

Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. The body uses an array of iron transport proteins to properly maintain iron levels in cells and tissues. Defects in iron transport proteins have been identified in humans, and result in too little iron or too much iron in the body. Strategies that restore normal iron transport in these conditions would be very beneficial to these people. Research described in this chapter shows that a compound called hinokitiol is able to correct an iron transport defect in zebrafish. In this figure, brown staining indicates hemoglobin production in (from left to right): normal zebrafish (+/+); zebrafish partially missing an iron transport protein (+/frs); zebrafish completely missing an iron transport protein (frs/frs); and (final two panels) zebrafish completely missing an iron transport protein (frs/frs) that were treated with hinokitiol.

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Kidney, Urologic, and Hematologic Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They afflict millions of Americans, 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 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, represents a life-threatening condition.

It has been estimated that more than 30 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 2015, over 701,000 patients received treatment for ESRD: over 493,000 received either hemodialysis or peritoneal dialysis, and over 207,000 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. Compared to non-Hispanic Whites and Asians, ESRD prevalence in 2015 was about 3 times greater in African Americans, 1.3 times greater in Hispanics, 1.2 times greater in American Indians and Alaska Natives.² All these represent reductions in relative risk of ESRD for these minority populations compared to non-Hispanic Whites over the past 15 years. In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The Institute supports a significant body of research aimed at understanding the biology underlying CKD and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. The NIDDK also supports studies

¹ Centers for Disease Control and Prevention. *National Chronic Kidney Disease Fact Sheet, 2017*. Atlanta, Georgia: U.S. Department of Health and Human Services, 2017.

² U.S. Renal Data System, *USRDS 2017 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, Maryland, 2017.

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

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 Urinary Stone Disease Research Network is: a) conducting a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conducting clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) providing data and collecting biological samples from the studies to create a resource for future researchers.

Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome—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.³ 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.⁴

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 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.⁵ Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as to provide new insights into non-surgical alternatives.

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

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

⁵ *Urological Diseases in America*. NIDDK, NIH Publication Number 12-7865, 2012.

As researchers continue to investigate treatment options, an equally important challenge is to identify and understand the important subgroups of patients with lower urinary tract symptoms (LUTS) through improved measurement of patient experiences of LUTS in men and women. To address this challenge, the NIDDK supports the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). LURN will develop new questionnaires that will improve diagnosis and more comprehensively assess outcomes of interventions. The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health and Office of Behavioral and Social Sciences Research established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal 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, which is currently focusing on regulatory determinants of hematopoietic stem cell fate; role of non-coding RNA in hematopoiesis; role of macrophages in blood cell development; effects of aging on hematopoiesis; metabolic modulators of hematopoiesis; and remodeling the hematopoietic stem-cell niche. The Institute's SHINE II program seeks to further catalyze research in basic or pre-clinical, proof-of-principle research projects that are tightly focused and directed at validating novel concepts and approaches that promise to open up new pathways for discovery in benign hematology research. The NIDDK is also keenly interested in the basic biology of stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research.

THE KIDNEYS AT WORK

Measuring How Well the Kidney Works—One

“Nephron” at a Time: Scientists developed a new method for calculating the average rate that a single kidney nephron filters blood—an important measure of kidney health. The basic functional unit of the kidney is the nephron, which consists of various cells and structures that work together to filter waste products, remove excess fluid from the blood, and balance various body chemicals. Of these structures, the glomerulus is the fundamental filtering apparatus. A common kidney function measurement called the glomerular filtration rate (GFR) is an estimate of blood filtered per minute by all the nephrons within the kidneys. However, calculating single nephron GFR is complicated for a number of reasons: there is substantial individual variation in the number of nephrons per kidney; there is variation in nephron size and in the amount of blood filtered per nephron; and availability of kidney biopsy samples is limited. Researchers have now developed a method for determining single-nephron GFR using biopsies from almost 1,400 people, with ages ranging from 20s to 70s, 58 percent of whom were women. These tiny biopsy samples were collected at the time of transplantation. People who donate their kidneys typically do not have chronic kidney disease or its major risk factors (e.g., diabetes, hypertension).

To calculate single-nephron GFR, the scientists first measured the total GFR and used a three-dimensional imaging technique that can determine the kidney's volume. Then they obtained a biopsy sample at the time of donation to determine the kidney's nephron density, as well as the average nephron size. With these measurements in hand, simple calculations revealed the number of nephrons per kidney (average of 860,000) and the single-nephron GFR for each individual. Several critical findings emerged from their analyses of the kidney donor cohort. The single-nephron GFR did not vary significantly by, sex, age (when under 70) or height (when under 6 feet, 2 inches). However, larger nephron size was associated with higher single-nephron GFR, as were hardening of glomeruli or blood vessels beyond what would be normally expected over time. In addition, some participant characteristics were linked to elevated single-nephron GFRs, including obesity, family history of end-stage renal disease, and height (over 6 feet, 2 inches).

Some of the characteristics found to be associated with elevated single-nephron GFR are known risk factors for kidney disease—seemingly contradictory findings, considering the clear association between lower total GFR and kidney disease. However, the scientists explain that some of these risk factors are often also associated with low numbers of nephrons in the kidney. This lower total number of nephrons could in turn cause individual nephrons to compensate by filtering blood at a higher rate, raising the total GFR to a normal level. However, in some people, over time the increased single nephron work load and other risk factors could lead to further nephron losses and kidney function deterioration, leading to eventual declining total GFR and kidney function. Further research would be needed to understand better how these characteristics affect the relationships between single-nephron GFR, total GFR, and kidney health.

The scientists recognize limitations in the study, such as the lack of diversity in the cohort. For example, only 2 percent of participants were Black. Also, the difficult and intensive process of kidney biopsy collection limited the study to donated kidneys. Due to the relatively healthy status of the kidneys selected through the donor screening process, additional research is needed to determine the generalizability of the results to those with reduced kidney function. Despite these limitations, this report establishes the first method for calculating single-nephron GFR from human kidneys, potentially leading to a greater understanding of the link between nephron GFR and overall kidney function and health.

Denic A, Mathew J, Lerman LO...and Rule AD. Single-nephron glomerular filtration rate in healthy adults. *N Eng J Med* 376: 2349-2357, doi: 10.1056/NEJMoa1614329, 2017.

Insights into Salt Handling, Water Balance, and Blood Pressure Regulation by the Kidneys: Two studies in mice have shed light on the complex relationships between kidney physiology, salt intake, water balance, and hypertension. One of the kidney's critical functions is to achieve electrolyte balance in the body by controlling urine salt concentration and water retention. Impairment of this essential function can lead to hypertension (high blood pressure). Two recent reports explored the links between salt, hypertension, and kidney function using rodent model systems.

Scientists have long believed that the body removes excess dietary salt through urination, leading to water loss that must be replenished—in other words, eating salty foods makes people thirsty. Recently, however, research has cast doubt on this simple relationship between salt and water consumption. In one previous study in 10 men, researchers found, surprisingly, that over time, increased salt consumption was associated with reduced water intake. In the present study, the team of researchers tested their previous observation experimentally using male mice that were fed either a low-salt diet with water or a high-salt diet with saline (salted water). Mice consuming a high-salt diet excreted more concentrated sodium in their urine than did mice on the lower-salt diet. Interestingly, over time the mice on a high-salt diet drank less fluid, retained more water, and consumed more food than did mice on a low-salt diet.

These results raised an important question: how does the body remove excess salt without simultaneously expelling too much water? The scientists considered that urea, a biological chemical abundantly found in urine, could be a key factor because urea in the kidney is known to drive reabsorption of water from developing urine. They found that the kidneys of mice on a high-salt diet contained higher levels of urea compared with those on a low-salt diet, helping to explain the observed water retention. Further examination of the mice revealed that additional urea was produced by muscle and liver tissue in response to increased salt. The muscle tissue appeared to be breaking down some of its molecular components as fuel to generate energy, likely to compensate for the energy-intensive process of urea production. This need for additional energy could also help explain the increased appetite observed in mice fed a high-salt diet. Together, these results uncover a novel coordinated, energy-intensive response to dietary salt by the liver, muscles, and kidneys to elevate urea levels, thereby conserving water.

In a separate study, scientists sought to gain a better understanding of the molecular basis of water maintenance and blood pressure regulation by the kidney. A segment of the nephron (the basic functional unit of the kidney) called the collecting duct fine-tunes the amounts of various essential substances, such as sodium, that can be retained in the body or excreted into the developing urine. The protein angiotensin II was

previously shown to control water reabsorption in the collecting duct. To better understand angiotensin II's role in the kidney, the researchers genetically engineered mice to lack the gene encoding the type 1 angiotensin (AT₁) receptor, its essential protein partner, specifically in the collecting ducts. AT₁ receptor-deficient mice had the same blood pressure as normal mice, and both groups experienced hypertension similarly when they were fed high-salt diets. Mice were then administered angiotensin II, which is also known to induce hypertension. Blood pressure in normal mice predictably increased, but surprisingly, blood pressure in AT₁ receptor-deficient mice rose even higher; this finding was unexpected because elimination of AT₁ receptors was expected to prevent angiotensin II's ability to raise blood pressure. These AT₁ receptor-deficient mice excreted less sodium than did normal mice when given angiotensin II, suggesting that the higher salt levels may have been responsible for the elevated blood pressure. The researchers then asked whether cyclooxygenase-2 (COX-2), a known regulator of angiotensin II function, was affected by AT₁ receptor deficiency. Drugs that inhibit COX-2 function have been shown to influence blood pressure, leading the scientists to ask whether there could be a link between COX-2 and AT₁ receptor activity in this segment of the kidney. They examined collecting ducts, and found that those of normal mice given angiotensin II contained higher levels of COX-2 than did their untreated counterparts, but the absence of AT₁ receptors attenuated this response. Finally, the scientists again treated mice with angiotensin II to induce hypertension, but also administered a chemical inhibitor of COX-2 function. The COX-2 inhibitor eliminated the difference between AT₁ receptor-deficient mice and normal mice, allowing the blood pressures of both groups to rise to similar levels, further implicating COX-2 as a mediator of angiotensin II-induced hypertension. Taken together, these results define a surprising, novel role in the collecting duct for the angiotensin II-AT₁ receptor-COX-2 molecular pathway as a regulator of blood pressure.

