH type 1. Moreover, dialysis does not adequately remove oxalate from the body.

A recent report describes how scientists explored the use of RNAi to block production of oxalate. RNAi is a widely used technique that allows researchers to target and “silence” a gene of interest so that the protein it encodes is not made. Recently, the technique has begun to be harnessed toward possible therapeutic applications. In this study, researchers took advantage of what is known about the multi-step pathway to oxalate production, in which a molecule called glycolate is converted to another molecule, glyoxylate, which can be converted to oxalate. Glycolate is converted to glyoxylate in liver cells by an enzyme called glycolate oxidase, or GO. Because people with PH type 1 do not metabolize glyoxylate normally—which causes it to build up and be converted to oxalate—the RNAi compound “ALN-GO1” was selected to silence the gene that encodes GO. The team hypothesized that, in the absence of GO, glycolate would accumulate, but be readily and safely eliminated from the body in the urine, while the remainder of the pathway would effectively shut down, preventing the excess oxalate production that causes harm in PH type 1.

Administration of ALN-GO1 significantly silenced the gene encoding GO in normal male mice, rats, and monkeys. In normal male monkeys and in a mouse model of PH type 1, ALN-GO1 increased glycolate levels in urine. Notably, and as predicted, ALN-GO1 significantly reduced urinary oxalate levels in both a mouse model of PH type 1 and a rat model that normally exhibits high levels of oxalate in the urine. These preclinical findings support further investigation into whether ALN-GO1 may have the potential to benefit patients with PH type 1.


KIDNEY TRANSPLANTATION

Promising Result Reported from Multi-center Kidney Transplantation Study: Researchers have reported a survival benefit for people who received kidney transplants from HLA-incompatible live donors compared with either those remaining on the kidney transplant waiting list or those who received kidney transplants from immune system-compatible deceased donors. Human leukocyte antigen (HLA) is a protein on the surfaces of human cells that identifies the cells as “self” or “foreign,” and performs essential roles in immune responses. There are multiple forms of HLAs, which vary among individuals and are analyzed in laboratory tests to determine whether one person’s organs and tissues are compatible with another person’s, and could be used in a transplant. The more closely the HLAs match between a donor and recipient, the less likely a transplant will be rejected by the recipient’s immune system. To overcome HLA-incompatible transplants, organ transplant recipients undergo “desensitization” protocols to remove antibodies in the blood that can harm the donated organ. Previous research from a single center indicated a survival benefit with kidney transplants from HLA-incompatible live donors as compared with those waiting for a compatible organ.

To assess whether the survival benefit seen in the single-center study is generalizable on a national scale, a 22-center study was designed and conducted. The researchers assessed the survival of people who received kidney transplants from HLA-incompatible live donors, at multiple time points up to 8 years after transplantation. They compared these outcomes with the survival of two control groups—those who remained on the waiting list or received a transplant from a deceased donor, and those who remained on the waiting list but did not receive a transplant. The multicenter study reported that a kidney transplant from an HLA-incompatible live donor was associated
with a significant survival benefit compared to the two control groups. As a compatible live kidney donor is rarely available, these results suggest that patients now could consider the option to undergo incompatible transplantation.


**INSIGHTS INTO UROLOGICAL PAIN SYNDROMES**

**Evaluating Nervous System Involvement in Women with Chronic Pelvic Pain:** Researchers have gained some new insights into nervous system contributions to chronic pelvic pain conditions in women. Nerves that are part of the autonomic nervous system (ANS) regulate bodily activities that generally run without conscious thought, such as heart rate, blood pressure, digestion, and bladder function. If ANS nerves malfunction or become damaged, however, a person can experience symptoms such as dizziness, increased or decreased sweating, and problems with urination. Researchers had previously detected possible malfunction, although not damage, in the ANS of women with interstitial cystitis/bladder pain syndrome (IC/BPS), a chronic urologic pelvic pain syndrome whose symptoms include urinary frequency, urgency, and pain with bladder filling. However, those initial results were complicated by the fact that many of these women also had another, distinct pelvic pain problem unrelated to bladder function, called myofascial pelvic pain (MPP).

In the present study, the same research team sought to distinguish ANS problems in women with IC/BPS versus other pelvic pain. Study volunteers included women with IC/BPS, women with MPP, women with both, and healthy women without either condition. These different groups of women all participated in a series of tests designed to detect the presence and extent of ANS abnormalities. For example, one test involved lying flat for several minutes on a tilt table that was subsequently raised partway upright, enabling the researchers to measure how effectively participants’ bodies adjusted to the tilt-associated drop in blood pressure. Other tests included breathing tests to measure heart rate and blood pressure, and a sweat test. The results of the tests suggest that women with MPP experience one type of ANS damage, autonomic neuropathy, more frequently than women with IC/BPS only.

Intriguingly, many of the women with any chronic pelvic pain—but none of the healthy women—reported symptoms such as dizziness when moved upright in the tilt-table test, but without any measurable changes in vital signs. This result suggests that the women’s symptoms were related to a heightened awareness of or sensitivity to environmental stimuli that can occur in pain conditions, rather than to ANS abnormalities. Finally, the researchers noted an increase in baseline heart rate among all the women with pelvic pain conditions compared to healthy women. Combining this result with observations from their earlier study, they suggest that women with chronic pelvic pain, particularly women with IC/BPS, may have systemic neural changes rather than nerve problems restricted to, for example, the bladder. The indicator of this systemic change, called vagal tone withdrawal, can potentially be addressed therapeutically.

Clarifying underlying causes of symptoms in chronic pain conditions is helpful for clinical treatment; thus, these new insights into the variable involvement of the ANS in chronic pelvic pain conditions in women could be helpful in future therapeutic strategies.


