Pediatric Insights

BEST CHILDREN'S HOSPITALS U.S.News & WORLD REPORT NEPHROLOGY 2019-20

Fall 2019

An Update From the Division of Pediatric Nephrology

In This Issue

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UPMC CHILDREN'S HOSPITAL OF PITTSBURGH

Division of Pediatric Nephrology Researcher Obtains New R01 Grant to Study Bladder Injury

4th Division NIH Grant in Last Six Months

UPMC Children's Hospital of Pittsburgh Division of Pediatric Nephrology researchers and division chief, **Carlton M. Bates, MD**, secured National Institutes of Health (NIH) R01 funding in July for a new investigation to study cyclophosphamide-induced injury to the bladder urothelium, which can lead to several problems including life-threatening hemorrhagic cystitis, chronic fibrosis, and bladder cancer.

Cyclophosphamide (CPP) is an immuno-suppressive chemotherapeutic agent used to treat various forms of cancer and autoimmune disorders, and it is sometimes used in the treatment of nephrotic syndrome when the condition proves to be refractory or the patient is unable to tolerate other medications. However, CPP carries with it significant toxicity and potential to damage the bladder.

Dr. Bates' study focuses on how a protein called fibroblast growth factor receptor 2 (FGFR2) mitigates the toxicity from CPP in the bladder. Specifically, his team has found that mice missing FGFR2 have an abnormal regenerative response in the urothelium following CPP administration. The mutant mice have prolonged injury and an inability to repair the damage to the lining of the bladder called the urothelium. What the team learns about the roles of FGFR2 will likely inform how mutations in other proteins act to mitigate the toxic effects of CPP on the bladder.

The full technical abstract of the grant is provided on page 3.

A Robust Research Program

Dr. Bates' new grant is the fourth NIH award to be secured by UPMC Children's nephrology researchers in the last six months, a fact that speaks volumes about the exemplary nature of the Division's research program and faculty. In addition to Dr. Bates' R01, Agnes Swiatecka-Urban, MD, was awarded an R01 grant to study pathogenic TGF beta activity in lung epithelial cells. Dana Fuhrman, DO, MS, another Division member, was awarded a K23 grant to support her studies to identify novel biomarkers to predict the severity of acute kidney injury in patients with congenital heart disease who undergo cardiac surgery. Sunder Sims-Lucas, PhD, secured a new R56 award to continue his acute kidney injury studies and the possible protective role that a family of genes called the sirtuins may play in AKI (see page 2).

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Grant to Study AKI Awarded to Sunder Sims-Lucas, PhD

Division of Pediatric Nephrology researcher **Sunder Sims-Lucas, PhD**, was awarded a new National Institutes of Health (NIH) R56 grant to continue his studies of acute kidney injury (AKI) and the significance of a family of genes called the sirtuins and their possible protective role in AKI. Sirtuin genes have been linked to a number of processes related to aging and metabolic defects, including tubule injury following AKI. The SIRT5 gene plays key roles in fatty acid oxidation, which is a major energy source for the tubules of the kidney.

"Knocking out SIRT5 in mouse models has led us to theorize two processes that may be driving a protective phenotype in AKI. SIRT5 interacts with SIRT1 and 3, both of which are known to be protective sirtuins. Knocking out SIRT5 may release SIRT1 and 3 from a competitive inhibition. In the models, we see this upregulation of SIRT1 and 3 driving a protective phenotype such that the animals are not very susceptible to AKI," says Dr. Sims-Lucas.

The R56 grant will allow Dr. Sims-Lucas to continue his research and gather data for R01 funding for his AKI studies. The aims of the new R56 are threefold. The first aim seeks to define the specific site of SIRT5 action during kidney injury with a particular focus on the proximal tubule epithelial cells (PTEC). The second part of the study will focus on the role of peroxisomal fatty acid oxidation during kidney injury. Lastly, Dr. Sims-Lucas' study will mechanistically define the molecular targets of SIRT5 during kidney injury. All three of the aims will utilize a rigorous, mechanistic approach that combines in vitro and in vivo models using both murine and human cell lines.

For complete information on Dr. Sims-Lucas' new grant, please see the technical abstract that follows.

Dr. Sims-Lucas also recently published new findings on his Sirtuin 5 research in the *Journal of the American Society of Nephrology.* Published in October, the new research findings show that Sirtuin 5 is a regulator of fatty acid oxidation in the proximal tubule, providing protection against acute kidney injury. This mechanism of protection may be a target against which new therapies can be developed to treat AKI.