These studies in mice challenge long-standing views and reveal the complexity of the kidney's role in salt and water balance, and in blood pressure regulation. If the molecular pathways described are found to work similarly in people, these two studies could pave the way for a more detailed understanding of how the human body maintains water balance in response to salt intake, and could generate novel therapeutic approaches for reducing the risk of hypertension.

Kitada K, Daub S, Zhang Y,...and Titze J. High salt intake reprioritizes osmolyte and energy metabolism for body fluid conservation. *J Clin Invest* 127: 1944-1959, doi: 10.1172/JCI88532, 2017.

Stegbauer J, Chen D, Herrera M,...and Coffman TM. Resistance to hypertension mediated by intercalated cells of the collecting duct. *JCI Insight* 2: e92720, doi: 10.1172/jci.insight.92720, 2017.

The Sweet Smell of Success—A Protein in the Kidney Helps the Body Retain Sugar: In a new study, scientists identified a protein in the mouse kidney that helps prevent the body from excreting glucose (sugar), potentially revealing a molecular target for novel treatment strategies for type 2 diabetes. This protein is one of a family of proteins called olfactory receptors. Decades ago, these receptors were discovered in mammals as the molecular sensors of odorants. Olfactory receptors were originally found in the nose, detecting chemicals to give us our sense of smell. Members of this protein family have since also been found in numerous other tissues throughout the body, recognizing a variety of substances and serving an array of physiological functions. Scientists previously discovered one particular olfactory receptor, called Olfr1393, in the mouse kidney, but its physiological function was unknown.

To extend their previous research, the scientists investigated the role of Olfr1393 in the kidneys of male and female mice. They determined that the *Olfr1393* gene was turned on specifically in the kidney's proximal tubules, basic functional units of the kidney. Tubules reabsorb various substances filtered from the blood to retain nutrients, such as glucose, in the body, so they are not excreted in the urine. The scientists then genetically engineered mice to lack the *Olfr1393* gene. In many ways, *Olfr1393*-deficient mice were similar to normal mice: their kidneys were of similar size and filtered blood at the same rate, and the mice had similar blood pressures and body weights. However, the researchers observed that the urine of *Olfr1393*-deficient mice contained significantly higher levels of glucose, suggesting that their kidneys did not reabsorb the sugar as well as their normal counterparts. Mice lacking *Olfr1393* also tolerated higher levels of glucose than the normal mice could.

Glucose reabsorption is a critical function of the kidney. Two related proteins in the kidney proximal tubule are responsible for glucose reabsorption: SglT1 and SglT2.

Sgt12 returns 90 percent of the filtered glucose to the blood, and Sgt1 returns the remaining 10 percent.* The scientists found that overall levels of Sgt1 and Sgt2 were normal in *Olfr1393*-deficient mice. They also examined some of the traits of the mice for potential differences between the sexes, and, interestingly, found that the kidneys of male mice that lacked *Olfr1393* contained higher levels of Sgt2 protein than did kidneys from female mice lacking *Olfr1393*; this finding is consistent with reduced levels of urine glucose observed in these male mice. Next, the researchers more closely examined the kidney tissue and found that while overall Sgt1 levels were similar in all mice, the protein was not found in its normal location, the cell membranes of proximal tubule cells, in mice deficient in *Olfr1393*. These data suggest that Sgt1 function was impaired due to its inability to reach its intended location within the cell.

These results demonstrate the critical role of *Olfr1393* in regulating glucose reabsorption by the kidney. Improved tolerance of glucose in *Olfr1393*-deficient mice suggests that the olfactory receptor could influence the body's ability to regulate glucose levels. If *Olfr1393* functions similarly in people, it could represent a potential new target for the development of therapeutic strategies for type 2 diabetes.

**Sgt2 inhibitors are a new class of diabetic medications indicated only for the treatment of type 2 diabetes. In conjunction with exercise and a healthy diet, they can improve glycemic control.*

Shepard BD, Cheval L, Peterlin Z,...and Pluznick JL. A renal olfactory receptor aids in kidney glucose handling.

Sci Rep 6: 35215, doi: 10.1038/srep35215, 2016.

INSIGHTS INTO LOST KIDNEY FUNCTION

Kidney Damage from High Blood Pressure Leads to New Lymphatic Vessel Formation: Scientists have discovered that in rodents, hypertension- or aging-induced kidney injury and inflammation leads to the development of new lymphatic vessels in the kidneys. Hypertension (high blood pressure) is a leading cause of chronic kidney disease and kidney failure. Previous research has shown that a type of immune cell, called the macrophage, accumulates in the kidney and contributes to inflammation—a process that can lead to hypertension, which in turn

increases risk for kidney disease. In a new study, researchers examined the effects of hypertension on lymphatic vessels, which drain fluid, immune cells (such as macrophages), and waste products away from tissues. Hypertension can lead to the formation of new lymphatic vessels, a process known as lymphangiogenesis, in various organs. However, lymphangiogenesis can either be harmful or beneficial depending on various factors, such as the timing of the inflammatory response. Because lymphatic vessels have been implicated in kidney disease, the scientists hypothesized that hypertension could affect the dynamics of their formation in the kidneys.

The researchers used well-studied rat models, including two types of rats that spontaneously develop hypertension, referred to as SHR (for spontaneously hypertensive rat). One of these types of hypertensive rats also develops kidney damage (a strain called SHR-A3), while the other does not (called SHR-B2). These two strains were compared with a third that does not develop high blood pressure under similar conditions (called WKY). By examining molecular signals of lymphangiogenesis from kidney biopsies, the researchers found that kidneys of male SHR-A3 rats (prone to kidney damage) had a higher density of lymphatic vessels and increased lymphangiogenesis than did the kidneys of male rats without hypertension (WKY). By contrast, male SHR-B2 rats' kidneys had fewer lymphatic vessels and reduced lymphangiogenesis compared to kidneys from WKY rats. Molecular signals indicating the presence of macrophages and inflammation were stronger in SHR-A3 rats' kidneys than in kidneys from SHR-B2 or WKY rats, suggesting that immune cells had invaded the injured kidneys.

The researchers then examined the effect of aging on lymphangiogenesis in the kidneys. They used Fischer 344 rats, which spontaneously develop kidney disease over time, but not hypertension. When 4-month old male rats were compared to 20- and 24-month old rats, the scientists found that kidney injury from aging led to lymphangiogenesis and increased immune cell invasion, similar to the SHR-A3 strain.

These findings suggest that kidney damage, due to either a hypertensive state or the course of aging, can lead to increased macrophage-induced inflammation and density of lymphatic vessels in the kidney.

However, the rodent models in the study were used because they exhibit particular traits (e.g., strong resistance to hypertension or kidney damage) that may not represent a real-world situation in people. A more likely explanation in patients, the scientists suggest, is that hypertension induces subtler kidney inflammation, injury, and lymphangiogenesis than is seen in these animal models, consistent with the slower progression of the disease that is often observed in chronic kidney disease. Additional research would help determine whether lymphatic vessels could be useful targets for the development of therapeutic strategies to reduce risk for hypertension and kidney disease.

Kneedler SC, Phillips LE, Hudson KR,...and Mitchell BM. Renal inflammation and injury are associated with lymphangiogenesis in hypertension. Am J Physiol Renal Physiol 312: F861-F869, doi: 10.1152/ajprenal.00679.2016, 2017.

Predicting Kidney Function Decline in People Who Are at High Risk for Kidney Disease: A new study has found that levels of the protein suPAR in the blood can help predict whether kidney function will deteriorate in people with high-risk genetic variants of the gene *APOL1*. Genetic variants of *APOL1*, which are found primarily in individuals of African ancestry, are arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and among the only established genetic factors contributing to the well-appreciated health disparities in kidney diseases in Blacks compared to Whites. Individuals with one or two copies of either the G1 or G2 variants of the *APOL1* gene are protected from a potentially deadly infectious disease (African sleeping sickness) compared to people with only the G0 variant. However, those with any combination of two G1 and/or G2 variants of the gene are at increased risk of developing kidney disease.

Previous research showed that high levels of a protein called suPAR in the blood is associated with decline in kidney function and progression to chronic kidney disease. Because many people with the high-risk G1 and G2 *APOL1* variants do not develop kidney disease, researchers explored whether blood suPAR levels can help predict whether African Americans with these genetic variants will experience declining kidney function. The scientists analyzed data that had been collected from participants of two other studies, which together included almost

1,100 African-American participants. Kidney function was determined by evaluating estimated glomerular filtration rate, which is a commonly used measurement of how well the kidneys are filtering wastes and extra fluid from the blood. The scientists found that in people with high-risk *APOL1* variants, kidney function was likely to decline more rapidly over time in those with elevated plasma suPAR levels than those with lower suPAR levels.

Using another series of tests, the researchers asked whether suPAR could physically associate with the *APOL1* protein, which is encoded by the *APOL1* gene, and found that the G0, G1, and G2 proteins all interacted directly and tightly with suPAR. They also examined whether *APOL1* and suPAR could bind to $\alpha_v\beta_3$ integrin, a protein complex known to mediate the action of suPAR in kidney cells. When in an activated state, $\alpha_v\beta_3$ integrin formed strong protein complexes with suPAR and the high-risk G1 and G2 *APOL1* complexes; by contrast, it formed very weak complexes with low-risk, G0 variant *APOL1* proteins.

The scientists then examined the functional effects of these protein interactions by measuring the stimulation of β_3 integrin, a component of the $\alpha_v\beta_3$ integrin protein complex, in cultures of a type of human kidney cells called podocytes. The G1 and G2 variants of *APOL1* could activate β_3 integrin, but only when suPAR was added to the culture; the *APOL1* G0 variant could not activate β_3 integrin under any condition. They then injected female mice with DNA encoding either human G0, G1, or G2 *APOL1* variants and found that mice with G1 or G2 excreted high levels of protein in their urine, and their podocytes appeared to be physically injured—two indicators of kidney damage. The G0 variant had no effect on these mice. However, when the *APOL1* G2 variant was produced in mice that were genetically engineered to lack suPAR, urine protein levels were the same as in normal mice.

Taken together, these findings reveal that high levels of suPAR may play an important role in the kidney function decline observed in some people with *APOL1* G1 and G2 variants, and thus may serve as a useful predictor of kidney disease. The direct interactions observed between *APOL1*, suPAR, and $\alpha_v\beta_3$ integrin proteins help provide a mechanistic explanation of how kidney damage develops in those with high-risk

APOL1 genetic variants, and thus may pave the way for new therapeutic strategies to prevent or treat kidney disease in these populations.