**Unique Microstructural Changes in Brains of People with Urologic Pain Syndromes Revealed:** Scientists using advanced imaging technology have identified a number of minute and specific brain changes associated with urologic chronic pelvic pain syndromes in people. Research has shown that people with chronic pain conditions, including people with the urologic chronic pelvic pain syndromes (UCPPS) interstitial cystitis/bladder pain syndrome or chronic prostatitis/chronic pelvic pain syndrome, exhibit changes in various brain regions, many related to pain perception. However, there is also reason to believe that unique sets of changes, or “brain signatures,” can be identified for specific chronic pain conditions, potentially leading the way to improved understanding, diagnosis, and therapy for these conditions.

Building on prior studies, researchers in the NIDDK’s Multidisciplinary Approach to the Study of Chronic Pelvic Pain Research Network used noninvasive magnetic resonance imaging (MRI) technology in two different ways to capture as much information as possible that might reveal such brain signatures in men and women with UCPPS.
Using these approaches, they compared brain images between people with UCPPS, healthy people, and people with a different visceral pain syndrome, irritable bowel syndrome (IBS). They found several microstructural differences throughout the brain between people with UCPPS and healthy people, including in areas involved in perceiving and responding to pain. By comparing the images from people with UCPPS and people with IBS, they could see that the changes were not identical between these two pain syndromes. Combining these analyses, the team identified at least three brain regions with changes that differed between people with UCPPS and the two other groups. They also looked to see whether any of the observed brain changes correlated with UCPPS symptom severity and/or duration, and the two other groups. They also looked to see whether any of the observed brain changes correlated with UCPPS symptom severity and/or duration, and found a few that did. This finding also suggests that the observed brain changes are a response to, rather than precede, chronic pain. Finally, because there are known sex-based differences in pain and pain syndromes, they also examined whether, with their approaches, they could see differences in the brain microstructure between women and men in any of the three groups. Interestingly, while a variety of male/female differences were observed within each group, the fewest differences were seen among those with UCPPS—raising the possibility that, if therapies emerge based upon “brain signatures,” men and women with UCPPS may benefit from similar treatments.

This exploratory research suggests that microstructural brain alterations specific to UCPPS exist. Future research may now focus on such questions as whether other changes can be detected, whether they and/or the changes observed in this study vary over time or in response to symptom changes or treatments, and whether one or more of these changes might emerge as a useful biomarker or “signature” for UCPPS versus other pain syndromes.


**UNDERSTANDING HEMATOLOGICAL DISEASES**

**Revving Up Human Red Blood Cell Production:** A recent study demonstrates that “turning off” a single gene significantly increased production of human red blood cells (RBCs) in the laboratory. The mechanisms controlling the transition from embryonic stem cells (ESCs) or early stage (progenitor) blood cells to mature RBCs are not well understood, but such knowledge could provide critical insight into how to produce blood cells in the laboratory.

While characterizing samples obtained from 4,678 volunteers, researchers discovered 11 rare mutations in the *SH2B3* gene associated with higher hemoglobin and hematocrit levels. Hemoglobin carries oxygen in RBCs from the lungs to the rest of the body, and the hematocrit measures the percentage of the blood that consists of RBCs. Thus, people who have a rare *SH2B3* mutation have higher levels of RBCs in their blood.

To confirm that the *SH2B3* genetic mutation was responsible for the increased RBC production, two different genetic approaches were used to essentially block the function of the gene in human ESCs and progenitor cells, which can mature to become various types of blood cells. The maturation of human ESCs and progenitor cells into RBCs is accomplished in the laboratory by the addition of a cocktail containing various factors identified from previous research. In the first approach, they used a technique called RNA interference to prevent cells from making the protein encoded by the *SH2B3* gene by disrupting a key intermediate in the protein-making process, a copy of the gene made of RNA. The scientists found that this approach successfully increased RBC production by at least three-fold. The increased RBC production was due to both increased maturation and increased production, and the newly produced RBCs appeared to have similar size and shape to natural RBCs. In the second approach, the *SH2B3* gene was inactivated in a line of human ESCs by the CRISPR/Cas9 system, which enables the gene’s DNA to be edited with unprecedented precision. (The NIH supports research using human ESCs within the *NIH Guidelines for Human Stem Cell Research.*) The results of this set of experiments showed that ESCs lacking a functional *SH2B3* gene produced approximately three-fold more RBCs than human ESCs having the intact gene. Although the RNA interference method would be difficult to scale-up to produce sufficient quantity of RBCs for clinical use, the CRISPR/Cas9 system could permanently shut-off the *SH2B3* gene in a renewable cell line, which could help enable larger-scale production of blood cells. Thus, the results of two different genetic approaches identify the *SH2B3* gene as having a mechanistic role in the ability of stem and progenitor blood cells to develop into RBCs in the laboratory.

*NIH Recent Advances & Emerging Opportunities: Kidney, Urologic, and Hematologic Diseases*
This newly acquired knowledge may contribute to future efforts to improve RBC production for medical applications such as replacement therapy during acute blood loss as a result of trauma or surgical procedures. This research may also lay the foundation to produce cells of rare blood types for people who need very specific types of blood not available via donated blood resources.


