National Institute of Diabetes and Digestive and Kidney Diseases
National Institutes of Health

Annual Meeting: Collaborating for the Advancement of Interdisciplinary Research in Benign Urology

Turf Valley Conference Center
2700 Turf Valley Road, Ellicott City, MD
December 12–14, 2018

DRAFT SUMMARY

WEDNESDAY, DECEMBER 12, 2018

Welcome

Christopher Mullins, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

On behalf of the NIDDK and the meeting planners, Dr. Christopher Mullins, Project Officer for the George M. O'Brien Urology Cooperative Research Centers (O'Brien Centers), welcomed attendees to the Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (CAIRIBU) meeting. He acknowledged the O'Brien Centers' NIDDK Project Scientist, Dr. Tamara Bavendam, and the Program Coordinator and Director of the Interactions Core, Dr. Kristina Penniston. The meeting participants included investigators in the NIDDK Urology Centers Program, which consists of the Developmental Centers (P20 funding mechanism) and O'Brien Centers (U54 funding mechanism); O'Brien Centers External Expert Panel; mentors and investigators supported through the NIDDK K12 Career Development Program (e.g., K12 Urologic Research [KURe] and K12 Urological Epidemiology [UroEPi]); and NIDDK program staff.

The Urology Centers Program actively is addressing the mission of the NIDDK, because it relates to urologic diseases and specifically addresses four key goals of benign urologic disorders and benign urology (BU) research as follows: (1) promotes research excellence, (2) builds interdisciplinary research collaborations; (3) ensures a robust research community; and (4) fosters high-quality, investigator-initiated (e.g., R01-funded) research studies. The NIDDK supports BU research through several funding mechanisms, including the R01, F and T series training grants, K-series career development awards, P20s, U54s, and U01s. The U01-funded grants support the urology-related clinical trials groups (e.g., Multidisciplinary Approach to the Study of Chronic Pelvic Pain, Symptoms of Lower Urinary Tract Dysfunction Research Network [LURN], Prevention of Lower Urinary Tract Symptoms, and Urinary Stone Disease Research Network). The NIDDK sees R01s as key to advancing its efforts in BU research. Dr. Mullins conveyed that the NIDDK wants investigators to contact program staff for guidance on research and training opportunities through the NIDDK website or via Google search.

Meeting Overview and Announcements

Kristina Penniston, Ph.D., University of Wisconsin at Madison (UW–Madison), and Tamara Bavendam, M.D., M.S., NIDDK

Dr. Bavendam explained that the new title for the Urology Program Director's meeting, CAIRIBU, was developed from the need to describe more clearly the meeting's purpose and the participation of the O'Brien and Developmental Centers and the K12 program investigators. From a historical perspective, the O'Brien Centers, which are named after Illinois Representative, the late George M. O'Brien, were

formed in 1987 and were awarded under the P50 funding mechanism as independent research centers. In 2010, the NIDDK recognized the need for interdisciplinary research and established the first U54 O'Brien Urology Cooperative Research Centers in 2013. Research incubators (i.e., P20 Developmental Centers) had been established in 2012. It is anticipated that the U54 and P20 structures will be incentives that drive the NIDDK R01 BU research applications. The goals of the O'Brien Centers are to conduct innovative science, bring new investigators and methods into BU research, support the new investigator pipeline within NIDDK's mission, and serve as a resource for the BU research community. The investigator-initiated research project grants, its three groups (P20s, U54s, and K12s), and researchers will be dynamic. The opportunity to thrive will exist and will be ongoing.

Dr. Bavendam acknowledged her Urology Program predecessors, Drs. Deborah Hoshizaki and Tracy Rankin, and thanked them for their vision in establishing the P20- and K12-funded programs. The Urology O'Brien Centers have sponsored meetings to share their scientific progress to date, built interdisciplinary teams at their respective institutions, used the Opportunity Pool Project funds to support new BU researchers and early-stage investigators, and established strong Educational Enrichment Programs and biomedical cores. She expressed appreciation to the O'Brien Centers Principal Investigators and Dr. Penniston for their role in organizing the meeting. The goal is to convene the CAIRIBU meeting annually and build trust. The next steps will be to increase O'Brien Centers' visibility through updating the website, attending conferences, matching researchers to the available resources, and avoiding duplications of efforts with other organizations.

Dr. Penniston provided an overview of the meeting agenda and logistics and reminded participants that the meeting was closed and confidential. The 2.5-day meeting was structured to maximize interdisciplinary cooperation, networking, and communication. The agenda consisted of a keynote lecture, four Scientific Sessions, two Emerging Leader Sessions, and three NIDDK Interactive Sessions. Participants also had other opportunities to network and communicate during the group lunches, dinners, and a moderated poster session. The next CAIRIBU meeting is planned tentatively for December 4–6, 2019, and the location is to be determined.

SCIENTIFIC SESSION 1—GENITOURINARY (GU) DEVELOPMENT: NORMAL AND ABNORMAL

Introduction and Overview

Moderators: Cathy Mendelsohn, Ph.D., and Jonathan Barasch, M.D., Ph.D., Columbia University (Principal Investigators, U54)

Dr. Jonathan Barasch explained that the Columbia University O'Brien Center addresses congenital defects of the lower urinary tract and that the principal investigators include human and mouse geneticists and cell biologists who will present their research later in the session. He announced that Dr. Simone Sanna-Cherchi is a new recipient of the P20 award at the Columbia O'Brien Center. Dr. Barasch noted that the speakers will answer participants' questions at the end of the session.

Developmental Origins of Congenital Malformations of the Urinary Tract

Cathy Mendelsohn, Ph.D., Columbia University (Principal Investigator, U54)

Dr. Cathy Mendelsohn described the Columbia O'Brien Center's approach to congenital malformation research, which focuses on congenital anomalies of the kidney and urinary tract (CAKUT) and the association of these malformations to urinary tract infection (UTI) and pyelonephritis. The Mendelsohn laboratory uses genetic mouse models for analysis and validation of mutations associated with these congenital malformations. She described focal points and common etiologies among lower urinary tract congenital defects, including vesicoureteral reflux (VUR), ureteropelvic junction obstruction (UPJO), and

posterior urethral valve (PUV). Although several theories exist into why these defects occur, the Mendelsohn laboratory has shown an association of apoptosis of the common nephric duct during human development. Other studies have linked the hedgehog-signaling pathway to UPJO and identified a role for hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in ureteral peristaltic dysfunction.

Aside from organ development, communication and signaling between the ureters and bladder also are important indicators in investigating congenital malformations. A mouse model of obstruction that focused on the urogenital sinus developed by the Mendelsohn laboratory shows that defective retinoic acid signaling results in squamous metaplasia in the developing urothelium and defective ureter insertion. In addition, congenital malformations and single-gene mutations have been linked to specific disorders. The incidence of PUV is associated with kidney disease in children, but the etiology is not well understood. Mutations in the *Sal-like 1* (*Sall1*) gene in humans are related to Townes-Brocks Syndrome, which is characterized by multiorgan abnormalities, including PUV. Mouse genetics could provide clues into the mechanism of PUV.

Dr. Mendelsohn summarized that syndromic defects involving the urinary tract and other organs can be attributed to single-gene mutations responsible for conserved functions. Communications between different organs in the body exist. Most urinary tract defects are observed in the first trimester of human development. Developmental and genetic pathways are conserved, and pediatric urology has gained insight into these pathways from the use of genetic mouse models.

Novel Genomic and Functional Approaches to Understand the Etiology and Consequences of Kidney and Urinary Tract Malformations
Simone Sanna-Cherchi, M.D., Columbia University (Principal Investigator, P20)

Dr. Sanna-Cherchi described novel genomic and functional approaches to understanding the etiology and consequences of kidney and urinary tract malformations, which is the focus of his recently awarded P20 Developmental Center grant. CAKUT conditions are genetically complex, familial aggregation exists, cases are rare, and sporadic early-age mortality occurs. In addition, 20 percent of CAKUT cases are misdiagnosed using clinical data. Dr. Sanna-Cherchi and co-investigators identified rare loss-of-function mutations in the growth regulation by estrogen in breast cancer 1-like (*GREB1L*) gene in 200 cases of kidney and GU malformations. To extend these findings, Dr. Sanna-Cherchi and his group are collaborating with Duke University on the functional modeling of *GREB1L* mutation in zebrafish. The copy number variants (CNV) in the congenital defects of kidney and urinary tract were assessed in 2,000 cases. Six syndromes were shown to be associated with 60 to 70 percent of the CNVs identified. Of the six, the 16p11.2 syndrome—a common disorder known to be associated with intellectual disability, obesity, and congenital defects of the urinary tract—was selected to be investigated further. In collaboration with Dr. Mendelsohn and Project 2, they were able to show that the *TBX6* gene is a genetic driver of urogenital malformations in the 16p11.2 syndrome. Dr. Sanna-Cherchi reviewed his future work involving CAKUT etiology, the disease penetrance, and initiating cell types, which is the basis of the P20 grant. Functional approaches will include exome sequencing, CNV analyses, and single-nuclei RNA Seq.

Novel Iron Metabolic System Controls UTI
Jonathan Barasch, Ph.D., Columbia University (Principal Investigator, U54)

Dr. Barasch is investigating the role of UTI in CAKUT obstruction and kidney injury. Approximately 13 percent of women and 2.5 percent of men present to the clinic annually with a UTI. Although not a medical emergency, chronic cystitis, kidney infection, or sepsis can result from a UTI. Immune defenses against UTIs include death of cells that shed to the urine, neutrophil recruitment, and production of antimicrobials. One component of innate immunity is nutritional immunity, in which, bacteria are challenged to obtain the necessary nutrient sources for replication and sequester minerals, including iron,

from invading pathogens. The amount of free iron is limited to support normal cellular processes and bacteria delivery systems. Studies have shown that siderophores, iron carriers, are upregulated in the bacterial colony-forming units (CFUs) from adult UTI patients. Alkalinized urine has been observed in CAKUT patients.