For more information, please see the new paper: Chiba T, Peasley KD, Gargill KR, Maringer KV, Bharthi SS, Mukherjee E, Zhang Y, Basisty N, Yagobian SD, Schilling B, Goetzman ES, Sims-Lucas S. Sirtuin 5 Regulates Proximal Tubule Fatty Acid Oxidation to Protect Against AKI. *J Am Soc Nephrol.* 2019 Oct 1. ASN.2019020163. Epub ahead of print.

About Dr. Sims-Lucas

Dr. Sims-Lucas is an assistant professor of pediatrics in the Division of Pediatric Nephrology at UPMC Children's Hospital of Pittsburgh. A developmental biologist by training with a focus on the kidney, Dr. Sims-Lucas also holds appointments in the University of Pittsburgh Department of Developmental Biology, the Clinical and Translational Science Institute, and the Center for Critical Care Nephrology. Dr. Sims-Lucas operates a broad portfolio of research interests, many with related and interrelated themes. His postdoctoral research at UPMC Children's focused on fibroblast growth factor receptors in kidney development, and upon joining the Division as full-time faculty in 2013, his laboratory began investigating aspects of the different types of developing vasculature, identifying a novel subset of endothelial progenitors that are critical for repair after an acute kidney injury (AKI). Since his early days with the Division, Dr. Sims-Lucas' research has progressed and expanded into a host of related themes that deal with vasculature development, acute kidney injury, hypoxia and nephrogenesis, and endothelial progenitors in the developing kidneys and lungs and their diseases, among others. The goal of much of this research is to ultimately develop new therapies that could be used to mediate acute kidney injury or help stimulate repair of injured kidney tissues following AKI.

R56 Grant Technical Abstract

Acute kidney injury (AKI) occurs in nearly 1 of 5 hospitalized patients and is associated with increased morbidity and mortality across all ages. Many AKI patients will recover kidney function post-injury but then progress to chronic kidney disease (CKD). The mechanisms are poorly understood, and there are currently no effective therapies to prevent, limit, or reverse the tissue damage. There is a critical need to identify mechanisms involved in the pathogenesis of AKI. Our long-term goal is to elucidate these mechanisms and leverage them for new therapies to limit AKI and prevent the transition to CKD. Proximal tubule epithelial cells (PTEC), a major site of damage during AKI, are very metabolically active and rich in mitochondria. Mitochondrial metabolism causes increased reactive oxygen species (ROS), which has been implicated in both ischemia-reperfusion injury (IRI) and cisplatin-induced nephrotoxicity. Modulating mitochondrial function during AKI is an attractive, but thus far unachievable, strategy. Our central hypothesis is that loss of the mitochondrial sirtuin lysine deacylase SIRT5 leads to shifts in PTEC metabolism that protect against AKI. This is supported by preliminary data showing protection against both IRI and cisplatin-induced AKI in global SIRT5 knockout (SIRT5-/-) mice in vivo and in vitro as well as in primary human PTEC with siRNA knockdown of SIRT5. Further data support our proposed mechanism of protection in which SIRT5-/- PTEC exhibit a form of metabolic preconditioning characterized by a shift of fatty acid oxidation (FAO) from mitochondria to peroxisomes. Peroxisomes have previously been linked to renoprotection in other animal models, most likely due to their ability to eliminate ROS. In SIRT5-/- kidneys, peroxisome number is increased at baseline and the peroxisomes are more resistant to damage during AKI. Our central hypothesis will be tested with three aims. The first aim will define the specific site of SIRT5 action during kidney injury with a particular focus on PTEC. While the second aim will drill down on the role of peroxisomal fatty acid oxidation during kidney injury.

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New R01 Grant to Study Bladder Injury *Continued from Page 1*

Technical Abstract

Cyclophosphamide (CPP)-induced injury to bladder urothelium can lead to life-threatening health conditions, including hemorrhagic cystitis and bladder cancer. The application's broad, long-term objectives are to identify mechanisms driving urothelial regeneration after CPP-induced bladder injury. CPP induces urothelial loss from apoptosis and necrosis within 48 hours, followed by proliferation and regeneration that finishes by around 28 days. Both Uroplakin-expressing cells near or at the lumenal surface and Keratin 14-expressing cells in the Basal layer of the bladder have been shown to have proliferative potential after CPP-injury. While fibroblast growth factor receptor 2 (FGFR2) is expressed throughout bladder urothelium, cell-specific roles of FGFR2 urothelial regeneration after CPP-injury are unknown.