Hayek SS, Koh KH, Grams ME,...and Reiser J. A tripartite complex of suPAR, APOL1 risk variants and $\alpha_v\beta_3$ integrin on podocytes mediates chronic kidney disease. *Nat Med* 23: 945-953, doi: 10.1038/nm.4362, 2017.

KIDNEY STONE PREVENTION

New Treatment Prevents Formation of Cystine Stones, a Type of Kidney Stone, in Mice: In a recent study, the antioxidant α -lipoic acid was shown to prevent the formation of cystine stones in mice. Kidney stone disease, also referred to as urinary stone disease, is a painful and increasingly common problem, with some people experiencing recurrent episodes. Despite the high prevalence and health and economic burden of the disease, little is known about how stones form or which are easily passed through the urinary tract. Advances in treatments in the past 30 years have evolved from open surgery to remove large stones, to new technologies. Unfortunately, these new advances have not benefited people who form cystine stones, and thus research efforts continue, toward preventing or slowing the formation of this type of stone.

Researchers recently used a mouse model of cystine stone disease to identify compounds that slowed the growth of this stone type. This genetically engineered model readily forms cystine stones in the bladder of the male mouse. Two compounds believed to have anti-cystine stone formation properties, tiopronin and L-CDME, were initially evaluated in this model system but were ineffective. In contrast to these two drugs, α -lipoic acid significantly slowed the growth rate of cystine stone formation compared with untreated animals in the model system. Prior to the time when these mice would begin to form stones, they were treated with or without α -lipoic acid to assess its ability to alter cystine stone formation. This experimental design demonstrated that α -lipoic acid significantly prevented stone formation, delayed the time needed for stone formation, and/or reduced the overall size of stones that did form. Notably, when α -lipoic acid treatment was discontinued, the mice subsequently resumed producing cystine stones, indicating that α -lipoic acid treatment is reversible.

Additional results of the study indicated similar amounts of cystine were present in the urine from both untreated and α -lipoic acid-treated animals in the model system. Given this finding, the investigators hypothesized that the urine obtained from α -lipoic acid-treated animals provided an environment in which cystine is less likely to crystalize and begin to form stones compared to untreated animals. The investigators confirmed this is a likely mechanism by demonstrating that the urine from α -lipoic acid-treated animals formed significantly less cystine precipitate (solid deposits that form from the compound in solution) during a 3-day period in the laboratory. Thus, it appears that the urine obtained from α -lipoic acid-treated animals can maintain larger amounts of cystine “in solution”—thereby shutting down the pathway that ultimately leads to stone formation.

Research efforts continue that ultimately seek to prevent cystine stone formation in people. One effort is assessing the efficacy of cystine mimetics (compounds with similar structure) to block the cystine crystallization process in human urine and stone formation in the model system used above. A second effort will evaluate how daily α -lipoic acid supplementation over 3 years affects cystine stone recurrence in 50 people.

Zee T, Bose N, Zee J,...Kapahi P. α -lipoic acid treatment prevents cystine urolithiasis in a mouse model of cystinuria. *Nat Med* 23: 288-290, doi: 10.1038/nm.4280, 2017.

ADVANCING UNDERSTANDING, TREATMENT, AND PREVENTION OF URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are common and occur more frequently in women, many of whom experience recurrent infections. UTIs are currently treatable with antibiotics. However, infections recur even after treatment in many women; and the emergence of antibiotic-resistant bacterial strains, as well as the personal burden and medical costs of care, make finding improved prevention and curative strategies a high priority.

The bacterium *Escherichia coli* (*E. coli*) is the primary culprit in UTIs. Normally an integral part of a healthy digestive tract, some *E. coli* acquire the ability to invade and wreak havoc in the urinary tract. These UTI-causing

E. coli, also referred to as uropathogenic *E. coli*, or UPEC, bind to and invade cells lining the inside of the bladder to initiate an infection. Researchers have been tackling the problem of UTIs from two main angles: seeking a better understanding of the factors produced by bacteria that enable them to thrive in the urinary tract; and infection-associated factors and defenses produced by the “hosts” (humans or animals). These factors could be targeted for therapeutic development. Researchers have also sought greater understanding of dynamic interactions between the bacterial and host factors. Several recent discoveries in animal models and humans about the biology of UTIs, including potential vaccine targets, summarized below, are taking scientists steps closer to new approaches to reduce the burden of this urologic disease in people.

Seeking a Genetic Signature for Urinary Tract Infections:

In a recent study, scientists sought to determine whether there is a universal “genetic signature(s)” defining the ability of UPEC to cause disease. Potential candidates for such a signature include so-called putative urovirulence factor (PUF) genes that have been found to be enriched in UTI-associated *E. coli* versus “regular” *E. coli*. To pursue this question, the researchers studied 21 representative *E. coli* strains obtained from the urine of 14 women who suffered from recurrent UTIs. They compared and “scored” these strains based upon how many PUF genes each one possessed. By comparing this score with how well each strain infected bladders in a standard female mouse model, the researchers determined that having a higher PUF gene score did not correlate with either strength (robust, variable, or deficient) of an acute infection, or with the ability to cause chronic infection in one mouse model. However, the investigators found that mice that differed genetically reacted differently to infection—for example, two bacterial strains that caused robust bladder infection in one mouse model were much less effective in a second mouse model. Rather than ignore the differences between the mouse models, the scientists investigated whether that could provide a clue to differences in infectivity. Using the first mouse model, they found that variation in the activity of certain genes regulating core *E. coli* functions correlated with how effectively the strains infected the animals. Bacterial strains that more robustly infected the mice correlated with markers of greater UTI severity in women, such as higher white blood cell counts in urine. Together, the

study findings suggest that rather than a signature set of genes consistently determining virulence, a more complex and dynamic interplay between *E. coli* strains and their host environment determines infection and subsequent disease.

Schreiber HL 4th, Conover MS, Chou WC,...Hultgren SJ. Bacterial virulence phenotypes of *Escherichia coli* and host susceptibility determine risk for urinary tract infections. *Sci Transl Med* 9 pii: eaaf1283, doi: 10.1126/scitranslmed.aaf1283, 2017.

Dynamic Bladder and Bacterial Changes Hold Clues to Therapies:

In two recent studies, researchers delved into changes that occur in the bladder and invading bacteria that affect both acute and recurrent infection. Repeated infections in women with a history of UTI are not always due to the same strain of UPEC or even to *E. coli*, and the majority of UPEC studies have been performed in “infection-naïve” mice—those that had no prior infection. Thus, there is a need to better understand how and what changes occur in the bladder during acute infection affect susceptibility to future infection, and how microbes have adapted to use these changes to persist in the bladder.

In one study, investigators examined more closely the impact of a history of UTI on risk for recurrent UTI. Similar to what is seen in human UTIs, mice infected with UPEC, in the absence of antibiotic treatment, have different outcomes—some spontaneously resolve the infection within a couple of weeks, while others go on to develop a chronic infection. To study the interaction of these outcomes with future infection, the researchers developed an experimental mouse model. They infected female mice with a standard UPEC strain commonly used in the laboratory, and separated mice in which the infection spontaneously resolved from mice that became chronically infected after 4 weeks. They then provided both groups with a 4-week convalescent period of antibiotic treatment. (A third, control group of mice was mock-infected but otherwise treated the same way.) Subsequently, the researchers exposed the three groups of mice to a fresh dose of UPEC. They found that the group of mice that previously developed chronic infections was predisposed to develop severe, recurrent infection, whereas mice from the spontaneously resolving group developed mild, acute infections that were quickly over, earning these groups the titles of “sensitized” and “resolved,” respectively. The researchers then compared how

sensitized, resolved, and naïve mice reacted to infection with several different strains of *E. coli* associated with urinary tract infections in people as well as a non-*E. coli* bacteria that can also cause UTIs. They found that the sensitized mice were highly susceptible to developing chronic infections from these strains, whereas virtually all of the resolved mice did not develop any chronic infection at all from these strains. These observations in mice suggest that a history of UTI and whether it resolved or became chronic could have an important impact on susceptibility to future infection.

Microscopic examination of the experimental model mouse bladders revealed dramatic structural changes. Cells at the surface of the bladder lining were smaller in both sensitized and resolved compared to naïve mice, whereas sensitized mice showed additional changes in deeper tissue layers that were suggestive of incomplete repair and regeneration of the bladder lining during convalescence. Intriguingly, results of other experiments suggested that this bladder remodeling also altered the dynamics of UPEC infection. Two further experiments shed light on possible targets for therapy. One experiment indicated that heightened susceptibility to chronic infection in sensitized mice is likely driven by a pro-inflammatory factor called cyclooxygenase-2 (COX-2). Treating sensitized mice with a COX-2 inhibitor prior to second infection with UPEC ameliorated the burden of infection. The other experiment demonstrated that vaccination against a UPEC protein essential to infection also protected sensitized mice from developing either acute or chronic infections. Important to potential translatability to humans, defects in the bladder lining have been observed in human chronic and recurrent UTI. Thus, such therapeutic targets could be useful if UTI-induced bladder changes and their impact as revealed in this study in mice turn out to occur similarly in some people.

In a second study, scientists focused on a specific UPEC factor and learned critical new information about its role in persistent UTIs. UPEC carry on their surface a number of protein fibers, called pili (singular: pilus), that enable them to attach to different surfaces. It has long been known that specific pili, called type 1 pili, are essential to UPEC's ability to initiate infection. The pili are tipped with adhesive domains that enable binding to specific receptor molecules in the bladder lining and facilitate bacterial invasion of bladder cells. The adhesive domains are referred to as "adhesins."

Through experiments in female mice, the researchers discovered that whereas the type 1 pilus adhesin, called FimH, enables UPEC to adhere to the cells at the surface of a healthy ("naïve") bladder lining at the outset of infection, UPEC then deploy a second type of pilus with a different adhesin, called FmlH, that adheres specifically to infected, inflamed bladder lining. This appears to occur as the superficial cells bound by FimH are shed during the bladder response to acute infection, exposing inflamed tissue bearing the target for FmlH binding. Notably, additional experiments indicated that the expression of the target is likely increasing as inflammation-induced remodeling of the bladder tissue occurs. The deployment of the second type of pilus provides an advantage in establishing chronic infection in a mouse model: UPEC lacking FmlH were much less effective at causing or maintaining chronic infection in mice. When the researchers tested a vaccine against FmlH in mice, they found that vaccinated mice initially had the same bacterial burden as mock-vaccinated mice, but within 2 to 3 days of infection the burden dropped significantly; the vaccine thus protected the mice against progression of infection. This study reveals a mechanism by which UPEC have adapted to and leveraged the host response to acute infection to their advantage, and a new therapeutic target that could help halt chronic or recurrent infection in its tracks.