To address the question of the source (e.g., kidney or tubules) of the iron in UTIs, the Barasch laboratory performed megalin receptor (i.e., proximal tubule protein uptake mediator) gene knock- down experiments. He found that a decrease in megalin protein resulted in an increase in iron containing proteins, including transferrin receptor and divalent metal transporter 1 (DMT1), in the urine, which are stimulatory to bacteria growth. The iron transporter proteins were absent from collecting duct, but the kidney duct-intercalating cells (ICs) contained high levels of ferritin. Fluorescent bacteria injected into lower urinary tract of female mice showed the 50 percent of the uptake occurred in collecting duct cells.

Using an Uracil phosphoribosyltransferase cell specific technique, the Barasch laboratory investigated the iron accumulation in ICs and the innate immune response in a mouse models for pyelonephritis and VUR. Bacteria are localized in the kidney in VUR and area of obstruction. The genetic signatures of bacteria present in the VUR model confirm the bacterial distribution in the pelvis of the kidney and collecting duct, on the surface of the ICs. Although the bacteria burden is removed, the genetic signature of the bacteria persists. The neutrophil gelatinase-associated lipocalin (*NGAL*) gene was upregulated in the presence of a UTI. The heme importer, *HGR1*, heme oxygenase, and heme exporters are expressed in the kidney duct. Heme injections into the kidney pelvis of mice confirm a mechanism of heme import/transport in the collecting duct in which carbon monoxide, the heme degradation product, kills the bacteria. This metabolic process dissipates after 24 hours. The HGR1importer, which is expressed in the kidney duct is upregulated in the circadian clock. The lesser known transcription factor CP2-like 1 (Tfcp2l1) was found to be associated with ICs.

Clinical Sequencing for Kidney and Urologic Disorders
Ali Gharavi, M.D., Columbia University (Principal Investigator, U54)

Dr. Ali Gharavi presented on the opportunities and challenges in precision medicine for kidney and urologic diseases from a clinical sequencing perspective and described his work on kidney genetics. Although the human genome has been sequenced, a wide range of genetic abnormalities, including chromosomal rearrangements and CNVs, have not been identified and could be involved in disease pathogenesis. The advent of sequencing technology has improved the capability of detecting genetic abnormalities, especially small chromosomal changes. Using genome sequencing, Dr. Gharavi and Dr. Sanna-Cherchi evaluated 3,000 children with congenital urinary tract defects and found 45 distinct genomic imbalances in 4 percent of the cohort, which previously had not been identified clinically. Genetic diagnoses—such as 17q12 syndrome, 16p11.2 microdeletion, and 22q11.2 DiGeorge syndrome—provide opportunities for personalized care (i.e., precision medicine) for renal diseases. Dr. Gharavi emphasized that microarray screening should be a first-line diagnostic approach in patients with congenital urinary tract defects.

With a focus on the coding region of the genome, Dr. Gharavi and co-investigators next used whole-exome sequencing (WES) for discovering genes for congenital kidney defects, which Dr. Sanna-Cherchi described earlier in the meeting. Clinical WES now is used widely in the diagnosis of many diseases and disorders, including cancer and Mendelian disorders. In a pilot study evaluating 92 adults with chronic kidney disease (CKD), clinical WES provided a diagnostic rate of 24 percent for patients with early-onset CKD, familial CKD, and CKD of unknown etiology. Thirteen distinct genetic disorders were detected in the 10 individuals with CKD of unknown etiology. The use of WES in adults with CKD was expanded to larger cohorts. The AURORA (A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Haemodialysis: An Assessment of Survival and Cardiovascular Events) and Columbia University

Medical Center cohorts were combined (AURORA-CUMA CKD cohort), yielding a total of 3,315 patients. The diagnostic yield was 9.3 percent for patients with CKD, and 66 genetic disorders were identified. Dr. Gharavi described case studies of the application of exome sequencing in the clinic, which revealed a diagnosis of Alport syndrome, one of most commonly misdiagnosed disorders in nephrology. The potential for misclassification of genetic variants and misdiagnosis also has been evident from WES analysis in healthy individuals, as the Gharavi laboratory has shown.

The interpretation of sequence variants is held to strict standards, and the American College of Medical Genomics and the Association for Molecular Pathology have issued standards and guidelines governing these interpretations. These findings provide compelling evidence that exome sequencing can identify genetic disorders in patients with kidney and GU disorders, with implications for diagnosis and management. Many opportunities for risk stratification, clinical care, selection for transplantation, and selection for clinical trials exist; however, many questions remain regarding the interpretation of variants of unknown significance and the assessment of the impact on clinical care and outcomes.

KEYNOTE LECTURE: CAKUT MUTATIONS—PRECISION MEDICINE AND HUMAN GENETICS

Friedhelm Hildebrandt, M.D., Harvard University, Boston Children's Hospital

Dr. Gharavi introduced the keynote speaker, Dr. Friedhelm Hildebrandt, who is the William E. Harmon Professor of Pediatrics at Harvard Medical School and Chief of Nephrology at Boston Children's Hospital. He has been a leader in the field of genetics for more than 20 years. His clinical work is focused on CAKUT, nephrotic syndrome, and retinal-renal ciliopathies; his laboratory has identified more than 50 novel kidney disease genes. The Hildebrandt laboratory also studies function in disease models of mice and zebrafish. Dr. Hildebrandt is a member of the National Academy of Medicine and has won numerous awards and research grants, mostly from the NIH, the Howard Hughes Medical Institute, and the Doris Duke Charitable Foundation.

Dr. Hildebrandt thanked the NIDDK and meeting organizers for the opportunity to discuss CAKUT mutations and present some aspects of his research. He reiterated that CAKUT are a diverse set of congenital malformations resulting in organ aberrations, including bilateral renal agenesis, renal hypodysplasia, multicystic dysplastic kidney, hydronephrosis, UPJO, VUR, and PUV. Dr. Hildebrandt described one example of a pediatric patient with a complicated clinical history of disease. A female, age 6 years, presented with a history of UTIs and eventually required surgery as a result of UPJO. Molecular data had already identified a single-point mutation in the T-Box 18 (*TBX18)* gene that is associated with UPJO, and this could have spared the patient the cumbersome clinical history to arrive at a solution. This is strong evidence of a cause and effect in which a single-gene mutation is sufficient to cause a disease, which in this case is CAKUT. These types of causative monogenic mutations can be dominant or recessive. Recessive diseases are monogenic disorders that occur because of damage to both copies of each allele. Dominant diseases are monogenic disorders that involve damage to only one gene copy. Truncated proteins are the indicators in monogenic diseases, and discovery is important to arrive at an earlier diagnosis, phenotype correlations, improved treatments, and identification of risk. The goal is establishing personalized medicine for CAKUT.

CAKUT has a high penetrance of genotype-phenotype correlations, and a strong stochastic effect has been observed in mice and humans. Renal agenesis and UPJO are different aberrations but could be the same genetically in monogenic CAKUT. The environmental influences and/or modifier genes are not different. Dr. Hildebrandt speculates that during kidney urinary tract development, morphogenesis is the major process in forming the ureter bud, which is related to the cell migration genes that result in renal agenesis if dysregulated. A ureter bud that stochastically is close to maturity could result in UPJO. In individuals requiring a kidney transplant for survival before age 25 years or who had CKD, the North

American Pediatric Renal Trials and Collaborative Studies, which evaluated 9,000 patients, found that CAKUT could be attributed to 50 percent of CKD causes. To date, 240 monogenic genes have been identified that are related to renal diseases in adults, including 50 in CAKUT. In patients age 25 or younger, CAKUT monogenic genes range from 20 to 30 percent. Because of these data, Dr. Hildebrandt emphasized that for any individual reporting for a renal ultrasound age 25 or younger in which the diagnosis is persistent bilateral renal agenesis, the decision will be compatible with renal disease. WES of the 240 genes will likely result in a 20 percent chance of finding causative monogenic mutations.

Dr. Hildebrandt detailed some of his research in CAKUT mutations. WES detected 49 monogenic causative genes in 236 patients with CAKUT. Similar genes with convincing mutations were detected in large cohorts of 1,000 individuals, which account for only one in 1,000 familial cases. To have genetically sound data, the study requires four families worldwide exhibiting four different mutations.
Dr. Hildebrandt and colleagues conducted a study in Ireland evaluating 114 patients with end-stage renal disease (ESRD) who had one relative with CKD. The findings revealed that 37 percent had mutations in known genes. Of the 37 percent, 13 percent had mutations in the CAKUT genes. These data suggest that monogenic causation is not restricted to childhood-onset CKD. In the European Renal Association-European Dialysis and Transplant Association (ER-EDTA) registry, patients with rare diseases also required a first renal replacement therapy in adulthood.

In the timeframe of his earlier studies on the characteristics of the monogenic causes of CAKUT, only five monogenic diseases in humans were identified, including thalassemia. To begin to address discovery of novel monogenic genes of CAKUT, worldwide sources were canvassed, and more than 10,000 cases were collected over a 20-year period and subjected to WES. Of the 10,000 sequenced, 4,000 had CAKUT and 170 of the 4,000 had convincing mutations. Dr. Hildebrandt collaborated with other laboratories during his research to further investigate CAKUT in families, and he detailed several hypotheses, which were investigated. Dr. Hildebrandt recently reported on 15 recessive and 25 dominant genes that, if mutated, represent monogenic causes of human CAKUT, a culmination of his 20 years of CAKUT genetics research. The identification of causative mutations will provide diagnostics for individuals and families, allow adequate stratification of clinical outcome studies, provide pathogenic insights, and possibly lead to preventative treatment for patients.