Preliminary data show that mice with conditional deletion of FGFR2 in all bladder urothelial layers have defective urothelial repair after injury. At three days post-CPP, controls had urothelial hyperplasia, significant restoration of Uroplakin staining and major resolution of inflammation and hemorrhage, while mutant bladders had less hyperplasia, attenuated Uroplakin staining, and ongoing hemorrhage and inflammation. While both mutants and controls had expansion of Keratin 14-expressing cells across Basal layers three days after CPP, mutant Keratin 14+ cells were hypertrophic with enlarged nuclei, suggesting a cell cycle defect. Cell cycle profiling and assays for DNA content suggest that mutant Keratin 14+ cells have aberrant endoreplication (DNA replication without completion of mitosis, leading to polyploidy). FGFR2 mutant cells undergoing apparent endoreplication also had evidence of increased DNA damage/replication stress three days after injury.

Given that FGFR2 stimulates ERK that can suppress cell cycle entry and endoreplication, ERK and its readouts were assessed, and both were reduced in mutants three days after CPP. Regeneration defects, including apparent endoreplication in Keratin 14+ cells, persisted 10 days after CPP. Together, the hypothesis is that endogenous FGFR2 signaling promotes regeneration after CPP by repressing endoreplication in Keratin 14-expressing urothelial cells via ERK.

To test this hypothesis, the following aims are proposed.

Aim 1: Determine how FGFR2 promotes regeneration of bladder urothelium after CPP-injury. The hypothesis is that FGFR2/ERK signaling suppresses pathological endoreplication and promotes regeneration after CPP injury. Whole animal and cell-based assays will elucidate roles of FGFR2/ERK to suppress endoreplication and drive regeneration, including genetic rescue with constitutively active ERK in mutants and chemical rescue to accelerate repair in controls.

Aim 2: Identify cell-specific roles of FGFR2 signaling in the bladder urothelium in regeneration after CPP injury. The hypothesis is that FGFR2 acts in Keratin 14+ Basal cells to promote regeneration after CPP injury. FGFR2 will be deleted specifically in Uroplakin-expressing and Keratin 14-expressing urothelial layers to identify cell-specific actions after CPP-injury.

Division News and Notes

New Clinical Trial for Hyperoxaluria to Begin at UPMC Children's

UPMC Children's Hospital of Pittsburgh is participating in the Dicerna PHYOX1 DCR-PHXC-201 and 301 clinical trials, and is the first site in the United States to be activated in the trial.

Dicerna's DCR-PHXC is a treatment for the genetic forms of primary hyperoxaluria, a rare condition in which the liver produces an over-abundance of oxalate, which tends to build up in the body and severely damage a number of organs, including the liver and the kidneys where oxalate injury leads to end-stage renal disease (ESRD).

The DCR-PHX-201 study is a multicenter, double-blind, randomized, placebo-controlled trial that will enroll patients with types 1 and 2 of primary hyperoxaluria. DCR-PHXC-301 will

be a longer-term study open to patients previously enrolled in prior DCR-PHXC studies to follow them long-term.

Michael Moritz, MD, clinical director of the Division of Pediatric Nephrology at UPMC Children's serves as the site investigator.

Clinical Director Recognized as International Leader in Water-Electrolyte Imbalances

Michael L. Moritz, MD, clinical director and director of dialysis for the Division of Pediatric Nephrology at UPMC Children's, was recently recognized as one of the top five experts in the world in water and electrolyte disorders by the website Expertscape.

Dr. Moritz has published extensively on electrolyte disorders with numerous papers, case studies, and guidelines involved with understanding and preventing hyponatremia. His work in studying and advocating for the use of isotonic fluids in hospitalized patients to prevent hyponatremia led to new published guidelines on the subject in 2018 from the American Academy of Pediatrics for which Dr. Moritz served as senior author on the committee that developed the new clinical guidance on maintenance fluids.

Dr. Moritz also recently co-authored a case report in the *British Medical Journal* outlining an intriguing case of fragility fracture and osteopaenia induced by chronic hyponatremia

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UPMC Children's Pediatric Nephrology Faculty Member Awarded New K23 Grant

Dana Fuhrman, DO, MS, assistant professor in the Division of Pediatric Nephrology at UPMC Children's Hospital of Pittsburgh, was recently awarded a National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) K23 career development award for a novel study of renal fitness in young adults with congenital heart disease (CHD). Dr. Fuhrman's study will assemble a multidisciplinary contingent of investigators from critical care medicine, nephrology, and cardiac surgery to unravel the mysteries of kidney "fitness" and how it can be augmented in patients undergoing complex heart surgery.