The Double-edged Sword of a Pro-inflammatory Protein:

New research delineates acute versus chronic effects of interleukin-1 (IL-1) exposure on blood stem cell fate. Inflammation is one of the immune system's responses to insults such as a bacterial infection or a splinter piercing a finger. Many different immune cells can take part during the process of inflammation. The immune cells release substances, also called inflammatory mediators, that direct blood stem (precursor) cells toward one of two pathways—production of more stem cells (self-renewal) or maturation into specialized cell types. One such mediator is IL-1, which plays a central role in fighting infections. IL-1 was previously shown to be an “emergency” signal to increase numbers of certain types of blood cells when needed. However, the consequences of acute (short duration) versus chronic (long-lasting) exposure of blood stem cells to IL-1 is largely unknown. Because chronic inflammation is a feature of a number of diseases and conditions, further understanding of inflammatory mediators, like IL-1, may lead to new ideas for treatment strategies.

To learn more about the way IL-1 exerts its effect, researchers designed studies using both isolated mouse blood stem cells and normal male and female mice. Under conditions of acute exposure, IL-1 significantly increased the rate of blood stem cell division into new daughter cells. IL-1 directed the new daughter cells to mature into blood cells called macrophages and granulocytes—members of the so-called myeloid family of blood cells—which have the ability to ingest and degrade invading bacteria. Consistent with findings using isolated cells, when the researchers injected mice once (acute exposure) with IL-1, they found a rapid increase in the percentage of myeloid cells in circulating blood, while levels of lymphoid cells—which include T cells and B cells of the immune system—and red blood cells declined. To assess IL-1 action on blood cell production on chronic, as compared to acute exposure, the researchers injected mice once or once daily for 20 days (chronic) and determined the levels of myeloid and lymphoid cells in bone marrow. The researchers reported a loss of early stage lymphoid cells following a single IL-1 injection. Following 20 days of IL-1 injections, there was a significant change in the composition of bone marrow cells—a significant increase in myeloid cells and a significant decrease in lymphoid cells. Thus, chronic IL-1 exposure alters the bone marrow’s ability to maintain a balanced blood cell population. The researchers also found that IL-1 impairs blood stem cell self-renewal. In humans, this could negatively affect the outcomes of anti-cancer procedures such as autologous stem cell transplants, which require the regrowth of a patient’s immune system from his or her own functional stem cells. Notably, the researchers found that upon IL-1 withdrawal, the bone marrow will “reset” to the numbers of myeloid and lymphoid cells and the capacity for stem cell self-renewal observed prior to IL-1 treatment.

This study, using isolated mouse blood stem cells and mice, identified IL-1 as a critical regulator of blood stem cell fate. Future research will be necessary to determine whether IL-1 underlies blood stem cell fate and changes in numbers and function of in blood cells in the context of chronic inflammation in humans.


Ramping Up Fetal Hemoglobin Production—Implications for Red Blood Cell Diseases: Research teams have provided new information regarding how DNA binding proteins help “turn off” production of fetal hemoglobin (HbF), which may lead to new ways to “turn on” HbF production to treat certain red blood cell diseases. People with one such disease, sickle cell disease, suffer from chronic anemia and episodes of bone, joint, and muscle pain, as well as other complications, because their red blood cells form rigid, “sickle” shapes in small blood vessels, leading to shortened red blood cell survival and impaired blood flow and oxygen delivery to tissues. This disease results from genetic mutations that affect the form of hemoglobin often called “adult” hemoglobin, even though its production begins soon after birth. Individuals with another genetic disorder of hemoglobin, β-thalassemia, also suffer from chronic anemia caused, in their case, by impaired adult hemoglobin production, which results in reduced numbers and viability of red blood cells.
Although HbF is mostly undetectable in adults and children (after about 6 months of age) in the general population, increased levels safely persist to varying degrees in some people. Researchers have observed that people with sickle cell disease who retain higher levels of HbF have less severe disease. Thus, one potential treatment approach is to reactivate HbF production, with the hope that, at sufficient levels, it could compensate for both the defective function of adult hemoglobin in sickle cell disease and the impaired synthesis of adult hemoglobin in β-thalassemia.

Previous research has shown that the DNA binding protein BCL11A prevents the production of HbF and that when BCL11A is absent, HbF levels increase. Building on these research findings, the current study explored the enhancer region of the BCL11A gene—a DNA element that helps “turn on” the gene—to see whether changes in the enhancer would “turn off” BCL11A’s production. To test systematically the importance of different parts of the enhancer, the researchers used a gene-editing tool to remove tiny sections of DNA along the length of the enhancer in stem cells or progenitor cells. These early stage cells were allowed to mature into red blood cells and tested for their ability to produce HbF. The deletion of a discrete region termed “h+58” within the human BCL11A enhancer resulted in decreased BCL11A production and increased HbF production. These results provide the foundation for the potential use of therapeutic genome editing of the BCL11A enhancer to raise the levels of HbF in people with hemoglobin-related diseases (hemoglobinopathies).

Another approach to “turn off” BCL11A production involves the use of small hairpin RNAs (shRNAs). The genetic information stored within DNA is encoded into messenger RNAs (mRNAs), which in turn are translated into proteins. Translation of mRNA into proteins can be “silenced” by synthetically produced, hairpin-shaped shRNA molecules which can be designed to cause the degradation of specific mRNAs. Targeting BCL11A mRNA in this way, for example, would prevent BCL11A protein from being made. In this recent study, investigators used an experimental system to deliver DNA encoding shRNAs targeting BCL11A to both human and mouse blood stem cells, where the shRNA was then produced. Initially, they designed the system for shRNA to be produced at high levels in many types of cells. The researchers’ findings showed that though this system reduced BCL11A protein levels, it also caused unwanted side-effects in both human and mouse cells: decreased numbers of blood cells—B cells, monocytes, and granulocytes—once the shRNA-treated cells were transplanted into living animals. The decreased numbers of these various blood cells were attributed to loss of BCL11A function in these cells and would be a major hurdle for future therapeutic use of this system.