Conclusions
Cathy Mendelsohn, Ph.D., and Jonathan Barasch, M.D., Ph.D., Columbia University (Principal Investigators, U54)

There were no additional comments.

Discussion

- From a drug development perspective, it is possible, in theory, to determine the master regulators of RNA or microRNA for a CAKUT-monogenic gene, but the signaling pathway details of most of these genes are not well understood. In addition, distinguishing background noise from a signal for the microRNAs would be challenging, and sequence variation exists within the patient population with CAKUT.
- A group of proteins in the intercalating cells of the collecting duct appears to exhibit antimicrobial properties, but these proteins have yet to be investigated.

EMERGING LEADERS IN BU RESEARCH—SESSION 1

Introduction
Mark Nelson, Ph.D., The University of Vermont

Dr. Mark Nelson remarked that the sessions on emerging leaders provide an opportunity for new (e.g., KURe grant awardees) and established investigators to interact and discuss emerging urology research. Details on the presentations were provided in the printed abstracts of meeting materials.

The Current State of Care of Adults with Urologic Congenitalism
Lindsay Hampson, M.D., M.A.S., University of California, San Francisco (UCSF)

Dr. Lindsay Hampson detailed a study addressing the current state of care of adults with urologic conditions to better understand the access to and utilization of care within the U.S. adult urologic congenitalism population. Although improvements in surgical and nonsurgical interventions have increased the life expectancy for patients with genitourinary (GU) congenital anomalies, specialized clinics that are dedicated to transitional urology care report that only 40 percent of patients had transitioned successfully from pediatric to adult care and that less than 50 percent of patients who had prior surgeries (e.g., bladder augmentation) that required continuing care had transitioned successfully. Using the Facebook advertising platform, Dr. Hampson and colleagues distributed a blind survey that assessed individuals who were 18 years of age and had congenital urologic conditions. Self-reported data on demographics, disease-related characteristics, and objective measures of care were collected on 271 participants from 48 states; respondents' median age was 39 years. Of the 271 responding participants, 70 percent completed the survey, 92 percent had a diagnosis of spina bifida, 72 percent were female; 83 percent were non-Hispanic whites; and a vast majority had a history of a prior urologic surgery. The study revealed the following: (1) significant discrepancies in the care among adults with congenital urologic conditions; (2) barriers that exist for these patients receiving routine urologic care; and (3) a high rate of emergency department and hospital visits. Dr. Hampson described the strengths and limitations of conducting an online survey distributed through social media outlets. The next steps will be to continue to improve access to care for this patient population; improved access to routine care should decrease emergency health care use.

Discussion

- Approximately 40 percent of patients who are treated by adult urologists had a direct referral from their pediatric urologist; the majority sought out a urologist on their own.
- In other disease conditions (e.g., cystic fibrosis) that are subject to care transition, an adult urologist often begins to see patients in the pediatric clinical setting to facilitate a clear transition from pediatric to adult care. Specialized urology clinics/centers could consider this to be a routine practice.
- A urology transition model that spans across pediatric subspecialties and incorporates patient electronic health records will be critical. The use of telehealth methodologies that can be implemented without promoting social isolation also would be helpful.

Kidney Motif and Ankyrin Repeat Domains 1 (KANK1) CNVs Can Be Associated with Urologic Birth Defects
Nannan Thirymavalavan, M.D., Baylor College of Medicine

Dr. Nannan Thirymavalavan discussed the role of KANK1 in male GU development, of which birth defects are common and involve disorders of sexual development and CAKUT. GU birth defects are associated with long-term morbidities, including sexual dysfunction, cancer, kidney dysfunction, and

decreased quality of life, but the etiology of these birth defects is multifactorial. Genetic researchers have shown that, in general, CNVs can lead to gene dosage changes, such as microdeletions (one copy) or microduplications (more than two copies). In a 2010 study, Dr. Delores Lamb at the Baylor College of Medicine and her laboratory evaluated chromosomal deletions and duplications regions in 114 patients with various GU phenotypes, including hypospadias, cryptorchidism, and ambiguous genitalia. Using array comparative genome hybridization methodology, the laboratory identified several hot spots that are associated with these GU defects, including CNVs at 9p23p24 that were present in four patients with ambiguous genitalia. The gene-mapping studies by Dr. Thirymavalavan and the Lamb laboratory revealed that patients with GU birth defects had increased CNVs of KANK1. They also observed renal dysfunction, glomerulosclerosis, and decreased penile size, sperm count and fertility in KANK1 knock-out (KO) mice. These data demonstrate that KANK1 plays a significant role in GU development and should be explored in the management of nephrotic syndrome in pediatric patients. Dr. Lamb is the recently appointed Vice-Chair for Research in the Department of Urology and Director of the Center for Reproductive Genomics at Cornell University.

Discussion

- KANK1 KO mice exhibiting altered testicular histology showed germ cell depletion in some tubules, but it was not a widespread effect.
- Dr. Sanna-Cherchi will share his data on the KANK1 KO mice.

Urinary Incontinence and Nocturia in Older Men: Cross-Sectional and Prospective Relationships with Body Composition and Muscle Strength in a Multicenter Cohort
Scott Bauer, M.D., Sc.M., UCSF

Dr. Scott Bauer explained that three-fourths of male lower urinary tract syndrome (LUTS) will be evaluated, diagnosed, and managed in the primary care setting. A diagnosis generally is based on patient-reported outcomes, rather than invasive measurements. The American Urological Association (AUA) has treatment guidelines and lifestyle advice on LUTS, but the quality of evidence is limited. The current cross-sectional study evaluated the impact of body composition, strength, and urinary incontinence (UI) in the National Institute of Aging (NIA) Health Aging and Body Composition (ABC) cohort. The study concluded that men between the ages of 70 and 79 years who have a lower body mass index (BMI), lower percentage of fat mass, and higher lean body mass are less likely to report prevalent UI; adjustments for physical activity did not change the overall results. Dr. Bauer and colleagues speculate that variations in muscle mass or function may be as important as weight in determining the susceptibility to male UI. Future work will include evaluating the association of body composition and strength on incident UI in older men in the Health ABC cohort and the association between physical activity and LUTS trajectory in the larger Osteoporotic Fractures in Men cohort. In addition, efforts will be focused on evaluating the prospective association between healthy dietary patterns and LUTS in conjunction with the Health Professionals Follow-up Study (HPFS). The all-male HPFS is complementary to the all-female Nurses' Health Study.

Discussion

- The most clinically relevant method for evaluating total fat mass and/or distribution across the body is waist circumference measurements, but imaging techniques such as magnetic resonance imaging (MRI)-computed tomography also are being considered.
- The role of psychosocial factors was not assessed in this study but could be considered in the future.

Predicting UI Status with Sacral Neuromodulation and Botulinum Toxin Treatments
James Hokanson, Ph.D., Duke University

Dr. James Hokanson described a K01 proposal that leverages the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development–sponsored Refractory Overactive Bladder: Sacral Neuromodulation (SNM) vs. Botulinum Toxin (Botox) Assessment (commonly called ROSETTA) study. The goal is to determine the best treatment strategy for urgency UI (UUI) using a predictive modeling approach that focuses on the individual, rather than an average response. Predictive models will be designed using ROSETTA clinical data without urodynamics (Aim 1) and all ROSETTA data with urodynamics (Aim 2). Previously used features (e.g., uroflow, cystometry, and micturition) will be expanded, and nonstandard and novel features, including quantified detrusor overactivity, will be added. Preliminary models using algorithm-based feature selection are being developed, and feasibility data will be compiled from existing ROSETTA sites.

Discussion

- Consider using counterfactual models of causation to assess treatment effects.

Chronic Monitoring of Voiding Function in a Novel Model of Detrusor Underactivity (DUA)
Eric Gonzales, Ph.D., Duke University

Dr. Eric Gonzales presented on developing a novel animal model of DUA to characterize voiding function. DUA is defined as a contraction of reduced strength and/or duration leading to prolonged or incomplete bladder emptying. The mechanisms involved in these symptoms are unclear, and the available animal models are limited. Dr. Gonzales' earlier studies conducted with Dr. Warren Grill at Duke University tested the hypothesis that obese-prone (OP) rats would have decreased contraction strength and bladder emptying. Acute and chronic voiding functions were assessed in the OP rat model after high-fat diet feeding. Cystometry data showed increases in bladder capacity and postvoid residual and decreases in micturition pressure and external urethral sphincter muscle electromyographic burst activity in OP rats after diet-induced obesity. There also was increased frequency and decreased void volumes in OP rats beginning at week 7 of the high-fat diet, as determined from the metabolic cage outputs. After demonstrating the functional difference in the OP rat bladder, the next steps were to determine the neurogenic and/or myogenic contributions to this underactivity in this diet-induced obesity OP rat model. In collaboration with Dr. Johanna Hannan at East Carolina University, muscle contractility tests were performed using KCl, carbachol, electric field stimulation (EFS), and caffeine. Preliminary data showed an initial decrease in bladder contractions in OP rats that was decreased further with EFS. In addition, neuromodulation via pudendal motor stimulation improved bladder emptying efficiency in OP rats.

Discussion

- Investigating the estrogenic effect in the high-fat diet OP rat model, investigating prostate changes, and reversing the effects of the diet might be worth pursuing.