"Patients' kidneys can take a beating from disease and treatment. Understanding how some patients can endure kidney insults while others become injured is a key unmet need," explains **John Kellum, MD**, director of the Center for Critical Care Nephrology in the Department of Critical Care Medicine at the University of Pittsburgh, who is an internationally recognized acute kidney injury researcher and Dr. Fuhrman's primary mentor. "The implications of her work will eventually be applicable to acute kidney injury, which affects the lives of 4 million Americans each year."

Children's hospitals across the country face an exponential increase in the numbers of young adults with congenital heart disease (CHD) who require corrective surgeries, since more and more children with CHD are able to survive into adulthood. Many of these patients are at high risk for poor renal outcomes, including acute kidney injury (AKI) and chronic kidney diseases, which require intensive intervention by nephrologists and intensivists.

"Unfortunately, we have had limited means to predict which patients will do poorly," says **Carlton Bates, MD**, chief of the Division of Pediatric Nephrology at UPMC Children's. "Dr. Fuhrman's research has the promise of identifying new and innovative biomarkers to indicate those patients at high risk of poor outcomes, allowing clinicians to gauge their level of scrutiny and care following cardiac procedures."

There are no readily available means to quantify renal fitness clinically, and there are no prior studies that have examined the long-term effects of glomerular reserve or preoperative tubular biomarker values in any patient group. Given this paucity of data, the goal of Dr. Fuhrman's research is to establish an objective method to quantify renal fitness in young CHD patients ages 18 to 28. Dr. Fuhrman plans to quantify glomerular reserve by measuring estimated glomerular filtration rate (eGFR) values using cystatin C before and after a protein load. Dr. Fuhrman piloted this easily replicated method during her nephrology fellowship years as a T32 NIH scholar at the University of Pittsburgh and UPMC Children's.

On admission to the hospital for coronary bypass (CBP) exposure, young CHD patients will be assessed for AKI risk in order to determine:

- The association of glomerular reserve and AKI risk
- The expression of tubular biomarkers that have been shown to be early markers of kidney damage
- The association of these biomarkers with AKI

Patients will be followed for one and two years in order to determine if preoperative glomerular reserve and tubular biomarkers estimate the risk of chronic kidney disease.

More information about Dr. Fuhrman's K23 grant can be found on the NIH RePORT site. Visit the UPMC Children's website to learn more about the Division of Pediatric Nephrology (**chp.edu/nephrology**) and the University of Pittsburgh Department of Critical Care Medicine's Center for Critical Care Nephrology website at **https://www.ccm.pitt.edu/content/center-critical-care-nephrology**.

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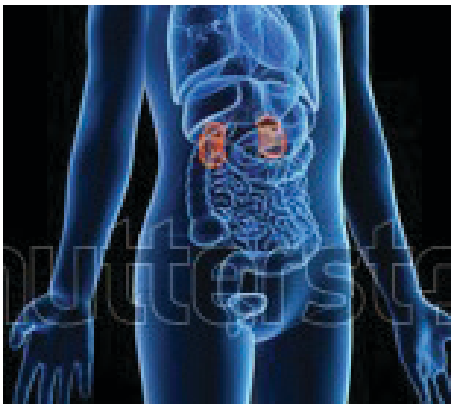
in a 16-year-old male patient. This is the first case in the literature of a young patient suffering a low impact fracture as a result of chronic hyponatremia and osteopaenia. Once the patient's hyponatremia was resolved, his bone mineral density eventually significantly improved over a period of 19 months. The case report suggests that "mild hyponatremia may have implications for future bone health in the pediatric population," and that hyponatremia "may be a modifiable risk factor for bone health in adult and pediatric patients." More research will be needed to fully understand these associations and pathways, but this case does open the door to new lines of investigation in the future.

More details about the case can be found in the following citation: Patel M, Ayus JC, Moritz ML. Fragility Fractures and Reversible Osteopaenia Due to Chronic Hyponatremia in an Adolescent Male. *BMJ.* 2019; 12:e229875. Epub ahead of print.

Pediatric Nephrology Chief Receives Society of Pediatric Research Service Award

Carlton M. Bates, MD, chief of the Division of Pediatric Nephrology at UPMC Children's Hospital of Pittsburgh has been selected to receive the 2019 Society for Pediatric Research (SPR) Thomas A. Hazinski Distinguished Service Award largely for his work to create and teach a grant writing course for researchers in the early stages of their careers. The course, "K-Grant 101 for Pediatric Researchers," is a six-month tutorial given to five selected applicants from the SPR Junior Section Members who are embarking upon writing proposals for K08 or K23 research funding.