Researchers hypothesized that they could overcome the negative impact of BCL11A loss in B cells, monocytes, and granulocytes by targeting the BCL11A-specific shRNA production only to red blood cells. To test this, they designed a system that would only produce shRNA in progenitor and mature red blood cells and thus would cause red blood cell-specific loss of BCL11A protein. Using this new “lineage-specific” approach, the investigators reported normal levels of transplanted B cells, monocytes, and granulocytes while maintaining the desired effect of reducing BCL11A protein and increasing HbF production in red blood cells. Furthermore, when they used this lineage-specific system to reduce BCL11A in mouse blood stem cells, and then transplanted those cells into a mouse model of sickle cell disease, they observed a substantial reduction in disease markers.

In addition to BCL11A, the DNA binding protein LRF has recently been identified as having the ability to modulate HbF production. LRF was initially discovered in this study for its ability to prevent production of HbF; when the gene that encodes LRF was genetically inactivated in red blood cells of adult mice, HbF levels increased. The investigators further showed that LRF binds to the HbF gene in a way that prevents production of HbF. Although both BCL11A and LRF bind to the HbF gene, they apparently do so in different ways, demonstrating that these proteins share common features yet have distinct mechanistic actions.

Together, these studies contribute new knowledge about BCL11A and LRF control of HbF production and may enable the development of safe and effective gene therapies to reactivate HbF production in patients with hemoglobinopathies.


Chronic kidney disease (CKD) is a major public health problem in the United States. The impact of CKD is substantial and includes increased risk of death, diminished quality of life, numerous co-associated diseases and conditions, such as cardiovascular disease (CVD, which includes heart disease and stroke), and significantly increased risk of progression to kidney failure (end-stage renal disease). As symptoms are few or non-existent, most people are unaware they have CKD until most kidney function has been lost. Understanding the risk factors for progression of CKD and associated CVD is necessary to design clinical trials, and identify candidate therapies to be tested in clinical trials to reduce the impact of CKD. This requires detailed evaluation and long-term follow-up of individuals with significantly diminished kidney function.

To identify the risk factors for loss of kidney function and the link between kidney and heart diseases, the NIDDK established the Chronic Renal Insufficiency Cohort (CRIC) observational study in 2001. This epidemiologic study seeks to study the distribution and determinants of health-related events in the CKD population and apply this acquired knowledge to improving health. Between 2003 and 2008, CRIC recruited nearly 4,000 men and women with CKD, about one-half of whom are African American and approximately one-half of whom reported they had diabetes. This cohort included 327 Hispanic Americans with CKD recruited through an ancillary project designed to augment the CRIC Study’s ability to assess this large and growing population. From 2013-2015, CRIC recruited a second cohort of nearly 1,600 men and women. This second cohort is older and on average has a higher level of kidney function than the first cohort, and enables the study of frailty, other features of accelerated aging, and the identification of risk factors for loss of kidney function earlier in the course of CKD. All study participants have annual in-clinic visits consisting of standard blood, urine, and other tests measuring kidney, heart, and vascular health as well as an interim telephone contact between clinic visits. Because very few of the participants have dropped out, the CRIC study has generated a very complete and robust database that is being used by the research community.

To leverage the NIDDK’s investment in CRIC, over 80 ancillary studies are either ongoing or have been completed. Most of these are investigator-initiated research projects to further analyze data and participants from the CRIC study. This effort has not only expanded the scope of the science conducted by CRIC investigators, but also has increased the number of investigators and institutions affiliated with this long-term epidemiologic study. To further educate and engage the research community, the CRIC study group conducts annual data workshops to facilitate the use/analysis of the CRIC data available through collaborative ancillary studies and through the NIDDK Central Repository.

Over 130 research papers have been published to date describing CRIC findings. Highlights include the following notable research advances:

**Key Link Discovered Between Kidney Disease and Heart Disease:** CRIC investigators have reported that high levels of a hormone called FGF-23, which regulates phosphate metabolism, are associated with an increased risk of CVD in patients with CKD. Elevated FGF-23 levels were shown to be associated...
with increased risk of structural heart disease (abnormal elevations in the size of the heart’s left ventricle) and heart failure (insufficient pumping of the heart that leads to retention of body fluids and congestion of the lungs). Experiments conducted in animal models supported the findings found in CRIC Study participants. For example, mice developed enlarged left ventricles following injection of FGF-23. This and other experiments suggest that FGF-23 may play a direct causal role in the heart disease seen so commonly in the setting of CKD.

**Genetic Variation and Progression of Chronic Kidney Disease:** CRIC investigators, in collaboration with other researchers, reported that APOL1 gene variants significantly contributed to the faster CKD progression in African Americans compared with Caucasians. This effect was observed regardless of whether participants had diabetes. This was the first report that showed APOL1 gene variants not only increase risk for CKD, but also affect kidney disease progression across the broad spectrum of CKD.

**Higher Urinary Excretion of Sodium Is Associated with Increased Risk of Cardiovascular Disease:** CRIC researchers have reported that higher levels of sodium in the urine are associated with increased risk of CVD. The analysis included the collection and analysis of urine samples and the CVD outcomes that ensued. CVD outcomes included congestive heart failure, stroke (death of brain cells due to inadequate flow of oxygen-rich blood to a portion of the brain), or heart attack.

The landmark CRIC study has provided invaluable insights into CKD risk and progression (and associated cardiovascular disease) in the United States. Its findings have important implications for understanding the differences in kidney disease risk across populations. Moving forward, physicians may be able to make better choices about when to start screening for kidney disease and how to choose an appropriate therapy by identifying which patients have risk factors for CKD and progression to kidney failure.