LUTS in Older Children with and without Diabetes Mellitus (DM): A Pilot Study
Maryellen Kelly, D.N.P., Duke University

Dr. Maryellen Kelly described data from a pilot study evaluating LUTS in pediatric patients with or without DM. Diabetic bladder dysfunction, which is experienced by 40 to 70 percent of adults with DM, is characterized by LUTS that could progress into chronic kidney disease, ESRD, nephrolithiasis, pyelonephritis, or UTIs. Less is known about the prevalence of LUTS in children with diabetes, its risk factors, and potential treatments/preventions. Prior studies revealed that diabetic adolescents show early

signs of diabetic bladder dysfunction, and known complications observed in adults with diabetes are now being observed in children with DM. This current study investigated the increase in LUTS in 120 children between the ages of 11 and 17 years, of whom 90 were without DM and 30 were with DM. The variables (e.g., age, sex, diabetes status, and LUTS survey score) were collected, and the Vancouver Symptom Score (VSS) was used to assess LUTS. Results showed that more than one-fifth of pediatric patients were reported as having LUTS, and the rates per the VSS were increased in patients with diabetes. The Hispanic/Latino pediatric patients had the highest rates of LUTS, regardless of DM status. A multicenter study currently is in progress at six different clinical sites in North America to expand on this pilot.

Discussion

- Some data on adults with diabetes in the Hispanic and African American populations show signs that increased prevalence of LUTS exists but is limited. The multicenter study will evaluate this finding further in a larger segment of the Hispanic population. The use of an English-language survey versus a Spanish-language survey could be a factor.

Mind Over Matter (MOM)—Healthy Bowels, Healthy Bladder: A Randomized Controlled Trial
Heidi Brown, M.D., M.A.S., UW–Madison

Dr. Heidi Brown explained that MOM, a group-based intervention to improve bladder and bowel incontinence, leverages the "Dare to Age Well" intervention designed by Dr. Cara Tannenbaum at the Canadian Institutes of Health Research. This intervention was adapted in partnership with the Wisconsin Institute of Health Aging. The intervention group focuses on building knowledge, skill, and self-efficacy for behavior change and consists of eight to 10 women and one community facilitator. This current study is an individually randomized group treatment clinical trial, with a wait-list control group, to evaluate the 3-month impact of the MOM-adaptive intervention program for patients with urinary and bowel incontinence and for those seeking care. Women ages 50 years and over who have had some form of incontinence within the past 3 months were recruited by community organizations, screened, and consented by the study team members, and they completed written questionnaires at three time points during the study. The primary outcome was the Patient Global Impression of Improvement in UI, which was measured at 3 months and again at the end of the intervention. One hundred twenty-two women from six different communities were enrolled and randomized to the treatment or control groups. The study findings suggest that bladder and bowel continence can be improved through an intervention program implemented completely without health care professionals. The adapted MOM intervention has high potential for sustainability, given the existing infrastructure for dissemination of other evidence-based chronic disease self-management programs in Wisconsin. The long-term impact of this intervention program, as well as its impact on other populations, remains to be seen.

Discussion

- In the individually randomized group treatment trial of MOM, no enrollment site-related differences were observed.
- The minimum criteria for community facilitators were based on comfortable interactions with older adults and groups. Those selected received a 2-day facilitator training and were evaluated on skills.

NIDDK INTERACTIONS—SESSION 1

Developing a Proficient Research Question

Robert Star, M.D., NIDDK

Dr. Robert Star, Director, Division of Kidney, Urologic, and Hematologic Diseases (KUH), discussed key aspects of formulating a proficient research question. All research begins with a question. Although there may be many interesting questions, the goal is to choose a question that addresses the NIDDK mission and is appropriate for funding. The process involves selecting a topic and refining that selection. To select a research topic worth pursuing, a clinician might start by focusing on gaps in data/knowledge for treating patients. Other investigators might focus on those subjects that seemingly are unclear or not well understood in their respective fields. When developing a research focus, deciding what not to consider is as important as deciding what to pursue; engaging mentors at this stage of the process will be helpful.

Dr. Star emphasized that establishing research filters to selectively screen ideas is essential. Filters can be as broad as a mission statement or narrow and pragmatic. The important screening determinants in his filter are that the research should be important, innovative, and feasible to translational research. Refining the research project involves sketching, rephrasing, or enhancing the question. Focus efforts on work that is doable with the available resources. The research project also should be important to the respective field, regardless of whether the outcome results in negative or positive data. Other aspects of refining the research project are to consider an assumed truth that does not have a clear meaning, develop an alternative paradigm, and design a simple experiment to test the assumed truth.

Participants held table discussions in an interactive exercise to refine their research questions. The following points were made:

- The specific aims should be developed and refined through a series of discussions with mentors and other colleagues.
- The language used to describe the research project should be specific, and the research setting should be clear.
- The research question should be adapted, whether it is broad or narrow, to fit the application or proposal type.
- The research should begin with a global question that can be crystallized with specific aims.
- Sufficient study models would be necessary to address the goals of the research question.
- The researcher should think big, but be precise.
- The predictors and outcome variables should be defined clearly.
- Consider ways to forge key collaborations with other disciplines.

THURSDAY, DECEMBER 13, 2018

SCIENTIFIC SESSION 2—MECHANICS OF BPH/LUTS

Introduction and Overview

Moderators: William Ricke, Ph.D., UW–Madison, and Zhou Wang, Ph.D., University of Pittsburgh (PITT) Medical Center (Principal Investigators, U54)

Dr. William Ricke welcomed participants to Day 2 of the meeting and provided an overview of the O'Brien Centers BPH research. Two centers focused on BPH have been funded since the inception of the Program: (1) UW–Madison/University of Massachusetts Boston (UMass Boston) in FY 2014 for fibrosis and BPH and (2) PITT in FY 2016 for inflammation and BPH. Many positive aspects of the O'Brien

Centers distinguish them from the P01, P50, and U01 funding mechanisms and structures, such as resources and training/education. Training is a major component of the O'Brien Centers mission, which the NIDDK has strongly endorsed and supported. Dr. Ricke explained that UW–Madison/UMass Boston speakers would present project updates and address barriers to progress in BPH research. He pointed out that sharing unpublished data in this closed meeting forum is a mandate from the NIDDK and reminded participants not to take pictures of PowerPoint slides or posters. The speakers will address participants' questions at the end of the session.

Dr. Zhou Wang also welcomed attendees and explained that the PITT O'Brien Center supports three main research projects and pilot projects. He introduced the two other principal investigators, Drs. Naoki Yoshimura and Donald DeFranco, and the two junior investigators, Drs. Pradeep Tyagi and Laura Pascal, who presented in this session.

Regulation of Prostate Epithelial Cell Function by Estrogen Receptor (ER) Beta (ß) Ligands
Donald DeFranco, Ph.D., PITT (Project Leader, U54)

Dr. DeFranco remarked on the relevance of ERß in maintaining prostate tissue homeostasis and noted the available treatment modalities for BPH/LUTS, which include alpha-adrenergic blockers (alpha blockers), 5-alpha (α)-reductase inhibitors, and surgery if drug treatments fail. Several studies have suggested that patients with high levels of inflammation in the prostate and high International Prostate Symptom Scores have worsening LUTS, but the beneficial effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are not long-lasting, according to clinical trial data. To address the research question of why NSAIDS are not ameliorating inflammation in prostate tissue, Dr. DeFranco focused his studies on investigating the limited clinical effectiveness of NSAIDs, such as cyclooxygenase 2 (Cox-2) inhibitors, in the treatment and prevention of BPH. This research leverages prior findings and current studies of O'Brien Center investigators on the estrogen-androgen balance in the prostate tissue homeostasis (Ricke laboratory); the upregulation of transforming growth factor (TGF)-β cascade genes (e.g., Cox-2) in a prostatic inflammation model (Yoshimura laboratory); and the therapeutic effect of ERß on prostatic inflammation (Yoshimura laboratory).

The DeFranco laboratory showed that reactive oxygen species (ROS) limit the protective effects of ERß in a human prostate cell line, BPH-1, but ERß did not inhibit the epithelial-mesenchymal transition. In another series of experiments, it was observed that Cox-2 has a role in maintaining ERß ligand production, suggesting a protective mechanism. Specifically, the inhibition of Cox-2 in BPH-1 cells reduced production of ERß ligands from testosterone, suggesting the potential disruption of prostatic steroidogenic pathways. Efforts next focused on investigating the regulation of steroid hormone metabolism in BPH-1 cells. Although inconclusive from ingenuity pathway analyses, Cox-2 ablation results in downregulation of ERß biosynthetic enzyme genes and upregulation of the metabolic genes. There also was a rapid loss of ERß ligand 3α-Adiol, an accumulation of dehydroepiandrosterone (DHEA) and dihydrotestosterone (DHT), and transient accumulation of delta-5-Adiol in BPH-1 cells. In addition, they observed a reciprocal regulation of Cox-1 and -2, which suggest a roles for aging.

The DeFranco laboratory also investigated the effects of ERß ligands on BPH-1 cell proliferation. Their findings showed that ERß ligands reduced cell survival in BPH-1 cells, which appears to be associated with the autophagy pathway. In a working mechanistic model of reciprocal cross-talk between ERß and Cox-2 pathways in prostate epithelial cells, Cox-2 has an opposing role in generating ROS, which block ERß, and in generating prostaglandin E2 (PGE2), thereby promoting ERß production.

Potential Mechanisms Underlying Bladder and Afferent Sensitization in Prostatic Inflammation
Naoki Yoshimura, M.D., Ph.D., PITT (Project Leader, U54)

Dr. Yoshimura pointed out the challenges in male LUTS with BPH and the rationale for treatments in addition to currently available bladder outlet obstruction–targeting (BOO) therapies (e.g., alpha blockers or 5α-reductase inhibitors). A positive correlation between LUTS severity and the level of prostatic inflammation in BPH specimens has been reported in the Medical Therapy of Prostatic Symptoms (MTOPS) and the Incidence of Prostate Cancer in Men Who Are at Increased Risk (commonly called REDUCE) trials. In addition, prostatic inflammation in BPH has been shown to be associated with focal regulation of Cox-2 in the glandular epithelium, leading to prostaglandin generation. The interactions between pelvic organs due to cross-organ sensitization, including bladder and prostate, are well known. Reports have shown bladder-afferent hypersensitivity induced by prostate-to-bladder cross-sensitization. These observations led Dr. Yoshimura to hypothesize that prostatic inflammation contributes to bladder dysfunction and enhanced afferent excitability in BPH-associated LUTS.