Conover MS, Ruer S, Taganna J,...Hultgren SJ. Inflammation-induced adhesin-receptor interaction provides a fitness advantage to uropathogenic *E. coli* during chronic infection. *Cell Host Microbe* 20: 482-492, doi: 10.1016/j.chom.2016.08.013, 2016.

O'Brien VP, Hannan TJ, Yu L,...Hultgren SJ. A mucosal imprint left by prior *Escherichia coli* bladder infection sensitizes to recurrent disease. *Nat Microbiol* 2: 16196, doi: 10.1038/nmicrobiol.2016.196, 2016.

Possible New Trigger for Recurrent Urinary Tract Infections Identified: Researchers have identified a naturally occurring component of the vaginal microbiota as a possible trigger of recurrent UPEC UTI and a subsequent kidney infection.

A current model of the "infection life cycle" for UPEC-caused UTIs starts with acute infection, in which UPEC attach to and invade cells lining the bladder. There, they are protected from immune responses and form intracellular bacterial communities (IBCs), expand in number, and then burst forth from

the bladder cells. As the bladder tries to clear the bacteria by sending out immune cells and sloughing off the superficial layer of infected bladder cells, remaining UPEC go deeper within the bladder tissue and form additional IBCs, contributing to the acute infection. In some cases, these IBCs, which are also protected from antibiotics, go on to exist in a quiescent state (latency) until a signal in the environment causes them to emerge and start a new infection. Together with other evidence, such as the detection of IBCs in human urine during infection, this model provides a possible explanation for some of the cases in which recurring UTIs in humans are caused by the same bacterial strain—complementing other likely sources of same-strain reinfection, such as the gastrointestinal tract and vagina. However, factors that can trigger the emergence of quiescent UPEC from intracellular reservoirs in humans remain unknown.

Building on evidence implicating vaginal bacteria in susceptibility to bladder and kidney infections, researchers sought to determine if certain bacteria might trigger recurrent UPEC infection. Using a female mouse model carrying latent UPEC reservoirs, they exposed bladders of these mice to one or the other of two different types of bacteria that can predominate in the vagina, providing two doses one week apart. They found that in the case of one of the bacteria, *Gardnerella vaginalis* (*G. vaginalis*), this transient exposure was sufficient to induce the appearance of UPEC in the urine of over 50 percent of the tested mice. Notably, mice that responded to *G. vaginalis* as a trigger for UPEC reemergence were more likely to have had a more severe UTI prior to its resolution. *G. vaginalis* is associated with bacterial vaginosis, a form of inflammation caused by bacterial overgrowth. Many risk factors for bacterial vaginosis in humans overlap with risk factors for kidney infection. Upon examination, the researchers found that *G. vaginalis* was detectable in about one-third of mouse kidneys within 3 hours of exposure, and was able to cause kidney injury in the absence of *E. coli*. Exposure of UPEC reservoir-containing mice to *G. vaginalis* also increased the risk for these animals to develop severe *E. coli* kidney infections. This result suggests that *G. vaginalis* is a trigger for recurrent UTIs in mice, and future studies could help delineate whether *G. vaginalis* exposure is also a trigger for recurrent UTIs in humans, and the potential relevance to clinical care and prevention of UTIs and possibly associated kidney infections.

Gilbert NM, O'Brien VP, and Lewis AL. Transient microbiota exposures activate dormant *Escherichia coli* infection in the bladder and drive severe outcomes of recurrent disease. *PLoS Pathog* 13: e1006238, doi: 10.1371/journal.ppat.1006238, 2017

Urinary Tract Infection Vaccines—Putting Metal

to the Pedal: Metals such as iron and copper are necessary co-factors in many microbial molecular pathways. Thus, during an infection, host organisms will try to limit access to these essential nutrients to help quash microbial growth, and microbes will try to acquire them to thrive and survive. Microbial acquisition of metals is usually accomplished by small metal-binding molecules, such as siderophores, which are small, high-affinity compounds that bind iron. In one recent report, investigators describe new evidence suggesting that UPEC use a siderophore called yersiniabactin (Ybt) more broadly to also modulate uptake of copper, preserving its availability while minimizing its toxicity to the bacteria. This multi-metal tasking aspect of Ybt adds to its importance to UPEC.

In a second study, researchers focused on using Ybt and another UPEC siderophore, aerobactin (Aer) as vaccination agents in mice, with the goal of raising host immune responses to UTI-causing bacteria. The mice were then challenged with exposure to a UPEC strain known to encode Ybt and Aer, which are not normally detected by host immune responses, and enterobactin (Ent), a siderophore that is targeted by innate immune host defenses, thus making Ybt and Aer essential to iron acquisition by this strain. The results showed that vaccination with Ybt or Aer reduced acute bacterial burden in the bladder by 12- and 19-fold, respectively. The mouse model they used is one that experiences “ascending” UTIs—i.e., infections that move into the kidneys—and the researchers observed that the siderophore vaccines also significantly reduced bacterial burden in the kidneys. Unlike innate immune responses, which act quickly, inducing adaptive immune responses—such as the formation of antibodies in response to a vaccine agent—takes time. Thus, although the researchers did not have the means to confirm that anti-siderophore antibodies were being generated, an additional experiment showed that their vaccination approach was not effective if the mice were exposed to bacteria only 1 week after administration, providing indirect evidence that the siderophore vaccines are inducing an adaptive immune response. These

encouraging results point toward an additional pool of candidate targets for vaccines to prevent UTIs.

Koh EI, Robinson AE, Bandara N, Rogers BE, and Henderson JP. Copper import in *Escherichia coli* by the yersiniabactin metallophore system. *Nat Chem Biol* 13: 1016-1021, doi: 10.1038/nchembio.2441, 2017.

Mike LA, Smith SN, Sumner CA, Eaton KA, and Mobley HL. Siderophore vaccine conjugates protect against uropathogenic *Escherichia coli* urinary tract infection. *Proc Natl Acad Sci USA* 113: 13468-13473, doi: 10.1073/pnas.1606324113, 2016.

Reducing Catheter-associated Urinary Tract Infections in Nursing-home Residents:

A recent report highlights a successful effort to prevent catheter-associated urinary tract infection (CAUTI) in people residing in nursing homes. CAUTI is a chronic, costly, and potentially dangerous problem for people living in managed care facilities. Residents of nursing homes may use a urinary catheter for several reasons including urinary incontinence, urinary retention, urinary obstruction, accurate measurement of urinary output, required immobilization following trauma or surgery, and hospice or palliative care. Unfortunately, CAUTI can lead to hospitalization, cause a life-threatening condition called sepsis, and result in the spread of bacteria that are resistant to antibiotic treatment in this vulnerable population. While efforts to reduce such infections have included approaches to reducing the frequency of catheter usage, strategies need to be developed and tested to prevent infections in people who continue to require the use of urinary catheters.

Researchers now have reported the success of a multicomponent strategy to reduce CAUTI, as tested in 404 community-based nursing homes in 38 states. The strategy included, for example, training in proper aseptic (germ-free) insertion of catheters, catheter care and incontinence care planning, and catheter removal. Other components of the strategy addressed empowering facility teams, offering solutions to overcome barriers, resident and family engagement, and effective communications. This multi-pronged strategy did not reduce the frequency of catheter use, yet it succeeded in decreasing urinary tract infection rates by 54 percent—as compared to the infection rate at the same facilities before the study began.

The compelling results of this Agency for Healthcare Research and Quality (AHRQ)-led study, to which NIDDK and other organizations provided additional support, suggest this approach would be highly beneficial for people in nursing homes nationwide. To facilitate adoption of these methods, AHRQ has developed a toolkit to reduce CAUTI and other healthcare-associated infections in long term care facilities.

Mody L, Greene MT, Meddings J, ...Saint S. A national implementation project to prevent catheter-associated urinary tract infection in nursing home residents. *JAMA Intern Med* 177: 1154-1162, doi: 10.1001/jamainternmed.2017.1689, 2017.

UROLOGIC CHRONIC PELVIC PAIN SYNDROMES

Novel Insights into Present and Future Pain in People with Urologic Pain Syndromes: Two recent reports have revealed important new information about pain patterns and other symptoms in people with the urologic chronic pelvic pain syndromes (UCPPS) interstitial cystitis/bladder pain syndrome (IC/BPS) and chronic prostatitis/chronic pelvic pain syndrome (CP/ CPPS), and the detection of future pain trends through noninvasive brain imaging. This research was conducted by investigative teams from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. The Network is using approaches that “look beyond” the bladder and prostate—the traditional focal points for study of these syndromes—to uncover meaningful information about the not-well-understood UCPPS and their relationships to other chronic pain conditions that could set the stage for future interventions.

In the first report, the research team set out to capture information about pain location, severity, and associated health and quality-of-life factors in women and men with UCPPS enrolled in the Network. To do this, 233 women with IC/BPS and 191 men with either IC/BPS or CP/ CPPS were surveyed using multiple questionnaires assessing urologic, nonurologic, and psychosocial symptoms, and quality of life. For example, questionnaire items included measures or ratings of urinary frequency and urgency, pelvic pain, sleep, commonly co-occurring chronic pain conditions such as irritable bowel

syndrome, and stress. They were also asked to look at a “body map”—two drawings representing the front and back of the body partitioned into 45 numbered sites, comprising eight body regions—and to check off any site in which they had experienced pain in the past week. From these data, the investigators found that, whereas a quarter of participants reported pain only in the pelvic region, 75 percent reported pain in both pelvic and nonpelvic regions. They then subdivided the latter group into two groups: “intermediate pain” for people who checked sites in one to two nonpelvic regions, and “widespread pain” for people who checked sites in three to seven nonpelvic regions. Intriguingly, there were no differences in pelvic pain severity or urinary symptoms across the three groups. In contrast, nonpelvic pain severity, prevalence of chronic overlapping pain conditions, worsening psychosocial health, and poor quality of life increased as the number of pain regions increased. Notably, more women than men experienced widespread pain, and women were more likely to report a greater burden of nonpelvic and nonurinary symptoms and conditions as their pain locations increased.