The CRIC study is one of the largest and longest ongoing studies of CKD epidemiology in the United States. It is a collaboration among 13 U.S. clinical sites—Case Western Reserve University, Cleveland; the Cleveland Clinic, Cleveland; The Johns Hopkins Medical Institutions, Baltimore; Kaiser Permanente Northern California, Oakland; MetroHealth Cleveland, Cleveland; St. Johns Medical Center, Detroit; Tulane University, New Orleans; University of California San Francisco, San Francisco; University of Illinois, Chicago; University of Maryland, Baltimore; University of Michigan, Ann Arbor; University of Pennsylvania, Philadelphia; and Wayne State University, Detroit. The study’s Scientific and Data Coordinating Center is at the University of Pennsylvania.
Delineating the Anatomy of the Developing Genitourinary Tract

The genitourinary (GU) tract is the organ system (kidney, ureter, bladder, urethra, prostate, testis, epididymis, vas deferens, penis, ovary, uterus, and vagina) most commonly affected by inherited birth defects, and many reports suggest that some of these birth defects are increasing. Thus, investigations defining normal embryonic development of the GU tract have significant clinical relevance, given the significant health impact of inherited as well as acquired diseases of this organ system. Understanding normal development can help researchers gain insights into what processes go awry in disease, and can lead to the design of therapies. Until recently, GU developmental research was limited by lack of cell-specific markers for key cell lineages (e.g., podocytes, parietal cells, and mesangial cells), incomplete understanding of the normal cellular structure of the major organs of the GU tract, and the lack of a detailed integrative database to understand complex data linked to developmental processes during specific locations in time and space.

The GenitoUrinary Development Molecular Anatomy Project (GUDMAP)

In 2004, the NIDDK and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) established the GUDMAP consortium to begin efforts to identify the cell types that comprise the developing organs of the GU tract and their locations and to generate tools to facilitate research. The major goal of this discovery science effort is to provide the scientific and medical community with a searchable molecular and cellular atlas and cell-specific transcriptional profiles (whether genes are “on” or “off”) to facilitate research. Examples of GUDMAP data include an atlas of gene expression in the developing mouse and human kidney, and illustrative anatomical subcompartments of the developing mouse lower GU tract including the associated male and female reproductive structures.

In addition to providing tools to the research community, GUDMAP investigators have made significant scientific contributions. For example, a recent study identified a potential role of the Six2 protein in the species differences between mice and humans in determining the duration of kidney development and the final nephron (functional unit of the kidney) number in the mature kidney. This will allow investigators to model and understand human development better.

Investigator-initiated Research

The Institute also supports a robust and productive investigator-initiated research program that includes studies into repair mechanisms and translational studies. Highlights include the following research advances.

Proteins FGF9 and FGF20 have been shown to be both necessary and sufficient for the maintenance of early stage (progenitor) cells that mature into the mouse nephron. Understanding the factors that regulate the size of the pool of nephron progenitor cells that gives rise to nephrons is important, as lower nephron numbers predispose an organism to higher risk of hypertension and kidney disease. In addition, investigators have identified a second pool of self-renewing progenitor cells that gives rise to the stromal tissue—the connective tissue of the kidney—and contributes to the nephron progenitor cell pool.

As progenitor cells mature into the specialized cells of the nephron, the pool of nephron progenitor cells...
decreases and the remaining “older” progenitor cells have a reduced capacity to self-renew (proliferate) and mature more quickly into nephrons. Investigators have shown that “older” progenitors can be rejuvenated when brought into contact with “younger” progenitors, thereby increasing the number of progenitor cells within the pool. These new findings indicate that progenitor cells are not “locked” into an aging process and provide a strategy to increase or replace the progenitors that mature into nephrons.

For the first time, investigators have developed laboratory-based procedures to isolate and expand numbers of mouse nephron progenitor cells by promoting their self-renewal, as well as to direct them towards a mature cell type. This “synthetic” niche sets the stage for studies of nephron development with the ultimate goal of tissue repair/replacement. Researchers reported that kidney mesenchymal-like stem cells isolated from the adult mouse kidney collecting duct can self-renew in the laboratory and, when injected into the mouse kidney, integrate back into the collecting duct.

Using information from GUDMAP that both the Vangl2 mRNA and Celsr1 mRNA are present in early-stage mouse kidney development, other investigators have shown that these two genes are necessary for kidney growth and proper maturation.

Taken together, these findings greatly advance knowledge of kidney development in the mouse.

Ongoing Consortia Efforts

Building upon these foundational research findings, the NIDDK is now supporting the following consortia to advance research progress through collective efforts.

- The Human GUDMAP (hGUDMAP) consortium builds upon the existing database and website infrastructure, and retains the basic long-term objectives of GUDMAP—the establishment of a comprehensive understanding of human kidney and urinary tract tissue development to inform the study of tissue maturation and aging, defective organ development, and changes that occur in disease.

- The (Re)Building a Kidney (RBK) consortium aims to develop new therapeutic options for kidney failure, including strategies to repair injured kidneys in the body or generate functional replacement tissue in the laboratory for transplant.