The Yoshimura laboratory developed an animal model of prostatic inflammation induced by intraprostatic formalin injections that recapitulates human BPH. The model demonstrates upregulation of androgen and TGF-β signaling; activation of nucleotide-binding oligomerization domain-like receptor with pyrin domain protein 1 (NLRP1) and interleukin (IL)-18; and low-grade prostatic inflammation and bladder overactivity that persist 8 weeks after formalin injections. Dr. Yoshimura remarked that although the formalin-induced rat model of prostatic inflammation is not a direct model of human BPH, it is one of the best models available to investigate inflammation related to BPH, as well as LUTS pathophysiology.

Dr. Yoshimura next detailed the specific aims, methods, and the progress to date of his PITT BPH Project 1, entitled "Afferent and Urothelial Plasticity Underlying Bladder Sensitization in Prostatic Inflammation." Prostatic inflammation was induced in rats by formalin injection into the rat prostate. Four weeks after injection, the effect of prostatic inflammation on bladder/prostate afferent pathways was evaluated. Results showed that voiding frequency increased in prostatitis rats compared to normal or untreated animals. Also, in dichotomized and bladder afferents, transient receptor potential ion channel (TRPV1) and ATP receptor $P2X_2$ were upregulated, and hyperactivity of bladder afferent neurons was indicated by the downregulation of the calcium channel component Kv1.4, results that align with published data.

Collaborating with Dr. Lori Birder (PITT School of Medicine) to evaluate the urothelial sensitization in prostatic inflammation, Dr. Yorshimura found that nerve growth factor (NGF), TRPV1, PGE2, and PGE2 receptor 4 (EP4) were upregulated in the rat bladder urothelium. Inhibition of EP4 in the bladder reduced bladder overactivity in prostatitis rats, suggesting the importance of the PGE2-EP4 mechanism. For Project Aim 3, the 5α reductase inhibitor dutasteride, which is used clinically to treat prostatic inflammation in patients, was tested in the rat prostatitis model. Results showed improved bladder overactivity, reduced inflammation, and increased ER expression in animals following daily administration of dutasteride for 4 weeks. These findings align with the Yoshimura laboratory's published data. Future work will include analysis of ERβ-related steroids and androgen-responsive molecules in collaboration with PITT BPH Projects 2 and 3.

Discussion

- Given that detrusor muscle overactivity has been correlated with age and also could lead to afferent sensitization, the effect of age might be worth pursuing in the prostatic inflammation rat model.
- Studies are in progress to determine whether nociceptive or stretch fibers are affected.

Dysfunctional Luminal Epithelial Tight Junction and Permeability Barrier in BPH Pathogenesis
Zhou Wang, Ph.D., PITT Medical Center (Principal Investigator and Project Leader, U54)

Dr. Wang described his research to evaluate the luminal epithelium dysfunction in BPH pathogenesis. He collaborated with pathologists Drs. Rajiv Dhir (PITT Cancer Institute) and Anil Parwani to obtain fresh treatment-naïve and symptomatic BPH human prostate tissue specimens. The area of BPH, normal adjacent tissue, and prostate zone were analyzed and labeled in each specimen, and an immunohistochemistry panel (IHC) and proteomic analyses were performed on isolated stroma and epithelial cells. Surprisingly, proteomics showed that prostate-specific androgen (PSA) protein, which is produced by luminal epithelial cells and secreted to prostate lumina, was present in the stroma of nodular BPH, which was confirmed by IHC and corroborated by UW–Madison investigators. Localization analyses showed that PSA mRNA is not expressed in BPH stroma. In addition, the PSA protein also was not detected in the stroma of prostate cancer, basal cell hyperplasia, or stromal hyperplasia specimens. Collectively, these data suggest that compromised tight junctions in prostatic inflammation and BPH exist.

In a series of fluorescein isothiocyanate (FITC)-permeability experiments, BPH explants, but not normal adjacent prostate explants, were penetrable. Further investigations showed that the tight junctions mediator E-cadherin was downregulated in nodules of BPH and displayed a discontinuous pattern in BPH epithelial cells. Dr. Wang collaborated with Dr. Donna Stolz (PITT) to perform imaging analysis of BPH samples, and the results revealed that the number of connecting points is reduced in BPH luminal epithelial cells. To determine whether E-cadherin downregulation affects permeability, human prostate epithelial cell line BPH-1 and immortalized adult non-tumorigenic human prostate epithelial cell line BHPrE1 were cultured in a transwell system.

Cell monolayer permeability was evaluated using trans-epithelium electrical resistance and FITC-permeability assays. E-cadherin knockdown was performed using small interfering RNA methods. Results showed that E-cadherin knockdown increased permeability in the BHPrE1 monolayer and reduced the number of connecting points, but did not affect cell density. Similar results were observed in the BPH-1 monolayer. In addition, E-cadherin was downregulated in BPH in rat prostatitis. The Wang laboratory next showed that inflammatory cytokine TGF-β increased monolayer permeability in both BHPrE1 and BPH-1 cells and decreased junction proteins E-cadherin and Claudin-1 levels. However, laser capture microdissection of BPH specimens showed only downregulation of Claudin-1. Further investigations revealed that Claudin-1 knockdown increases permeability in BHPrE1 and BPH-1 monolayers. Dr. Wang concluded that inflammation in BPH increases luminal epithelial permeability, releasing secreted proteins into the stroma, which further exacerbates the inflammation in a cyclic manner.

Molecular Correlates in Urine for the Obesity and Prostatic Inflammation of BPH/LUTS Patients
Pradeep Tyagi, Ph.D., PITT (Investigator, U54)

Dr. Tyagi reported results from his work that culminated in a recent publication on the molecular correlates in urine for the obesity and prostatic inflammation of BPH/LUTS patients. Most of the association of BPH and inflammation is based on biopsy or prostatectomy, which are invasive methods that can be biased regarding the specimen size, gene expression changes, and clinical site. This study proposes urine samples as a noninvasive alternative to a prostate biopsy. Although the findings from the MTOPS and REDUCE trials aligned with prior studies showing that age and metabolic syndrome increase the risk for BPH, urine samples from those trials are not available. In collaboration with Dr. Jay Fowke at the University of Tennessee, Dr. Tyagi evaluated frozen urine samples from 207 BPH patients in the Nashville Men's Health Study collected from 2009 to 2012. The chart review included age, race, BMI, waist circumference, and needle biopsy data. Chemokines and secretory interleukin-1 receptor

antagonist (siL-1RA) were measured. Lymphocytic infiltration markers CD3 and CD20 were assessed in peripheral zone prostate biopsy of BPH patients.

Results showed that siL-1RA was detected in all 207 patient samples and positively correlated to obesity measurements. There was a mild relationship between increased CD3 staining in the peripheral zone and urine levels of CKCL-10 and CCL5. Dr. Tyagi summarized that the association of siL-1RA with centralized adiposity supports siL-1RA as a molecular correlate of obesity in BPH patients. Urinary chemokines were weakly associated with inflammation markers in peripheral zone biopsy and with the AUA Symptom Score Index (AUASS) of BPH patients.

Inducible Prostate Luminal Epithelial Cell–Specific Deletion of *CDH1* Induces Prostatic Hyperplasia, Inflammation, and Stromal Fibrosis
Laura Pascal, Ph.D., PITT (Investigator, U54)

Dr. Pascal presented preliminary data on *CDH1* deletion and the induction of prostatic hyperplasia, inflammation, and stromal fibrosis, which leverages the prior Wang laboratory findings that E-cadherin is downregulated in BPH tissues. An E-cadherin transgenic mouse strain (PSA CreERT2 $Cdh1^{-/-}$) was generated using the inducible Cre technology and then genotyped. Mice with E-cadherin deletion had increased proliferation and stromal fibrosis at age 150 days, which persisted for 365 days. Future work will focus on increasing the study sample size, assessing bladder function, and evaluating phenotypical behavior.

Hormones, Fibrosis, and Mouse Anatomy in the Study of Lower Urinary Tract Dysfunction (LUTD)
William Ricke, Ph.D., UW–Madison (Principal Investigator and Project Leader, U54)

Dr. Ricke reported on the success of the UW–Madison/UMass O'Brien Center, including experimental approaches aimed at benefiting the BU community and efforts to build the necessary infrastructure (e.g., Biomedical Core). He explained that barriers to BPH research include a lack of consensus on (1) the definition of BPH, (2) assessment techniques for urinary events, and (3) suitable animal models. When defining BPH, UW–Madison investigators have adopted the concept that BOO leads to symptoms (i.e., LUTS), and treatment depends on the type of BPH. For example, alpha blockers are used to treat smooth muscle BPH; 5α-reductase inhibitors are used in cases of hyperplasia BPH; and surgery, when drug treatment fails. There are no current treatments for fibrosis-related BPH, which is the focus of the UW–Madison/UMass O'Brien Center. The goals are to develop suitable preclinical animal models (e.g., canine, nonhuman primate, or mouse) to better inform and improve treatment for these patients.