The investigators then compared just the intermediate and widespread pain groups to see if there were any significant differences among study participants with UCPPS who reported nonpelvic pain. This comparison yielded results similar to the three-group comparison for pelvic pain and urinary symptoms, nonpelvic pain, and several other health measures, but also revealed more gender-specific differences. For example, compared to individuals in their respective intermediate pain groups, men with widespread pain were more likely to have migraines and anxiety, while women were more likely to have irritable bowel syndrome and sleep disturbance. The differences found in this study among people diagnosed with UCPPS have significant implications for better understanding the cause(s) and/or development of these syndromes, for research studies on potential treatment approaches, and for clinical diagnosis and personalized care.

Numerous studies have uncovered brain changes in people with UCPPS compared to people without these syndromes, and a second Network research team investigated whether there was a brain “signature” in people with UCPPS of several years duration that could predict future changes in pain. Such knowledge could potentially identify brain

factors involved in worsening or relief of pain and reveal a therapeutic target(s) for study. Using a brain imaging technique that measures the strength of functional interactions among different brain regions while at rest, researchers conducted voluntary brain scans on a subset of participants shortly after their enrollment in the Network. These scans and subsequent analyses yielded 13,530 brain connectivity measures per person. The team then compared analyses of the brain imaging data from 34 women and 18 men to their pelvic pain symptom status—categorized as “improvement” or “nonimprovement”—at 3, 6, and 12 months, to see if the brain scans could predict symptom trends. They found that their imaging analyses correctly predicted symptom trends in 73 percent of participants for the 3-month period following the scan. Further analyses revealed that the top 100 brain region connections that were more robust in “improvers” versus “nonimprovers” fell predominantly within a part of the brain associated with attention to sensory information.

Additional experiments will need to be performed in larger groups of people and under varying conditions to confirm, optimize, and extend these findings. However, this study is the first to demonstrate the feasibility of predicting pain symptom changes in women and men with UCPPS and has provided preliminary insights important to understanding the biology of pain and improving symptom management in these people.

Kutch JJ, Labus JS, Harris RE,...Mayer EA; for the MAPP Research Network. Resting-state functional connectivity predicts longitudinal pain symptom change in urologic chronic pelvic pain syndrome: a MAPP network study. Pain 158: 1069-1082, doi: 10.1097/j.pain.0000000000000886, 2017.

Lai HH, Jemielita T, Sutcliffe S,...Landis JR; for the MAPP Research Network. Characterization of whole body pain in urological chronic pelvic pain syndrome at baseline: a MAPP Research Network study. J Urol 198: 622-631, doi: 10.1016/j.juro.2017.03.132, 2017.

UNDERSTANDING AND TREATMENT OF BLOOD DISEASES

A Personalized Medicine Treatment Plan Developed After Identification of a Rare Pathogenic Mutation: After discovering a rare genetic mutation responsible for a previously unknown severe blood disorder in a 6-year old boy, researchers developed a personalized treatment plan for his newborn sibling, also born with

the same blood disorder. Doctors at first diagnosed the boy when he was 1 year of age with Diamond-Blackfan anemia (DBA). DBA is a serious medical condition characterized by insufficient level of red blood cells, also known as anemia. Red cells carry oxygen from the lungs to the body's organs and tissues. The boy was given standard therapy for DBA. He was treated initially with blood transfusions, which provide a source of needed red cells, and then underwent a bone marrow transplant with a fully matched donor at 6 years of age. Although the doctors hoped the bone marrow transplant would be curative, as is typically the case for people with DBA, early signs showed that the transplant was not working as expected, and unfortunately the boy subsequently did not survive due to complications of the procedure.

Clinical scientists became aware of this 6-year old boy while they were conducting research to discover yet unknown genetic causes of DBA. Given the unanticipated transplant outcome, the researchers were interested to learn the cause of the boy's severe anemia, thinking it might be different from typical DBA, and that new insights might help in developing a better treatment approach for others. An analysis of the boy's genes did not reveal mutations known to cause DBA but did identify, for the first time, a mutation in the gene encoding the small protein hormone erythropoietin (EPO). The genetic mutation alters one of the 160 amino acid building blocks of the EPO protein; it changes an arginine amino acid to a glutamine amino acid. EPO normally stimulates red cell production by interacting with other proteins, called EPO receptors, on the surface of early stage (progenitor) red cells in the bone marrow.

The investigators determined that although mutant EPO had slightly less attraction for the EPO receptor than normal EPO had, the EPO mutation decreased its ability to remain attached to the EPO receptor, apparently resulting in significantly diminished ability to stimulate red cell proliferation (*i.e.*, an increase in number of cells) in culture, as compared to normal EPO. These laboratory findings are consistent with the inability of mutant EPO to produce adequate levels of mature red cells in the young boy.

While these research findings were undergoing review for publication, the researchers learned that the parents of the 6-year old boy had a newborn child, who also had anemia. Further testing confirmed that the newborn carried the same arginine to glutamine mutation in EPO. Equipped with new knowledge gained from research, the clinical scientists

developed a treatment strategy. They obtained appropriate permissions to initiate a personalized medicine treatment plan consisting of injections of normal EPO produced in the laboratory, also called recombinant EPO. Recombinant EPO is used often to increase levels of red cells in patients whose red cells have been depleted by a different condition, for example as a result of kidney disease or chemotherapy for cancer. The researchers reasoned that recombinant normal EPO might also work for this child, by latching onto EPO receptors more productively than the child's own EPO, and thus restore red cell production. After 11 weeks of treatment, the child's red cell production had increased—eliminating the need for blood transfusions.

This study underscores the benefit of research to greatly improve the life of a patient. Additionally, this personalized medicine treatment plan potentially could help others with the same disorder.

Kim AR, Ullirsch JC, Wilmes S,...Sankaran VG. Functional selectivity in cytokine signaling revealed through a pathogenic EPO mutation. Cell 168: 1053-1064, doi:10.1016/j.cell.2017.02.026, 2017.

Correcting Iron-delivery Defects in Animals:

Scientists recently identified a small molecule compound capable of correcting iron-delivery defects in animal models. Iron is essential to the body's oxygen-delivery system. Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. Iron is shuttled to various tissues of the body by an exquisitely well-controlled series of proteins called iron transporters. Defects in iron delivery can lead to too little iron (also called iron deficiency anemia) or a buildup of too much iron (also called hemochromatosis) and are associated with more than 25 human diseases. Thus, approaches that restore normal iron delivery would be beneficial to those with these conditions.

The researchers began by studying iron transport in baker's yeast, an organism readily amenable to experimentation. While evaluating a series of candidate compounds for their ability to restore growth capacity to yeast missing a specific iron transport protein, researchers discovered that the natural product hinokitiol—originally isolated from the essential oil of a tree—restored normal growth rates. After showing that hinokitiol binds tightly to two forms of iron (ferrous and ferric) found in cells, the investigators demonstrated that this small molecule had the inherent ability to transport both ferrous and ferric iron across a model cell membrane. Further studies suggested that hinokitiol dissipates iron buildup by

transporting iron from areas of high iron concentration to areas of low concentration. In a set of experiments in mice and rats genetically engineered to lack specific iron transport proteins in the gut, the researchers showed that orally administered hinokitiol restored iron uptake in the animals. The researchers also demonstrated that hinokitiol restored normal hemoglobin production in zebrafish lacking a specific iron transport protein. This suggests that hinokitiol can work with the body's existing regulatory systems to supply iron where it is needed without interfering with other cell functions.

The findings from this study suggest that small molecules like hinokitiol may one day be used to substitute for missing or defective human iron transport proteins. More research into hinokitiol's function and safety will be needed to explore this fascinating possibility.

Grillo AS, SantaMaria AM, Kafina MD,...Burke MD. Restored iron transport by a small molecule promotes absorption and hemoglobinization in animals. *Science* 356: 608-616, doi: 10.1126/science.aah3862, 2017.

Small, Yet Powerful Mitochondria and Blood Stem Cells:

Two recent studies of blood stem cells highlight the importance of cellular components called mitochondria in determining how these cells function. Mitochondria are referred to as the “powerhouses” of the cell because these small organelles take energy that is ingested in the form of sugars or fats and convert it to fuel for the cell in a process called respiration. Blood stem cells (also called hematopoietic stem cells) have the potential to self-renew into two identical daughter stem cells or give rise (mature) to specialized cell types: red blood cells, white blood cells, or platelets. Scientists have sought to discover the cellular players that tip the balance in favor of self-renewal versus maturation.

In one report, scientists discovered that blood stem cells that retain the capacity to self-renew have a protein on their cell surface called Tie2, a feature distinguishing them from blood stem cells that may be on a path to become mature blood cells. When examining human and mouse blood stem cells with and without Tie2, they found that those with Tie2 have superior ability to grow and repeatedly give rise to new daughter stem cells over longer periods of time in the laboratory, creating many generations of blood stem cells. In

experiments in mice, blood stem cells with surface Tie2 were also more effective at migrating to the bone marrow, where they normally reside and self-renew. Further analysis of mouse Tie2-containing blood stem cells showed that these cells “turn on” several genes involved in the degradation of mitochondria, which helps cells remain healthy by selectively removing damaged mitochondria. Based on additional experiments, the researchers proposed that the process of mitochondrial clearance may be a key determinant that commits these cells to the self-renewal pathway.