- New knowledge acquired through hGUDMAP and RBK, including the development of technologies and methods of studying tissue, will help jumpstart the Kidney Precision Medicine Project (KPMP). The KPMP aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury or chronic kidney disease, create a kidney tissue atlas, define disease subgroups, and identify critical cells, pathways, and targets for novel therapies.
SCIENTIFIC PRESENTATION

Dr. Joseph Bonventre—Kidney Repair and Regeneration Shop

Dr. Joseph Bonventre is Samuel A. Levine Professor of Medicine at Harvard Medical School, Boston. He is also Chief of the Division of Renal Medicine and the Chief of the Division of Engineering in Medicine at Brigham and Women’s Hospital. Dr. Bonventre earned his B.S. from Cornell University, followed by his M.D. from Harvard Medical School and Ph.D. in biophysics from Harvard University. After his medical internship and residency at Massachusetts General Hospital (MGH), Dr. Bonventre completed a clinical nephrology fellowship at MGH. He is a widely recognized scientist, clinician, and teacher, known for his research in various aspects of the cellular injury and repair mechanisms in the kidney. Dr. Bonventre has been recognized by his peers for numerous accomplishments, including elected membership to the American Society of Clinical Investigation, the Association of American Physicians, and the American Institute for Medical and Biological Engineering. He has been awarded the Osler Medal of the Royal Society of Physicians and the Bywaters Award from the International Society of Nephrology for his contributions to the field of acute kidney injury. Dr. Bonventre currently serves as Editor of Seminars in Nephrology and is on the Editorial Board of a number of journals. The NIDDK has supported his research for more than 30 years. At the January 2016 meeting of the NIDDK Advisory Council, Dr. Bonventre presented his laboratory’s recent research findings. The following are highlights from his presentation.

Dr. Bonventre has a longstanding interest in various aspects of the cellular injury and repair mechanisms in the kidney, with a special emphasis on the role of inflammation, biomarkers, and stem cells. Chronically impaired kidney function—also called chronic kidney disease (CKD)—often leads to end-stage kidney disease. CKD results in dysregulation of many body systems and is a major risk factor for cardiovascular disease. A considerable public health concern as well as a financial problem for the United States, CKD is increasingly important globally. This is partially driven by the growing epidemic of diabetes, of which CKD is a major side effect.

Over the past few years an underlying theme has emerged—a close relationship between CKD and acute kidney injury (AKI). In contrast to CKD, which usually progresses slowly over time, AKI is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. Dr. Bonventre pointed out that AKI leads to CKD, and CKD clearly predisposes to AKI. Additional research is needed to understand the mechanisms of this relationship, and to learn how to prevent AKI and prevent development and/or progression of CKD after AKI.

Kidney Repair and Regeneration

AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction usually caused by infection), decreased perfusion of blood, or kidney damage from drugs or toxins. AKI is associated with high in-hospital mortality rates. Even though most people with AKI who survive will regain some degree of kidney function, many do not. There is no effective drug therapy to mitigate or reverse AKI. Dr. Bonventre explained that under normal conditions the kidney tubule, made up of intact epithelial cells, functions by reabsorbing water and salts. When the mouse kidney proximal tubule is experimentally injured, the epithelial cells can be lost or damaged.
**SCIENTIFIC PRESENTATION**

Damaged epithelial cells lose their polarity—meaning that proteins that are assigned to be in one part of the cell become randomly dispersed. When the healthy kidney proximal tubule initially sustains an injury, several tissue and cellular responses can occur, including the death of epithelial cells that form the renal tubules, and injury to blood vessels resulting in stickiness of the endothelial cells that line the capillaries. White blood cells (called leukocytes) adhere to the cells and move across the damaged endothelium. Filtrate is blocked as it flows through the tubule, and there is an increased number of immune cells called “M1” macrophages that migrate into the kidney. Following injury, the tissue begins a process to repair itself. The tissue repair process can be “adaptive” or “maladaptive,” depending on factors such as the severity of the injury and the age of the organ.

In adaptive tissue repair, the repair process is successful, and a fully functional kidney is restored. Several cellular responses contribute to adaptive repair in the kidney, including the presence of immune cells called “M2” macrophages, which support an increase in the number of epithelial cells from pre-existing epithelial cells remaining in the tubule, reduced numbers of inflammatory cells, and an increase in the number of endothelial cells. Dr. Bonventre explained that his lab discovered that new epithelial cells derived from pre-existing epithelial cells during repair. These results were obtained from lineage-tracing studies—where tubule cells are biologically labeled, and daughter cells identified by the persistent presence of the label.

Maladaptive tissue repair is characterized by persistent inflammation, and an increased number of myofibroblasts, which are responsible for an increased deposition of extracellular matrix (also referred to as fibrosis) with corresponding organ dysfunction. To better understand the pathophysiology of kidney injury, a new mouse model of acute kidney injury was used to study the proximal tubules. The tubules reabsorb about two-thirds of the fluid filtered by the glomeruli, the filtering units of the kidney’s nephrons. After inducing a one-time injury in a specific region of these tubules, Dr. Bonventre and his colleagues observed severe tubular injury, along with the proliferation of tubular epithelial cells and the appearance of inflammatory cells. Following this single injury, the kidney recovered completely. However, when the researchers induced three injuries at 1-week intervals, they observed diminished cellular repair, with significantly increased kidney tissue fibrosis both in the glomerulus (glomerulosclerosis) and in the area of the tubules, as well as leakage of protein into the urine (proteinuria). The kidney tubule was unable to repair itself after three successive injuries, and a chronic disease process ensued.

These findings have been extrapolated to other situations, for example, in studies using the “Akita” mouse model of type 1 diabetes. After inducing a one-time kidney injury in these diabetic mice,
there is increased kidney fibrosis, proteinuria, and glomerulosclerosis, indicating progressive chronic kidney disease. When the researchers induced three injuries (one per week) to the proximal tubule at 1-week intervals, the consequences were so severe that the animals did not survive. From these results, Dr. Bonventre commented that animals with pre-existing conditions, such as diabetes or hypertension, may be much more limited in their ability to repair an insult to the kidney when compared to animals with no underlying diseases.