The use of mouse models of BPH/LUTS in preclinical studies has been controversial for decades. Because rodent and human prostate anatomy are different, the normal adult urinary tract of the male mouse also is different, which Dr. Ricke and co-investigators have shown. The ongoing 4-year study of the anatomy of normal urinary tract and prostatic urethra of the male mouse has resulted in new insights into BPH. Since its inception, the UW–Madison/UMass O'Brien Center has functioned to disseminate its science, consensus, and resources for BPH and has provided education and outreach to the BU research community. In this next phase of the U54, communication is bidirectional between the Center and the community. This translational approach engages the community, fosters new collaborations, and extends BPH research. Efforts have expanded to three-dimensional (3-D) reconstruction of the mouse prostate and urethra anatomy, improved urethral measurements using MRI and ultrasound, and new preclinical animal models. Dr. Ricke emphasized that new BPH targets are needed and remarked that the research strategies and infrastructure of the UW–Madison/UMass O'Brien Center have moved the needle forward toward a BPH consensus.

Cell-type Lineages and Their Potential Roles in LUTD
Chad Vezina, Ph.D., UW–Madison (Project Leader, U54)

Dr. Chad Vezina provided an update on the project, of which he and Dr. Paul Marker at UW-Wisconsin are co-investigators, addressing the role of β-catenin in urinary dysfunction. One of their major findings is that collagen accumulates in the prostate periurethral region of some men with BPH and fibrosis, but not in the epithelial BPH nodules. Similar results were observed in canines and mice. Biopsy samples obtained from the MTOPS cohort showed an increase in collagens that correlated with symptom severity, suggesting that prostatic fibrosis is a risk factor for LUTS. Furthermore, in male canines, the Vezina and Marker laboratories revealed that aging increases collagens across the intact prostate and urethra. However, these collagen effects were not observed in castrated animals. The efforts next focused on evaluating the signaling pathways involved in collagen production. Overexpression of β-catenin, which has been shown to accumulate in the prostatic epithelial cells of men with BPH/LUTS, also drives epithelial hyperplasia in mice. The Vezina and Marker laboratories developed a new collagen quantification method to evaluate collagen structure and associated pathologies in BPH that leverages the UW–Madison Curvelet-Denoising Fiber Extraction (CT-FIRE) software. They demonstrated that β-catenin locally changes collagen fiber length, but not fiber density, in a mouse model of LUTD. Increased fiber length correlated with an increase in tissue fibrosis. Notably, β-catenin depletion in mouse prostate significantly decreased voiding function, especially in mixed genetic backgrounds.

Additional in-depth studies into the signaling pathways involved investigating the cellular anatomy of human BPH. The Vezina laboratory and Dr. Strand at the University of Texas at Southwestern Medical Center collaborated and analyzed lower the urinary tract tissue specimens obtained via the Southwest Transplant Alliance. The results showed increased collagen production in 3-oxo-5α-steroid 4-dehydrogenase 2-positive ($SRD5A2^+$) human prostatic fibroblasts. Because collagen causes urinary dysfunction in aging men, fibroblasts are a source of collagen production, and human fibroblasts are $SRD5A2^+$, the next logical steps were to generate a mouse model to mark *Srd5a2* stromal cells, follow them through time, and investigate cell-lineage types. The results show that in mice, *Srd5a2* cells mark a stromal lineage with progenitor characteristics. In addition, forced or involuntary connective tissue growth factor (*CTGF)* expression in $Srd5a2^+$ cells increases prostatic collagens and causes voiding dysfunction in mice. In this model, prostatic inflammation drives collagen accumulation, but the question remains whether inflammation recruits $Srd5a2^+$ cells to produce collagen.

Prostate Transition Zone (TZ) Fibrosis in Men Who Failed Doxazosin, Finasteride, or Combination Therapy in the MTOPS Study
Jill Macoska, Ph.D., University of Massachusetts Boston (Project Leader, U54)

Dr. Jill Macoska reported on the findings of the MTOPS study, an NIDDK-funded prospective, randomized, double-blind, multicenter, placebo-controlled clinical trial, which is a key component of her O'Brien Center project. The objective was to determine whether the long-term medical therapy of BPH using 5-α-reductase inhibitor finasteride or α-adrenergic receptor antagonist doxazosin or the doxazosin-finasteride combination would prevent or delay the clinical progression of BPH. The study population consisted of 3,047 men who were randomized to one of four groups: (1) placebo, (2) doxazosin, (3) finasteride, or (4) combination therapy. The randomization period lasted from December 1995 to March 1998, and the mean duration of follow-up was 4.5 years, ending in 2001. BPH clinical progression was evaluated by AUASS, UI, renal insufficiency, and/or recurrent UTI.

The findings showed that doxazosin, finasteride, and combination therapy each resulted in significant improvement in AUASS and that combination therapy was superior to doxazosin or finasteride alone. BPH clinical progression assessed by AUASS alone was 10 percent in therapy groups and 5 percent in the combination group. Four to 13 percent of participants failed to improve on therapy, and 4 to 7 percent

failed to improve on combination therapy, which suggests that pathobiologies other than those targeted by 5-α-reductase inhibitors or α-adrenergic receptor antagonists contribute to clinical progression. The Macoska laboratory analyzed left and right TZ biopsy samples obtained from the MTOPS Biopsy Substudy and found that an increase in TZ collagens correlates with combination treatment failure. The TZ collagen content was higher in stromal tissue than in epithelial tissue, and collagen disorganization analyzed using CT-FIRE correlated with combination therapy failure. Other researchers also examining the MTOPS Biopsy Substudy specimens found high levels of inflammatory markers (e.g., CD45, CD4, CD8, or CD68), which could be correlated with BPH clinical progression. The MTOPS findings are consistent with the hypothesis that inflammation promotes fibrosis, which contributes to LUTD. Dr. Macoska emphasized the idea of exploring antifibrotic agents as medical treatment options for men who fail to improve with doxazosin-finasteride combination therapy.

Conclusion
Will Ricke, Ph.D., UW–Madison, and Zhou Wang, Ph.D., PITT (Principal Investigators, U54)

Dr. Wang commented on the research efforts and the complex nature of prostatic inflammation. He invited speakers and participants to the panel discussion.

Discussion

- Because detrusor muscle overactivity has been correlated with age and also could lead to afferent sensitization, the effect of age might be worth pursuing in the prostatic inflammation rat model.
- Studies are in progress to determine whether nociceptive or stretch fibers are affected.
- The organoid models are promising predictors of human disease and could benefit signaling studies.
- Experiments to determine the permeability of the normal prostate have not been conducted, but could be considered for the future.
- The Ricke laboratory has not performed experiments that evaluate the dynamic properties of the urethral wall when the prostate reduces in size with BPH but could consider doing so in the future, especially with improved urethral measurement techniques.
- Because of the advent of new tools and techniques, neurologic and bladder remodeling is now possible. The goals of the BPH research should be to stimulate interest in the BU research community, such that both domains are incorporated into future studies.
- A model of exercise/intervention or investigating the strains of mice that are hyperactive could be considered in the BPH studies.
- In the cell-lineage studies, the cell of origin, with regard to collagen production and collagen expressing, is well understood. Additional research would be necessary to draw a conclusion.
- The Jackson Laboratory already has data on OP mice and high-fat diets and might consider sharing this data with the O'Brien Center investigators.

NIDDK INTERACTIONS—SESSION 2

Developing Resources to Support BU Research
Kristina Penniston, Ph.D., UW–Madison

Dr. Penniston explained that the session would consist of a review of the definition of a resource and informal reports of O'Brien Centers resources. The K12 scholars were encouraged to lead breakout tables, and teams would, as a group, discuss the following three questions:

1. What are the major unmet needs for available resources to support BU research?

2. What are the existing local or national roadblocks for developing or sharing research resources?
3. What would be some improved strategies for developing and ways to improve resource sharing?

Table leaders planned to summarize the discussion, parlay the key points into a list, and report back to the larger group.

O'Brien Center Resources

- MRI for investigating BPH
- Opportunity Pool Projects
- BPH clinical specimens
- Prostatic inflammation animal model
- Conditional E-cadherin KO mouse model (PSA CreER *Cdh1*)
- Lower urinary tract tissue specimens via the Southwest Transplant Alliance
- Whole-mouse slides for IHC analyses

Table-by-Table Discussion Cross-Cutting Themes

- Facilitate data sharing and improve investigator access to data.
- Improve access to clinical trial data and patient clinical data.
- Establish a database for RNA sequence data.
- Develop and preserve animal models.
- Establish biobanking repositories for diseased and control tissue specimens.
- Establish a link from NIDDK's website of a list of existing resources found in all cores.

SCIENTIFIC SESSION 3—BRAIN-BLADDER REGULATION AND FUNCTION

Introduction and Overview

Moderator: Mark Nelson, Ph.D., The University of Vermont (Chair, U54 External Expert Panel)

Dr. Nelson introduced the speakers and summarized this session as covering new tools in neurobiology to understand the physiology of voiding, from the bladder to the brain and back. Research includes understanding the fundamental process of sensing fullness in the bladder and the bladder's communication with the central nervous system. He mentioned a recent research paper in *Science* identifying ATP-sensitive ion channels in the vasculature and cited recent tools that allow viewing cell circuitry *in vivo* and *ex vivo*.

Communication of Bladder Fullness to the Central Nervous System (CNS)

Nathan Tykocki, Ph.D., The University of Vermont (U54 Investigator-Collaborator)

Dr. Nathan Tykocki began by delineating unknown factors in the field of bladder communication. The molecular identity of stretch receptors in the bladder wall has yet to be identified. Most incontinence drugs try to decrease the bladder's ability to contract, but targeting the stretch receptors would be a more preferred option. Bladder filling is not a passive process, because the bladder wall constantly moves and shifts. Dr. Tykocki's study dissected a mouse bladder *ex vivo* with the major pelvic ganglion, measuring outflow. Results showed that small changes in pressure correlate with maximal afferent nerve activity in a regular pattern. Scientists have yet to discern how bladder fullness is sensed, how the sensation is transduced to the CNS, and the makeup of the sensor in the bladder wall.