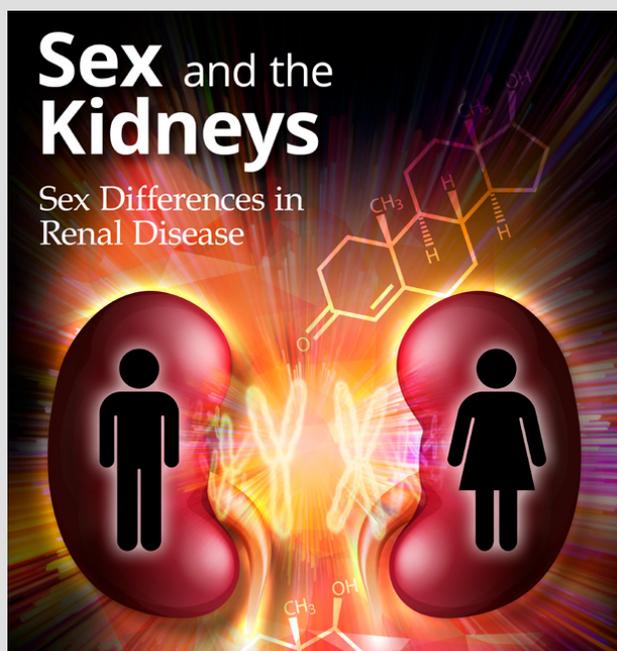
A second advance assessed whether intact, functional mitochondrial respiration is required for the function of mouse fetal and adult blood stem cells. The investigators found that deficiency in a protein component of the mitochondrial respiration process, called RISP, resulted in a mouse fetus that developed fewer red blood cells than normal and subsequently died. In addition to the lack of red blood cells, the late-stage mouse fetus contained fewer white blood cells and platelets—an indication that the blood stem cells were unable to mature into other critical blood cells. Another set of experiments confirmed that intact mitochondrial respiration is also essential for adult mouse blood stem cell functions. In contrast to normal adult blood stem cells, those lacking RISP initially generated more cells, but soon these cells died—resulting in an inadequate number of blood stem cells to self-renew or mature into specialized blood cells. These findings, in mice, suggest that mitochondrial respiration is required for normal blood stem cell function.

These two studies serve to underscore the significance of mitochondrial activity in normal blood stem cell function. Approaches to engineer these pathways may enable investigators to direct these cells to undergo self-renewal or maturation, which could be useful for future study of these important cells and for potential therapeutic strategies.

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Ansó E, Weinberg SE, Diebold LP,...Chandel NS. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nat Cell Biol* 19: 614-625, doi: 10.1038/ncb3529, 2017.

Sex Differences in Kidney Disease



Women now account for roughly half of all participants in NIH-supported clinical research. However, basic and preclinical biomedical research has often focused on male animals and cells. An over-reliance on male animals and cells may obscure understanding of key sex influences on health processes and outcomes. Accounting for sex as a biological variable begins with the development of research questions and study design. It also includes data collection and analysis of results, as well as reporting of findings. Consideration of sex may be critical to the interpretation, validation, and generalizability of research findings. Adequate consideration of both sexes in experiments and separation of data by sex allows for sex-based comparisons and may inform clinical interventions. Appropriate analysis and transparent reporting of data by sex may therefore enhance the rigor and applicability of preclinical biomedical research.

The NIH has recently re-focused the research community on the role of sex as a biological variable—requiring all research grant submissions to deliberately consider sex as a modifier of biological response. As part of its efforts to address this important issue, the NIDDK sponsored a workshop on “Sex

and the Kidneys: Sex Differences in Renal Disease,” held on July 13-14, 2017, on the NIH campus in Bethesda, Maryland. This workshop follows a previous conference held in 1999, “Women in Renal Disease,” which focused on identifying the unique risks of chronic kidney disease (CKD) and progression to end-stage renal disease present in women across their lifespans. At the conclusion of that conference, the organizers proposed several avenues of research to advance our understanding of the pathophysiology of sex differences in CKD and improve clinical care of women with CKD. While some advances have been made since then in both clinical and basic research, much remains poorly understood, both at the molecular and clinical level.

The purpose of the 2017 workshop was to afford the kidney research community an opportunity to re-visit the role of sex in disease risk and etiology, in light of the advances made in our understanding of sex hormone action in the body’s tissues. Basic and clinical scientists, clinicians, and industry and regulatory representatives gathered to assess the state of the science, identify needed research, and set priorities for future efforts. A wide range of presentation topics were discussed, including the molecular mechanisms of sex hormone activity; lessons of sex difference epidemiology learned from other disease systems; basic mechanisms and clinical manifestations of kidney disease; and approaches and tools to identify, understand, and treat sex differences. In-depth discussion among experts in four breakout sessions led to a series of recommendations to address needs in basic, translational, and clinical science, and to develop necessary tools and methodologies. These recommendations are expected to inform experimental design and interpretation, ultimately leading to a better understanding of sex differences in kidney disease and improved clinical care.

The Kidney Precision Medicine Project: A Bold Vision for the Future of Nephrology

Acute kidney injury (AKI) and chronic kidney disease (CKD) impose a significant global health burden; however, only a few drug therapies are available for CKD, and none currently exist for AKI. Development of pharmacologic agents for AKI and CKD has been hampered by non-predictive animal models, the inability to identify and prioritize molecular factors in human kidneys that could be targeted with medication, and an underlying poor understanding of human AKI and CKD. A growing consensus suggests that CKD and AKI are not homogeneous diseases; rather, they are heterogeneous disorders that contain specific subgroups that are driven by different disease pathways. Thus, a better understanding of disease heterogeneity will likely inspire the development of more effective individualized treatment options. In 2017, the NIDDK began the Kidney Precision Medicine Project (KPMP)—a bold initiative that will begin to chart a course toward a more personalized approach to clinical care for people with kidney disease.

One might envision a more individualized future for clinical practice, where each person with kidney disease can find answers to important, patient-centered questions: “What do I have?” “What will happen to me?” “What can I do about it?” A nephrologist (kidney disease specialist) in this vision of the future might evaluate the person’s disease profile using blood and urine tests, image the kidney in real-time to identify and biopsy areas of kidney damage, then analyze the biopsy tissue using a kidney tissue atlas (a tool designed to classify the location and health of kidney tissue components), and select the appropriate drug to start individualized treatment.

How will this individualized approach to patient care be achieved? The KPMP outlines several goals with the overall objective of bringing

precision medicine to AKI and CKD: to ethically and safely obtain and evaluate human kidney biopsies from research participants with AKI or CKD; create a kidney tissue atlas; define disease subgroups; and identify critical cells, extracellular components, and pathways that can be targeted for novel therapies.

The kidney biopsy is essential to this vision of the future, as it will identify the specific subtype of AKI or CKD to provide the information needed to answer the patient-centered questions. However, advancing science to a point where subtypes are well defined will require technological leaps that can only be made by analyzing biopsy tissue from people with kidney disease. Today, kidney biopsies are of limited benefit to an individual, and the biopsy procedure carries some risk for well-defined complications (e.g., bleeding, pain). A central component of the KPMP will be to build a strong case to explain to patients and clinicians how biopsies could have long-term benefit because of their critical role in advancing research progress toward precision medicine.

Human kidney biopsies obtained through the KPMP will be analyzed using sophisticated technologies that have matured over the past few years, identifying new molecular markers that will reveal cellular and tissue heterogeneity in exquisite detail to define specific kidney structures. Markers characterized by the KPMP will help establish a complex kidney atlas that can classify and locate different cell types, cell states (healthy, injured, dying, recovering, undergoing repair, etc.), and important molecules. These new markers will then be linked to important patient clinical outcomes. The emerging kidney tissue atlas will be used as a foundation to better understand kidney disease heterogeneity and will be able to inform decision-making by pathologists, nephrologists, and patients with AKI and CKD.

Because the current kidney biopsy procedure is risky, with well-defined complications, ethical and participant safety considerations must be a primary concern. The NIDDK is also supporting research through its small business research programs to develop safer biopsy methods and novel techniques to analyze human kidney tissue. All resulting resources from the KPMP will be public, open, and transparent, and will be made available to everyone (e.g., patients, academic researchers, industry scientists). These findings and resources will help nephrologists better understand human kidney disease, and will invigorate kidney research, attract top talent from inside and outside nephrology, and seed new investigator-initiated research. To achieve maximal success, the KPMP will foster

partnerships among patients, academic researchers, private industry, advocacy organizations, and the NIDDK.

This patient-centered, individualized vision for the future of nephrology aims to keep the patient voice front and center in the design and implementation of the KPMP. Over time, results and resources from the KPMP are expected to drive the evolution of nephrology toward this future. An increased understanding of human kidney diseases is likely to catalyze the development of new therapies. Biopsy results will likely become more informative to clinical care as pathologists and nephrologists can better predict a drug's effectiveness based on an individual's specific kidney profile.

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APOL1 Gene Variants: Unraveling the Genetic Basis of Elevated Risk for Kidney Disease in African Americans

African Americans have higher rates of end-stage renal disease (ESRD) than European Americans, but for many years the reasons for this health disparity were largely unknown. In 2008, researchers reported that genetic variations on chromosome 22, later revealed to be in the *APOL1* gene, were linked to a greater incidence of non-diabetic kidney disease among African Americans. The identification of *APOL1* kidney disease risk variants, which are found primarily in African Americans, is arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and these variants are among the only known genetic factors contributing to the well-appreciated health disparities in kidney diseases. In the years since the initial groundbreaking discovery of *APOL1* risk variants, numerous NIDDK-supported studies have shed light on their important roles in disease risk and the underlying mechanisms of *APOL1* protein function, creating new research avenues to improve health in populations at risk.

The Heavy Burden of Kidney Disease in Some Populations

Early-stage kidney disease often has no symptoms. Left unchecked, however, it can silently progress to kidney failure, a condition in which the kidneys are no longer able to filter waste and excess fluids from the blood. Millions of U.S. adults are estimated to have chronic kidney disease (CKD), and despite

recent advances in preserving kidney function in individuals with early-stage kidney disease, serious health complications are common.

The two most common causes of kidney failure are diabetes and hypertension (high blood pressure), which account for a majority of new cases. Both conditions are more frequent in minority populations, and African Americans bear an especially heavy burden of kidney disease. African Americans are more likely to develop diabetic kidney disease and kidney failure than Whites. One cause is a form of kidney disease called focal segmental glomerulosclerosis (FSGS), in which the glomeruli—the tiny filtering units of the kidneys—are damaged and scarred. Most FSGS arises from unknown causes and is termed “idiopathic” FSGS. African Americans are significantly more likely to develop idiopathic FSGS compared to individuals of other racial backgrounds. The health disparity increases dramatically with HIV infection: African Americans are far more likely than Whites to develop FSGS related to infection with HIV, the virus that causes AIDS. These rather striking disparities represent a serious public health problem, not only because of the kidney disease itself, but also because people who have even mild- to moderately-severe kidney disease typically have high blood pressure and other risk factors for serious complications such as cardiovascular disease.

What accounts for this dramatically increased risk of severe kidney disease in African Americans? Scientists and physicians have long known

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that kidney disease tends to run in families and to cluster in racial/ethnic groups. These observations indicate that kidney disease is likely to have a genetic component. It is also almost certain that environmental and other factors also play a role in disease susceptibility and outcomes. However, studies that have attempted to identify genes that confer susceptibility to kidney disease and kidney failure had not generally been successful.