Dr. Bonventre further examined the state of kidney cells just after injury, to see whether or not the cells were replicating. Cells replicate themselves through an organized, step-by-step process called the cell cycle, which consists of phases for growth (referred to as G1, S, and G2 phases) and division (M phase). The cycle has checkpoints, which allow the cell to halt the cycle for repairs. Dr. Bonventre’s laboratory has provided evidence that injury to tubular epithelial cells, by three different experimental approaches, stalls the cells at the G2/M checkpoint—inhaling their ability to progress through the cell cycle to produce two daughter cells. Further examination elucidated the existence of a strong correlation between G2/M arrest in tubular cells and fibrosis. For example, the longer the tubular cells remain in the G2/M checkpoint, the greater was the production of molecules that promote fibrosis. Thus, under conditions when the injury is sufficient to prevent the normal process of DNA damage repair, the cells arrest in G2, leading to maladaptive repair. The progression to the maladaptive repair process in these animal model systems resembles the transition from acute kidney injury to chronic kidney disease in humans.

Dr. Bonventre explained that his research team was interested to learn why humans are unable to generate new kidney nephrons after birth, with the hope that such knowledge may lead to strategies to induce kidney nephrons in the body to regenerate after injury. Fish are capable of regenerating nephrons after birth, but mammals are unable to do this. Rats and mice can generate new nephrons up to 2 to 3 days after birth but it was not clear whether these were nephrons generated de novo (starting from the beginning) or nephrons that matured from early committed developmental structures present at birth. In an attempt to gain insight into whether the mouse kidney could be coerced to generate entirely new nephrons after birth, the kidneys of newborn mice were experimentally injured to remove nephrons at the day of birth. The finding, unfortunately, was that injured nephrons were not replaced even if the other kidney was removed at the time of surgery.

Another interest of Dr. Bonventre and his team is the formation of new kidney organoids in the laboratory. A kidney organoid is a three-dimensional, laboratory-grown set of cells that mimics characteristics of normal kidneys. Building on previous knowledge of how the normal kidney develops, the team began their quest to form kidney organoids using human induced pluripotent stem cells (iPSCs). iPSCs are cells that have been experimentally induced to revert to an earlier stage of development (embryonic stem cell-like) and are capable of developing into all the different cell types of the body. By trial and error (adding various compounds in a three-dimensional cell culture system), the iPSCs formed spheroids. By inhibiting a protein called glycogen synthase kinase 3β and adding growth factors in specific sequences, the spheroids developed into kidney organoids—possessing tubule- and glomerulus-like structures. To test whether the kidney organoids respond similarly to injury as do actual kidneys, the organoids were treated with a toxic compound (cisplatin) often used in cancer chemotherapy in humans and then assessed for injury by expression of the clinical biomarker kidney injury molecule-1 (KIM-1) previously discovered and characterized by Dr. Bonventre. As would be the case with
actual kidneys, the tubule-like structures within the organoid that expressed characteristics of the proximal tubule produced KIM-1 upon injury with low levels of cisplatin, whereas uninjured spheroids did not, indicating the response was specific to injured kidney organoids.

The kidney organoids were further investigated to determine their potential to serve as a functional model of polycystic kidney disease (PKD). PKD is a genetic disorder that causes numerous cysts to grow in the kidneys. Kidney cysts are abnormal sacs filled with fluid that result in chronic kidney disease (reduced kidney function overtime). Using a gene-editing technology called CRISPR/Cas9, genetic mutations were introduced into the genes known to contribute strongly to PKD cyst formation in iPSCs, and then the cells were subjected to the process to form organoids. Organoids containing mutations in PKD cyst-forming genes, that were exposed to a compound that is known to increase cellular cAMP, formed balloon-like, fluid-filled sacs that appear to model the human disease. This finding suggests that these genetically mutated organoids may serve as a faster way to screen drugs for potential therapeutic use in humans.

**Future Directions**

Dr. Bonventre closed by thanking his research team for their contributions. His research efforts on repair and rebuilding or replacing the kidney serve as a foundation on which to increase our knowledge to ultimately develop new effective therapies for people with kidney disease.
PATIENT PROFILE

Paying It Forward—A Physician-scientist with Type 1 Diabetes Participates in a Clinical Trial To Prevent Kidney Disease

As a pediatric emergency physician and research scientist who has type 1 diabetes, Robert Truckner has personally witnessed, as both a patient and a doctor, the remarkable technological advances made over the past few decades that have improved the lives of people with type 1 diabetes. However, he has been concerned about developing kidney disease and other diabetes-related complications since shortly after he was diagnosed with type 1 diabetes as a child in the 1970s. “You’re worried about kidneys,” he says, as well as other organs that could be adversely affected by type 1 diabetes, such as the eyes and the heart. These fears “have always been in my mind,” Robert remembers.

Living with Type 1 Diabetes, and the Fear of Kidney Disease and Other Complications

Robert was diagnosed with type 1 diabetes when he was about 10 years old. He recalls one year around Christmas, his observant mother noticed that he was excessively thirsty and hungry, and was losing weight—characteristics that he later learned were classic signs of type 1 diabetes. After visiting his equally sharp family doctor, followed by a trip to the hospital, Robert and his family were told the difficult news that he had type 1 diabetes. Because Robert was so young, it took some time for him to fully understand what this diagnosis meant. But soon he began to realize how managing his health would change his life. He
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learned to give himself insulin shots, to monitor his urine glucose levels, and to help maintain his health by living a very active lifestyle.