New techniques in spatio-temporal processing and analysis include calcium imaging with calcium-encoded biosensors, 3-D multiplane video imaging of bladder contraction, and images of bladder wall micromotions. Dr. Tykocki and colleagues found that these micromotions throughout the bladder wall muscles occur constantly, but the output in terms of global pressure changes is regular and phased. In a 3-D model, they evaluated bladder filling, emptying, contracting, and relaxing over time. They found that the pressure changes are linked with afferent activity and angular displacement, while microcontractions move or ripple along the bladder wall. This research led Dr. Tykocki and colleagues to conclude that both local stress and global pressure contribute to bladder sensation. Increases in calcium ions cause local contraction, which, in turn, causes pressure changes, activating afferent nerves and bringing information directly to the CNS.

Questions for future research include determining how micromotions become pressure events, uncovering how a micromotion activates sensory afferents, elucidating the driver of bladder wall micromotions, and determining whether the sensor is a pressure, stretch, or tension sensor. Dr. Tykocki noted a recent discovery of TRPV1-positive interstitial cells that did not appear to be nerve-related. He speculated that these cells could be acting to coordinate the microcontractions of smooth muscle in multiple areas of the bladder simultaneously.

Central Nervous Circuity
Mark Zeidel, Ph.D., Harvard University (Principal Investigator, P20)

The goals of the Beth Israel Deaconess Medical Center Benign Urology Research Program are to develop state-of-the-art tools and approaches to better understand normal and unbalanced bladder function and disseminate these approaches to the BU research community. Functional MRI, including cystometry, is a major tool in this effort. Dr. Mark Zeidel described his research focused on the Barrington's nucleus, also known as the pontine micturition center (PMC), which contains 25 different neuron types and transversing axons. Cre-expressing neurons were located and visualized by optogenetics, in which light-activated channelrhodopsin (ChR2) is expressed. This process enables the activation of the cell, providing cell-type specificity and geographic specificity. During bladder fill and contraction, neurons in mouse brains activate in the periaqueductal gray (PAG), the PMC, and hypothalamus. Deactivation occurs in the hippocampus, presubiculum, and medulla. Similar patterns were observed in male and female mice. Further research will compare these activation and deactivation patterns across different strains of mice to determine the patterns' physiological significance. Comparing changes in the MRI to changes in the actual neurons shows time-linked connectedness in the same locations.

Dr. Zeidel reviewed experiments that injected the protein calcium sensor GCaMP6 into specific neurons. Results showed time-linking between the firing of neurons and an increase in bladder pressure. He also performed an experiment using a calcium indicator to detect the neural subtypes activating between the PAG and the PMC during bladder filling and emptying. Researchers also have mapped the neurons that drive bladder voiding. Dr. Hanneke Verstegen at Harvard Medical School has studied the afferent nerves traveling from the PAG to the PMC by ChR2 imaging, which demonstrated synaptic connections between the PAG neurons and the PMC. A goal of further research is to sequence and map individual neurons to identify subpopulations of cells in the PMC, particularly those that express glutamate, and then study those subpopulations with Cre recombinase technology.

The Zeidel laboratory has begun studying bladder-brain function in disease states, while also further elucidating the normal bladder-brain neural connections. The differences between normal mice and mice subjected to head trauma were evaluated. The data showed no difference in the bladder or the PAG or PMC sections of the brain in either group. In the trauma group, degeneration was found in the urodynamic cystometry frontal input that disturbed normal bladder function. Future research will

investigate the details of these processes and also discern what may be bladder stretch signals along the sacral cord to the PAG.

Concepts, New Techniques, and Concluding Remarks
Mark Nelson, Ph.D., The University of Vermont (Chair, U54 External Expert Panel)

Dr. Nelson noted that increased fibrosis or changes in bladder wall composition alter in unhealthy ways the dynamics of micromotion, afferent nerve activity, and sensory outflow. He opened the floor for discussion about the preceding talks.

Discussion

- Administering an antibody to the cis-tau protein at the time of traumatic brain injury inhibits brain degeneration.
- Determining whether wave motions in urination are related to bladder micromotions is a useful variable for further study.
- Urination can be induced by compressing a section of bladder wall. After initiating a misfiring, the next voiding was disturbed, but function returned to normal the third time.
- The bladder's length-tension relationship is very dynamic.
- It was speculated that urothelial cells could be acting as pacemakers, but these cells do not indicate activation.
- In Dr. Tykocki's research, mouse bladders are filled via the urethra.
- The hypothalamus is being studied in relation to the connection between bladder fullness and thirst, including its neural connections with the PMC. Much more room for research exists to thoroughly describe these neural circuits.

EMERGING LEADERS IN BU RESEARCH—SESSION 2

Introduction
Tamara Bavendam, M.D., M.S., NIDDK

Dr. Bavendam welcomed participants to the second emerging leaders session and reflected on the changes from prior years and the now-diverse representation among the NIDDK Urology Program investigators.

Phenotypic Segregation of Orgasmic and Concomitant Erectile Dysfunction in Men with Type 1 Diabetes
Nnenaya Agochukwu, M.D., University of Michigan

Dr. Nnenaya Agochukwu reported on her work on a retrospective study that focused on the prevalence and risk factors for orgasmic dysfunction (OD), its relationship to erectile dysfunction (ED), and its natural history in men with type 1 diabetes. The study population included 563 men from the NIDDK-sponsored Diabetes Control and Complications Trial/Urological Epidemiology of Diabetes Interventions and Complications (Uro-EDIC) at Uro-EDIC year 17. ED was evaluated based on responses to the International Index of Erectile Function (IIEF) question 15, and OD was based on IIEF questions 9 and 10. The severity of OD was assessed on a 5-point scale from 0 to 5, in which no sexual stimulation was rated as a 0. Risk factors (e.g., alcoholism and depression) were assessed using the age-adjusted odds ratio. Of the 563 participants, 303 had no OD or ED; 177 had ED only; 21 had OD only; and 62 had dual OD and ED. The OD-only group exhibited a psychogenic phenotype in which there was an association with depression, decreased alcohol intake, and low sexual desire. The OD/ED group were

mixed phenotype (physiological and psychogenic) and had some associations as observed in prior ED research. The mixed phenotype profile also showed increased diabetes severity and metabolic dysfunction. Future work will include examining the natural history in these phenotypes using longitudinal models and leveraging ongoing projects, including the LURN.

Discussion

- Testosterone levels did not appear to be a risk factor or predictor for OD or ED in the study population of Uro-EDIC men at Uro-EDIC year 17.

Trajectories of ED Subphenotypes in Men with Longstanding Type 1 Diabetes
Karandeep Singh, M.D., University of Michigan

Dr. Karandeep Singh presented his work on categorizing trajectories of ED into subphenotypes (i.e., clusters) based on symptom severity. Efforts also focused on predicting trajectories for men with type 1 diabetes using baseline data and identifying risk factors (e.g., age, hemoglobin A1c, or smoking) influencing the long-term trajectory of ED in this population. Data from the Uro-EDIC II (male participants) were used in this study; Uro-EDIC year 17 was used as the baseline period and years 18–22 as the outcomes period. ED trajectories from the outcomes period data were categorized into subphenotypes using a k-means clustering approach based on responses to IIEF question 15 and assessed on a 5-point scale. A multinormal random forest model was fitted to predict subphenotype assignment using baseline data. Factors associated with ED trajectory were identified using permutation methods. Dr. Singh developed a computational model to define clusters that integrated identifiable risk factors, the random forest, and the AUASS scores. The analyses identified three subphenotypes of ED in men—Cluster A (low ED), Cluster B (moderate ED), and Cluster C (high ED)—from Uro-EDIC years 18–22. The AUASS scores were stable over the 5-year period. The random forest ED subphenotype predictor model achieved a multiclass area under the curve of 0.649 using a tenfold cross-validation. Statistically significant predictors of ED included age, autonomic dysfunction, smoking status, and time-averaged A1c.

Discussion

- The contributing factors to the variation of ED reflect prior data collected for the Uro-EDIC cohort.
- The algorithm kml: k-means for longitudinal clustering was used for determining ED trajectories and predicts clusters, rather than increases or decreases in severity. This could be a limitation to data interpretations of this study.
- Approximately 2 to 3 percent of the Uro-EDIC male participants who fit the criteria for ED also were taking medicines, but were not excluded from the study.
- LUTS is a strong predictor of ED, but occurrences are low in the Uro-EDIC male cohort because of the young ages.

The Impact of Age on the Lower Urinary Tract of Mice
Teresa Liu, Ph.D., UW–Madison

Dr. Teresa Liu described her work investigating the role of ERs in BPH disease. Under normal conditions, the ERα/ERβ actions/functions are in homeostasis, whereas in BPH, ERα-mediated proliferation is increased and ERβ-mediated apoptosis is decreased. The hypothesis is that selective estrogen receptor modulators (SERM) can re-establish the ERα/ERβ homeostasis and reverse the ERβ-mediated apoptosis in BPH progression. Many of the preclinical animal models of BPH do not translate

into clinical efficacy; age could be a factor because of differences in human/mouse age equivalents. The young mice used most often in studies, 1 to 6 months of age, correlate to ages 20 to 30 in humans, but the average age of BPH onset is 50 years. In addition, the normal measures of BPH (e.g., bladder mass and volume) increase with normal aging. This study evaluated the effect of age on the lower urinary tract of C57B/L6 mice. The void spot assay (VSA) and ultrasound techniques were used to measure voiding/flow, and MRI techniques were used to examine obstruction. The results showed that age worsens LUTD, which was further exacerbated by steroid hormones. Steroid hormone metabolism changes with age. Future work will combine VSA, ultrasound, and MRI to examine dysfunction alone and in the presence of SERM treatment. Multiplex IHC will be used to determine the contribution of the ER and hormone enzymes.