Moreover, it is not clear that all forms of kidney disease originate from a common starting point or progress through a shared pathway. For example, while patients with diabetes or those with hypertension are at increased risk of developing kidney disease and kidney failure, not all patients at risk go on to develop kidney disease. In addition, it is not clear that the underlying disease mechanisms which initiate injury and facilitate progression in diabetic and hypertensive kidney disease are the same. If, in fact, these two conditions cause kidney disease through different pathways, then treatment strategies for people whose kidney disease is a consequence of diabetes could be very different from those for people whose kidney disease is attributed to hypertension. Because of these considerations, it has been especially important to identify the genetic contribution to disease development and progression and characterize the biological pathways that lead to diminished kidney function.

A Foundational Discovery

In 2008, members of the NIDDK-supported Family Investigation of Nephropathy and Diabetes (FIND) Consortium, along with scientists in the NIDDK's Intramural Research Program, reported that genetic variations on chromosome 22 were linked to greater incidence of non-diabetic kidney disease

among African Americans. Initially, attention focused on the region surrounding the *MYH9* gene. Further analyses revealed that much of the increased risk of kidney disease is actually due to two variations in the adjacent *APOL1* gene, which encodes the protein apolipoprotein L1 (also designated as APOL1 protein,) a component of so-called "good" cholesterol that is found circulating in the blood and in kidney cells. Two specific variants of this gene, termed G1 and G2, have been shown to account for nearly all of the excess risk of kidney failure in African Americans arising from causes other than diabetes—a major breakthrough in understanding the increased burden of the disease. The low-risk *APOL1* genetic variant is termed G0.

NIH Scientists Identify Critical Associations Between *APOL1* Variants and Kidney Diseases

Soon after this association was reported, scientists found that African Americans with two copies of *APOL1* high-risk variants are at increased risk of developing kidney disease, particularly FSGS and kidney disease related to infection with HIV. This finding comes from collaborative research led by scientists in NIDDK and NCI's intramural research programs. Investigators in the United States and Europe were also part of the research team. The scientists studied patients with kidney disease who came to the NIH Clinical Center or other collaborating medical centers and provided blood samples for genetic studies.

Human cells typically have two copies of each gene—one inherited from each parent. African Americans with no normal copies of the *APOL1* gene, but instead two kidney disease risk variant copies, have about a 4 percent lifetime risk of developing FSGS.

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

The persistence of *APOL1* variants in people of African descent may be partly explained by the ability of the *APOL1* protein to destroy certain parasites. Although the normal *APOL1* protein can destroy the parasite *Trypanosoma brucei brucei* (*T. b. brucei*), it is unable to destroy two related parasites, *T. b. rhodensiense* and *T. b. gambiense*. These parasites cause African sleeping sickness, a hematologic and neurological disease, spread by the tsetse fly, that kills thousands of people in sub-Saharan Africa each year. However, people with at least one copy of the G1 or G2 variants are protected against infection because they are able to destroy *T. b. rhodensiense* and *T. b. gambiense*. These two *APOL1* variants appear to have evolved relatively recently—in the past 10,000 years or so. Their relatively recent appearance and high frequency in chromosomes in individuals of African descent suggest that the variants may support protection against parasitic infection.

It should be noted that most people with two *APOL1* variants do not develop kidney disease.

Indeed, the much higher risk of kidney disease in patients with HIV suggested that a second triggering event, or “hit,” either with a virus or another factor, contributes to kidney injury in people who have two high-risk *APOL1* variants. Nevertheless, the observed increased risks of FSGS and HIV-associated kidney disease were the strongest effects yet discovered for common variants in a complex disease.

A New Understanding of Kidney Disease Progression and Treatment

The link between *APOL1* gene variants and kidney disease risk led scientists in different studies to seek associations between these variants and measures of kidney disease severity. One NIDDK-supported 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 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 was associated with a faster decline of kidney function compared to study participants without this variant. Another NIDDK-supported study examined over 400 African Americans with kidney failure and asked whether the presence of one or two 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

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of the G1 variant began hemodialysis at a significantly younger age (approximately 49 years old), than those with one copy of the variant (about 56 years old). People with two normal copies of the *APOL1* gene began hemodialysis at around 62 years of age.

***APOL1* Gene Variants and Cardiovascular Disease**

Because hypertension is a leading cause of kidney failure, researchers have also sought to determine the relationship between the *APOL1* variants and cardiovascular disease. Scientists analyzed data from people enrolled in AASK and the Chronic Renal Insufficiency Cohort (CRIC) Study. The CRIC study, also supported by the NIDDK, is one of the largest and longest ongoing studies of CKD epidemiology in the United States; it is examining the health of both White and African American people with CKD, about half of whom also have diabetes. In their analysis, the researchers found a correlation between the presence of high-risk variants of the *APOL1* gene and an increased risk of CKD progression among African Americans. This effect was seen regardless of whether patients maintained good blood pressure control or had diabetes. In a separate study, over 2,500 African American volunteers in the Systolic Blood Pressure Intervention Trial (SPRINT) clinical study agreed to undergo genetic testing to allow researchers to examine their *APOL1* status as it related to their kidney function and risk of developing cardiovascular disease. SPRINT was led by the National Heart, Lung, and Blood Institute (NHLBI), and co-sponsored by the NIDDK and other NIH institutes. In the genetics study, which also was supported by the NIDDK and other NIH Institutes, researchers found that SPRINT study participants with two risk variants of the *APOL1* gene were more likely to have mild kidney disease than people with a single risk

variant or none. However, they were not more likely to have cardiovascular disease.

More recently, scientists supported by the NIDDK analyzed data from multiple independent studies, overall including over 5,000 African American study participants, to explore whether *APOL1* variants affect the age of hypertension onset. They found that in young African Americans (20-29 years old), high-risk *APOL1* variants were linked to higher blood pressure levels and younger age of hypertension diagnosis than low-risk variants. Another NIDDK-supported research team asked whether *APOL1* gene variants were linked to differences in blood pressure trajectories over time. The scientists analyzed data from NHLBI's Coronary Artery Risk Development in Young Adults (CARDIA) Study, which began in 1986 and has been examining the development and determinants of cardiovascular disease and its risk factors by following study participants for 25 years. The scientists found that while blood pressure levels over the years rose to higher levels in African Americans than in Whites, blood pressure trajectories in those with high-risk *APOL1* variants did not appear to be different than in people with the low-risk variants.

Cellular and Molecular Mechanisms of *APOL1* Protein Function

Great strides have been made over the past few years in defining various aspects of the genetic risk of kidney disease associated with *APOL1* variants in some populations. In addition, understanding the mechanisms of *APOL1* protein action in the kidney that underlie disease risk could inform clinical decisions and the development of new therapeutic strategies for people with high-risk variants. To begin exploring these mechanisms, one important experimental challenge had to be overcome by scientists—the *APOL1* gene is only found in humans and some primates. Thus,

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researchers needed to develop innovative strategies for investigating APOL1 protein function in animal models and other laboratory systems. Recent reports have shed light on the proteins and pathways that mediate APOL1 action in the kidney.

In one study, NIDDK-supported researchers genetically engineered mice to produce the human APOL1 G0, G1, or G2 variants in specialized cells in the kidney, called podocytes, that wrap around the glomerulus and are key components of the filtration apparatus. Mice that produced the G0 variant appeared normal, but those with a high-risk G1 or G2 variant exhibited hallmarks of human kidney disease (e.g., high levels of protein in the urine, physical injury to podocytes). These results in mice lend strong support to the hypothesis that these human APOL1 variants cause kidney disease. Another team of scientists supported by the NIDDK used the *Drosophila melanogaster* fruit fly system to investigate cellular and molecular mechanisms of APOL1 activity. The researchers engineered flies to produce human APOL1 G0 or G1 variants in their nephrocytes, which are cells in *Drosophila* that have a similar function as human podocytes. Both variants over time led to increased protein uptake by nephrocytes and impaired acidification of critical cellular compartments. These cellular changes over time were accompanied by decreased function, increased size, and premature death of nephrocytes. In each experiment, the results were much more severe in flies with the G1 variant than in those with the G0 variant. Together, these findings suggest that APOL1 variants are causative agents of kidney disease, and point to cellular toxicity as a potential mechanism for decline in kidney function.

High levels in the blood of a protein called suPAR are associated with decline in kidney function and progression to CKD. Because

many people with the high-risk G1 and G2 APOL1 variants do not develop kidney disease, an NIDDK-supported research team explored whether blood suPAR levels can help predict whether African Americans with these genetic variants will experience declining kidney function. The scientists analyzed data from the Emory Cardiovascular Biobank (EmCAB) and the AASK trial, which together included almost 1,100 African American participants. The scientists found that in people with high-risk APOL1 variants, kidney function was likely to decline more rapidly over time in those with elevated plasma suPAR levels than those with lower suPAR. To gain additional insights, the researchers studied APOL1 variants and suPAR in mice. Female mice engineered to express either the G1 or G2 variant showed evidence of kidney damage, but mice with the G0 variant appeared normal. However, when suPAR was genetically deleted from mice, the kidney damage caused by the G2 variant was gone. Taken together, these findings suggest that high levels of suPAR may be necessary for the kidney function decline observed in some people with APOL1 G1 and G2 variants, and thus may serve as a useful predictor of kidney disease. (For more details about this study, please see the advance earlier in this chapter).

Looking Forward

A clear picture is emerging that links APOL1 gene variants to kidney diseases under a range of conditions that need further study, such as HIV-associated nephropathy, pediatric kidney disease, sickle cell nephropathy, and kidney transplantation. The NIDDK continues to support research at multiple levels to understand these relationships in African Americans and other populations. For example, the NIDDK held a conference in June 2015 on APOL1 and kidney disease to assess gaps in knowledge, including

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the function of the *APOL1* protein and its role in kidney transplantation. The conference developed new ideas regarding how *APOL1* gene variants lead to disease susceptibility, what kidney and cardiovascular outcomes are associated with these variants, which additional genetic variants or environmental factors play a role in differences in disease symptoms, and the possible role of determining whether patients have *APOL1* gene variants in guiding treatment as well as preventive strategies for patients.