Dr. Bates started the course in 2017, which is a part of the SPR Peer Mentoring Group. Dr. Bates' course begins with an initial face-to-face meeting with the selected applicants at the annual spring Pediatric Academic Societies (PAS) Meeting. Dr. Bates and the participants then have monthly teleconferences in which he provides guidance for how to write individual grant sections. The participants then submit grant section drafts for review by Dr. Bates and a peer, after which Dr. Bates and the peers provide oral and written critiques at a follow-up meeting. Two classes of participants have completed the course to date and increasing numbers of Junior Section Members are applying to take the course each year. The SPR also is tracking past participants' successes in obtaining K awards.

Dr. Bates intends to continue the course after he completes his term as a SPR Council member in 2019.

More About Dr. Bates

Dr. Bates received his medical degree from The Ohio State University College of Medicine and completed a residency in pediatrics at Nationwide Children's Hospital. He then completed a fellowship in pediatric nephrology at the University of Texas Southwestern (UTSW) Medical Center in Dallas.

After one year as nontenure track faculty at UTSW, Dr. Bates became an assistant professor of pediatrics at The Ohio State University/ Nationwide Children's Hospital in 1999. He was tenured and promoted to associate professor in 2007. In 2008, Dr. Bates accepted the position of division chief of pediatric nephrology and the director of the pediatric nephrology fellowship program UPMC Children's Hospital of Pittsburgh and the University of Pittsburgh School of Medicine. Dr. Bates was promoted to professor of pediatrics in 2014. In 2016, Dr. Bates assumed the role of Vice Chair of Basic Research in the Department of Pediatrics at the University of Pittsburgh School of Medicine.

Dr. Bates' research is focused on the role of fibroblast growth factor receptors (FGFRs) and their adapter binding proteins in kidney and lower urinary tract development and disease in murine models. Utilizing conditional and global knockout approaches to manipulate expression of FGFRs 1 and/or 2 and their adapter proteins, his lab has uncovered many novel roles for the receptors in multiple lineages and at different stages of kidney and lower urinary tract development. These murine lines often mimic many of the congenital forms of kidney and bladder disease that are leading causes of renal and lower urinary tract disease in children. Recently, the lab has identified novel roles for FGFR2 in protecting against bladder urothelial injury and driving early regeneration of outer urothelial layers after cyclophosphamide infusion.

Dr. Bates was elected to the Society for Pediatric Research in 2002 and was elected to the SPR Council in 2016. Since beginning his tenure on the SPR Council, Dr. Bates has been a member of the Mentoring Work Group and the Finance Committee. He also is actively participating in writing an SPR perspectives manuscript that will offer strategies for early stage pediatric clinician-scientists (and their institutional leadership) to increase their chances of success in obtaining external research funding.

New NIH Grant to Study AKI *Continued from Page 2*

Finally, the third aim will mechanistically define the molecular targets of SIRT5 during kidney injury. All of the three aims will utilize a rigorous, mechanistic approach that combines in vitro and in vivo models. In vivo studies in mice will use both global SIRT5-/- and inducible, PTEC-specific knockout of SIRT5, as well as global LCAD-/- (key mitochondrial FAO enzyme). In vitro studies will use isolated primary mouse and human PTEC, as well as genetically manipulated mouse and human cell lines. Human AKI will be modeled in mice by unilateral ischemia-reperfusion injury and single-high dose treatment with the nephrotoxin cisplatin. In both models of injury, the role of SIRT5 in mediating the progression from acute to chronic kidney disease will be studied. This project will significantly advance the field by opening up new therapeutic avenues where SIRT5 can be pharmacologically inhibited in the context of AKI to protect against injury and block the progression to chronic kidney disease.

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About the Division

The Division of Pediatric Nephrology at UPMC Children's Hospital of Pittsburgh provides a full range of services for the evaluation and management of children with simple or complex nephrologic or urologic disorders. UPMC Children's is ranked 13th nationally by *U.S. News and World Report* in pediatric nephrology.

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For a referral or consultation, please contact us at 412-692-5182. Visit us online at **CHP.edu/nephrology.**

About UPMC Children's Hospital of Pittsburgh

Regionally, nationally, and globally, UPMC Children's Hospital of Pittsburgh is a leader in the treatment of childhood conditions and diseases, a pioneer in the development of new and improved therapies, and a top educator of the next generation of pediatricians and pediatric subspecialists. With generous community support, UPMC Children's Hospital has fulfilled this mission since its founding in 1890. UPMC Children's is recognized consistently for its clinical, research, educational, and advocacy-related accomplishments, including ranking 15th among children's hospitals and schools of medicine in funding for pediatric research provided by the National Institutes of Health (FY2018) and ranking on *U.S. News & World Report's* Honor Roll of America's Best Children's Hospitals (2019–20).

USNW513095 AS/MP 10/19