Type 1 diabetes presented a variety of challenges throughout life. As a child, Robert spent years trying diligently to control his blood sugar as best as was possible at the time, but without the benefit of a robust community nearby to provide support. “It was a very isolating experience,” Robert remembers; “I didn’t know anybody who had diabetes—no one.” But that changed when he was 19 years old, and he began working at Camp Midicha—a summer camp run by the Michigan Children’s Diabetes Association—where he interacted with kids ages 6 to 16 who had diabetes. Initially he was a counselor, and later became its Director for a couple of years. “This was mind-opening for me,” says Robert of his time working with these youths at Camp Midicha, “just a phenomenal experience.”

Robert remembers always planning a career in medicine in the back of his mind. As a young teenager, he wrote a letter to a prominent diabetes center asking a physician there for advice about whether he should become a physician himself, considering his diabetes. He remembers the response: “I got a letter back … but they discouraged me, really, from becoming a physician,” noting that the hours are long and will take a toll on his health. While the letter was somewhat dispiriting, Robert credits his parents with instilling in him the perseverance to pursue his dreams. This was a value that “my Mom and Dad ingrained in me from a very young age … that there was nothing I couldn’t do, even with diabetes.”

Even with the new and improved technologies currently available to help Robert and other people with type 1 diabetes manage their disease, it is difficult even for the most vigilant patients to achieve levels of blood sugar control that research has shown can reduce the risk of long-term complications, including kidney disease. Thus, in addition to providing support for studies that have contributed to technology development and improved understanding of the long-term effects of diabetes, NIDDK is also funding research to identify other approaches to prevent and treat diabetes-related complications. Toward this goal, in fall 2015, Robert enrolled in an NIDDK-funded study investigating whether an inexpensive drug, called allopurinol, can help slow the decline of kidney function in people with type 1 diabetes and very early kidney damage.
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Diabetic Kidney Disease and the PERL Clinical Trial

Diabetes is the most common cause of kidney disease and can lead to kidney failure, requiring dialysis or kidney transplantation—both highly invasive treatments. A growing number of research studies suggest that reducing the level of the bodily waste product uric acid in the blood could help curb the deterioration of kidney function. The drug allopurinol has been used for decades to lower uric acid levels in patients with gout, which is a painful condition that occurs when uric acid is deposited as needle-like crystals in the joints and/or soft tissues. Allopurinol exhibits an excellent safety profile and is a generic, relatively inexpensive drug—attractive characteristics for a potential preventative therapy for a prevalent condition.

The NIDDK-supported Preventing Early Renal Function Loss in Diabetes (PERL) clinical trial is investigating whether allopurinol can prevent or delay the loss of kidney function in people with type 1 diabetes and very early kidney damage. Several centers around the United States and Canada have enrolled patients so populations from different geographic locations will be included in the study. The primary outcome measurement for the study is glomerular filtration rate (GFR)—the rate at which kidneys filter wastes and extra fluid from the blood, serving as a measure of kidney function.

Patients enrolled in the trial, including Robert, have relatively high serum uric acid levels but only mildly or moderately decreased renal function. PERL is a double-blind randomized clinical trial, which means that study participants receive either allopurinol or a placebo control, but neither the participants nor the scientists who interact directly with them know which patient receives which treatment through the course of the study. Thus, because the trial is still ongoing, Robert does not yet know whether he is receiving allopurinol or placebo. Trial participants receive the treatment for 3 years, after which their GFR levels, as well as other conditions, will be compared to see whether there are differences in health between those who received the different treatments.

A Unique Perspective: Patient, Physician, and Scientist

As a physician and scientist who has been involved in conducting clinical trials, Robert is well-aware of logistical and other issues participants face when enrolling in clinical research studies. Based on his previous experiences, Robert feels that the PERL trial is not as demanding as some other clinical trials. “As studies go,” he says, “this is a piece of cake.” In addition to taking daily pills (either allopurinol or placebo), participants visit the clinic every few weeks for weight measurement and blood sugar analysis, and for kidney function tests. Due to Robert’s schedule as an emergency physician, coordinating these visits is fairly easy for him, but he acknowledges that “people who work 9-to-5 jobs… may have more difficulty.” He also notes that while he lives in Spokane, Washington, near one of the sites where the trial is being conducted, study participants are being recruited from a larger area of the Pacific Northwest, so some people may have to travel longer distances.

Interestingly, through conversations with research staff, Robert was made aware of a potential unexpected benefit of participation in the PERL study for some people. He learned that by simply engaging frequently with the well-trained staff, some
participants were given valuable advice in passing, such as tips on making healthy dietary choices, that they might not have otherwise received. “I think a secondary effect of these studies for people who have poorly controlled blood glucose levels,” he says, “is that they get some diabetes education along the way.” In this way, study nurses and others help improve diabetes care for some study participants.

Hope Through Research

Robert recognizes that improvements in technology—made possible through scientific research—have led to improved quality of care for people with type 1 diabetes over the past few decades. By participating in the PERL trial, he will be a part of the ongoing research efforts to develop the next potential wave of life-improving or life-saving therapeutics. The idea of preventing kidney disease with allopurinol gives Robert hope for all people with type 1 diabetes, but particularly for today’s children. “There’s this whole generation of kids” growing up with the fear of developing kidney disease, and “if I can use ... an old medication to save my kidneys,” he ponders, or “to at least study if it does, and it works—wouldn’t that be wonderful?”

From his varied experiences, Robert knows that clinical trials aimed at addressing important health issues, such as preventing kidney disease, require commitment on many levels, by many groups of interested people. Robert expresses great appreciation to all those involved. “I want to say thank you ... thank you to the scientists. I want to say thank you to NIDDK, and the other study participants,” he reflects. “Thank you for caring.”