Additionally, in 2016 the NIDDK, the National Institute on Minority Health and Health Disparities, and the National Institute of Allergy and Infectious Diseases began the *APOL1* Long-term Kidney Transplantation Outcomes Network (APOLLO) initiative. This initiative aims to determine the impact of *APOL1* genetic variants as risk factors in U.S. kidney transplant recipients who received kidneys from African American donors. The consortium will examine the rate of change of kidney function in recipients, and rates of acute rejection of

the kidney transplant, graft failure, and return to maintenance dialysis in the recipients, who received kidneys from patients of African descent, in the presence and absence of the *APOL1* genetic variants. Outcomes in kidney donors, including vital and renal functional status, will also be assessed.

These seminal studies, as well as many others over the past several years, have revealed the importance of *APOL1* in understanding some key differences in kidney disease risk across populations. Unraveling the molecular mechanisms by which *APOL1* variants contribute to kidney injury could provide key insights into the causes and possible treatments for kidney disease in African Americans. Moving forward, clinicians may be able to make more informed choices about when to start screening for kidney disease and how to choose an appropriate therapy by identifying which patients have these gene variants and are therefore at increased risk of developing kidney disease and progressing to kidney failure.

Olivier: Participating in Clinical Research To Help Others with Benign Prostatic Hyperplasia Down the Road

Symptoms began gradually for 57-year old Olivier, so it is difficult to pinpoint exactly when he began dealing with urinary urgency, but he estimates he first noticed the problem 10 years ago. Urinary urgency is the sudden, strong need to urinate immediately. Although he has been dealing with urgency for the better part of a decade, he only learned the source of his symptoms—a condition called benign prostatic hyperplasia (BPH)—relatively recently. He admits that he is not good at going to the doctor right away when anything happens. “You kind of ignore what you have, and then you don’t want to think of the worst. There’s always the question of prostate cancer... Initially, you try to cope with it.” Although Olivier put off going to the doctor to find out what the problem was, he explains that he could not control or influence his symptoms very much. As he says, a natural way “was to drink less water, which is really not what you want to do ... because you’re thirsty all the time. Definitely not good for your metabolism to restrict your water intake. You realize that’s not the solution.” To better understand the nature of the symptoms that characterize his urinary problems, Olivier recently began participating in an NIDDK-supported research study—turning his own experiences with this difficult condition into a way to help others.

It’s Progressive

After Olivier’s urinary urgency started, it got progressively worse over time, and began to

seriously interfere with his life. It was hard to predict when the urinary urge symptom would start, complicating the family’s social life and his everyday activities. “It’s not that you can choose when you go—you have to go right now.” Describing these challenges further, he says that with urinary urgency, “basically you cannot be in a room for more than an hour. Sometimes less. You have a diminished ability to interact with people and [need to] leave at specific moments.” Olivier, a husband and father of two boys, comments that it became very apparent to the people he interacted with that he had this pretty serious issue. “Everyone knew in my family that when I have to run out—it was mostly a joke. But,” he adds with a laugh, “I guess a good way to take it.”

Urinary urgency affected his life in other ways, too. He notes that air travel is especially difficult, “because once your seatbelt is fastened and you’re told you cannot move, that becomes a serious issue. You know ... sometimes ... it takes a long time before the plane taxis out from the gate and you actually take off [and finally] you are in the air and able to go use a restroom.” An owner of a business that imports specialty foods from Europe, he noticed that when he’s in a food warehouse, which is kept at about 40 degrees Fahrenheit, that really triggers the need to urinate. “I couldn’t stay in the cold environment more than 40 minutes. I would need to run to use the restroom.” Olivier also has struggled to get a good night’s sleep because of his night-time urinary symptoms.

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"Start waking up at night once. And then it becomes two. And then it becomes three.... You don't get any rest." As the nightly urinary symptoms became worse, exhaustion became an additional challenge during the day.

To complicate things further, he also developed another urinary problem. "Going to the bathroom to urinate became a real struggle, too. It's not just going more often—suddenly you're having a harder and harder time to try to urinate. It's increasingly hard to handle. Hard on your body too, just to go through that process."

Benign Prostatic Hyperplasia Diagnosis and Treatment Plan

The combination of these and other symptoms significantly contributed to Olivier's diminished quality of life and work experiences, and finally brought him to a tipping point. He says, "That's what started to tell me that I need to do something about it, because it was

"That's what started to tell me that I need to do something about it, because it was getting out of control" says Olivier of his decision to finally seek medical help for his severe urinary symptoms.

getting out of control." After about 5 years of urinary urge and urinary stream problems, he made an appointment with his general practitioner, who referred him to a urologist, who diagnosed Olivier with BPH. BPH is a condition in which a man's prostate gland is enlarged but not cancerous. The prostate surrounds the urethra, which is the tube that carries urine from the bladder to the outside of the body. In BPH, the enlarged prostate presses against and pinches the urethra, causing urinary urgency, trouble starting a urine stream, and/or other symptoms. BPH is a fairly common condition in men around Olivier's age and older, and men with a family history of BPH are more likely to develop the condition. Olivier eventually

realized that his father probably developed this condition, although with his father it didn't occur until much later in life.

"I was put on tamsulosin (a medicine that relaxes the muscles in the prostate and those that connect the bladder to the urethra in the bladder neck) for several years" to treat the BPH, Olivier says. Initially, there was a slight improvement with tamsulosin, but, he adds, "there were a lot of side effects [from the medicine], which were not really emphasized. These side effects were marked enough to lead to some deterioration of quality of life" which led him to completely stop taking the medicine.

The approach to Olivier's treatment changed in the summer of 2016, when he received a transurethral resection of the prostate (TURP), a surgical procedure that removes some of the

prostate gland; the procedure is performed by a urologist. Olivier says "the surgery was a tremendous improvement. I

am thankful to Dr. John L. Gore and his team [at the University of Washington Medical Center] for the excellent care and surgery procedure that I received."

Participating in the LURN Clinical Study

Olivier learned of the Symptoms of the Lower Urinary Tract Dysfunction Research Network (LURN) observational study from his urologist at the time of his surgery, and agreed at that time to participate in the study. To hasten advances in assessing and treating lower urinary tract dysfunction, whether caused by prostate-related or other conditions, the NIDDK started LURN in 2012. Expanded in 2013, LURN

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includes six clinical research sites and one data coordinating center. LURN was designed for people like Olivier, to better understand lower urinary tract conditions. Knowledge gained from LURN could then be used in other research efforts to develop and test new treatments to improve the health and quality of life of people with lower urinary tract symptoms (LUTS).

LUTS are highly prevalent in both males and females, but many people who seek help from healthcare providers for LUTS experience neither total nor permanent resolution of their symptoms with current management approaches. One of the barriers to improving diagnosis and management of LUTS is incomplete knowledge and imprecise classification of subtypes of LUTS and their associated causes. There are a wide variety of lower urinary tract symptoms that people can experience, which may be caused by problems in the urinary tract or may originate elsewhere in the body. Even people with similar symptoms may have different underlying urinary tract conditions. The researchers aim to identify and understand the different subgroups of people with LUTS based on their urinary symptoms, other health conditions they may have, and other characteristics; improve measurements of people's experiences with urologic symptoms; share novel findings with other researchers, clinicians, and patients; and generate data, samples, and research tools for future studies. Ultimately, the researchers hope that knowledge gained from this study will help researchers and healthcare providers improve prevention, management, and treatment strategies.

During his participation in LURN, Olivier underwent a standardized clinical examination and medical

history, and completed questionnaires designed to gather information about urinary and other symptoms and health-related quality of life. He also provided blood, urine, and other samples for storage in the NIDDK Sample Repository for future study by the LURN investigators and the broader research community.

Olivier speaks well of his participation in the LURN study. "I wouldn't say there was a hard part at all. I just have to go to the hospital a few times, and they were very flexible. The appointments were no more than a half hour

at a time." And he describes the LURN study staff as "very good. Very fine. They are

"I wouldn't say there was a hard part at all," Olivier says of his participation in the LURN study.

understanding. Good interactions." Olivier also mentions that his wife, a medicinal chemist by training, also thinks his participation in LURN is a good thing because the more people participate, the more data is collected and analyzed to improve understanding of these conditions. Asked whether other men who are experiencing urinary tract symptoms similar to his should consider enrolling in a study like LURN, Olivier says "I would encourage them to do so. That way, there would be more information [collected to help improve] counseling in how to manage this."

Additional Efforts To Help People with LUTS

As part of the overall LURN research efforts, there are several sub-studies designed to examine certain aspects of urinary tract problems in smaller groups of participants. For example, one of these is a neuroimaging and sensory testing study to provide additional data to help identify subtypes of LUTS. In this effort, researchers are using functional magnetic resonance imaging (fMRI) to examine brain

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structure and function as it relates to an empty versus full bladder. This approach may identify differences in brain areas involved in bladder control in participants with LUTS compared with those who do not have this condition.

With other procedures, the researchers plan to explore whether people with LUTS have

different central nervous system responses to auditory (hearing) and pain stimuli.

LURN also plans to compare how participants report LUTS experiences over different time periods (e.g., 3-day, 7-day, and 30-day recall). These “recall” data will help LURN investigators determine the most appropriate reporting period for specific symptoms.

A LURN pilot study effort will test the feasibility of using urodynamics—procedures that look at how well the bladder, sphincters, and urethra are storing and releasing urine—in healthy, asymptomatic women to inform the design of a larger study to identify subgroups of people with LUTS. In the long term, LURN seeks to develop diagnostic organ testing protocols for both women and men with LUTS to identify different mechanistic causes that may give rise to similar LUTS profiles.

A second LURN pilot study seeks to discover potential biomarkers in women and men that could ultimately be used in clinical practice as a tool to measure the presence, severity and/or subtype of LUTS. In addition to providing

Asked whether other men who are experiencing urinary tract symptoms similar to his should consider enrolling in a study like LURN, Olivier says “I would encourage them to do so. That way, there would be more information [collected to help improve] counseling in how to manage this.”

potential insights into the biological mechanisms underlying LUTS, these biomarkers

could one day be used as tools by clinicians to initiate more effective treatments and monitor the response.

For People with LUTS

The NIDDK is committed to acquiring new knowledge that will help lead to future strategies to better manage and, when possible, prevent the development of LUTS. Olivier's struggle to attain an effective treatment plan is a reminder that more research needs to be conducted to help people with this burdensome condition. And Olivier's participation in research, along with the many other study volunteers, is key to progress toward improving people's lives.