Discussion

- Given the effects of mouse strain on the interpretation of results from age-related studies, age might be worth pursuing in this research.
- Age-related immune cell changes have been observed in female mice and could be considered in this study in the future. The multiplex IHC method, which is being optimized, will support conducting these types of analyses.

MRI Shows Bladder Wall Thickness and Detrusor Muscle Volume Increase in Association with Age-dependent Increase in Prostate Volume
Alejandro Roldan-Alzate, Ph.D., UW–Madison

In collaboration with O'Brien Center investigators to conduct small animal MRI studies, Dr. Alejandro Roldan-Alzate was able to visualize urological MRI biomarkers in healthy and BPH animals and showed a correlation of visceral adipose tissue to prostate volume. The next steps were to perform a retrospective analysis of 117 pelvic MRI studies from healthy people. The data set consisted of 59 women and 58 men, and the studies were divided into four age groups stratified by decade from 30 to 60 years. The data showed that the bladder wall thickness was greater in males than in females and was statistically significant in the 60-year age group. In males, the increase in bladder wall thickness correlated to an increase in prostate volume. Similar patterns were observed for the detrusor muscle volume. Dynamic contrast-enhanced magnetic resonance angiography was next used to measure detrusor muscle perfusion over time in four subjects, two females, both 26 years of age, and two males, one 59 years of age and another 70 years of age. Results revealed differences in perfusion between the two males, but not the females. A new project, MRI urodynamics, is being planned to visualize the prostate and urethra during voiding and institutional review board approval is pending.

Discussion

- Assessing urodynamics using a multi-positioning MRI in which patients will be in a seated rather than supine position will closely mimic normal voiding.

Are White Matter Tract Integrities Different in Multiple Sclerosis Females with Voiding Dysfunction?
Rose Khavari, M.D., Houston Methodist Hospital

Dr. Rose Khavari presented data on brain control over the bladder in voiding dysfunction, which is a morbid and costly health condition characterized by hesitancy, intermittency, or absence of urine flow. Voiding dysfunction could lead to UTIs, sepsis, urinary stones, and/or permanent renal failure and is present in diseases with neurogenic as well as non-neurogenic etiologies. Multiple sclerosis (MS), a chronic neurogenic disorder that affects the CNS, is characterized by chronic inflammation and neuron

demyelination and neurogenic LUTD. In fact, voiding dysfunction occurs in 34 to 79 percent of MS patients. MS lesions primarily affect brain white matter, and two white matter tracts (WMT)—anterior thalamic radiation (ATR) and superior longitudinal fasiculus (SLF)—are involved in lower urinary tract function.

This prospective observational study investigated ATR and SLF tracts in adult female patients with clinically stable MS for at least 3 months prior to screening. Diffusion tensor imaging methods were used to measure fractional anisotropy (FA) and mean diffusivity (MD) to assess ATR and SLF tissue damage associated with voiding dysfunction. The study population consisted of 28 MS patients and 11 healthy females as controls (HCs). Baseline assessments and evaluations were conducted, and MS patients were divided into two groups: Group 1 (patients without voiding dysfunction) and Group 2 (patients with voiding dysfunction).

Dr. Khavari explained the imaging scanning protocol and summarized the study results. Overall, MS patients have significantly higher mean MD values and lower mean FA values compared to HCs in both WMTs. Group 2 had the higher mean MD and lower mean FA of the two groups. Group 2 had significantly higher MD and lower FA values than Group 1 in the left ATR. These data demonstrate that damage to ATR and SLF tracts exists in MS patients and provides further evidence of their importance in the lower urinary tract function of this population. Future efforts will focus on targeted cortical stimulation of the ATR and SLF regions in voiding dysfunction, using transcranial magnetic stimulation and/or transcranial rotating permanent magnet stimulation.

- These findings may have broader application to voiding dysfunction in other neurogenic-related diseases, which is being proposed as a future direction of this research.

Nocturnal Bladder Symptoms and Sleep Disruption Among Women with Overactive Bladder (OAB) Enrolled in a Randomized Trial of Slow-paced Respiration
Alison Huang, M.D., M.A.S., UCSF

Dr. Alison Huang reported results of the Controlling Urgency with Relaxation Exercise (CURE) randomized trial and ancillary sleep study. More than 40 percent of women diagnosed with OAB report repeated trips to the bathroom to urinate during the night, which disrupts their sleep. The causes of OAB are challenging to uncover because the relationships between nocturnal OAB symptoms and sleep dysfunction (e.g., insomnia) are complex and bidirectional. Stress/anxiety and autonomous dysfunction are possible contributors to OAB and insomnia. Behavioral interventions, such as slow-paced respiration (i.e., slow guided breathing), are approaches to alleviating OAB-related symptoms and overlap with existing urge suppression techniques used to manage OAB and UUI. CURE is a randomized, single-center, parallel-group 12-week clinical trial designed to evaluate whether device-guided slow-paced respiration can improve OAB symptoms and sleep quality, duration, or efficiency and to examine the associations of nocturnal OAB symptoms and sleep outcome in women with OAB.

Dr. Huang explained that 161 women were enrolled and randomized to one of two treatment arms, paced respiration (experimental intervention) or music-controlled (control intervention). The experimental intervention involved a slow-paced exercise guided by RESPeRATE (Intercure, Ltd.), a commercially available portable guided-breathing device approved by the U.S. Food and Drug Administration for treatment of hypertension. In the control intervention, RESPeRATE was reprogrammed to play non-rhythmic musical tones, and spontaneous breathing, rather than guided respiration, was monitored. Data on urinary symptoms, sleep outcomes, stress/anxiety, and autonomic function were collected. Findings showed that sleep dysfunction was common in women enrolled in the trial of slow-paced respiration for OAB. Women with OAB attributed most nocturnal awakenings to using the bathroom at night. Greater nocturnal voiding frequency was associated with decreased sleep efficiency, but not necessarily global

sleep quality or duration. Clinical implications are that nocturnal bladder symptom frequency, global sleep quality, and sleep efficiency improved modestly in both intervention groups over the 12 weeks.

Discussion

- Sleep apnea was not prevalent in the CURE cohort.
- Other factors not directly related to OAB could impact the frequency of trips to the bathroom and might have long-term effects on the autonomic nervous system.
- University of Michigan researchers have discovered that pelvic damage negatively affects bladder function.

Conclusion
Tamara Bavendam, M.D., M.S., NIDDK

There were no additional comments.

CAIRIBU MODERATED POSTER SESSION

All meeting participants were invited to view the posters submitted to the NIDDK KUH CAIRIBU Annual Meeting and to converse with their presenters. Judges examined the posters and discussed the described research with each poster's presenter (s). In a moderated session, select presenters were invited to present 2-minute talks of their research. Winners were selected for each of six categories: Animal Models, Bladder, Genetics, Prostate, LUTS, and Stones. Poster presenters were thanked for their time and willingness to share their research with the urology community. The 11 winners of the poster session awards were then announced and congratulated:

Animal Models Award

Hannah Ruetten, B.S., Doctoral Candidate, University of Wisconsin–Madison
"Impact of Age and Castration on Canine Prostate Collagen Organization"

Bladder Award

Indira Mysorekar, Ph.D., Professor, Washington University in St. Louis
"Effect of Vaginal Estrogen Therapy on the Postmenopausal Inflammatory Bladder State"

Pradeep Tyagi, Ph.D., Associate Professor, University of Pittsburgh
"Prejunctional M1 Receptor: A Target for Detrusor Underactivity Secondary to Bladder Outlet Obstruction"

Gregory Wiessner, B.S., Doctoral Candidate, Columbia University
"Retinoid-dependent Signals in the Cloaca Regulate Urothelial Differentiation and Common Nephric Duct Remodeling during the Development of the Lower Urinary Tract"

Genetics Award

Lindsay Hampson, M.D., M.A.S., Assistant Professor, University of California, San Francisco
"Understanding Caregivers and Caregiver Burden among Those Caring for Patients with Congenital Urologic Conditions"

LUTS Award

Bryce MacIver, Ph.D., Instructor, Harvard University
"Traumatic Brain Injury–related Voiding Dysfunction in Mice Is Caused by Damage to Rostral Pathways, Altering Inputs to the Reflex Pathways"

Chelsea O'Driscoll, B.S., Doctoral Candidate, University of Wisconsin–Madison
"Exogenous Hormone Exposure: A Potential Platform for Testing Interventional Therapies for Lower Urinary Tract Dysfunction in Male Mice"

Prostate Award

Wei Chen, Ph.D., Associate Professor, University of Pittsburgh
"Primary Stromal Cells from BPH, But Not Normal Adjacent Prostate, Stimulates Benign Prostatic Epithelial Cell Growth in 3-D Culture"

Douglas Strand, Ph.D., Assistant Professor, The University of Texas Southwestern Medical Center
"Building a Comprehensive Cellular Anatomy of the Normal and Diseased Human Prostate"

Stones Award

Elena Wilson, Undergraduate Research Fellow, Mayo Clinic
"Characterization of Renal Inflammatory Cell Populations in Nephrectomy Patients with and without a History of Kidney Stone Disease"

Benjamin Wood, Undergraduate Research Fellow, Mayo Clinic
"Imaging Kidneys Using Doppler Ultrasound as a Method of Detecting Kidney Stones via Twinkling Artifacts"

FRIDAY, DECEMBER 14, 2018

P20 ROUND-UP

Leukocytic Phenotypes Associated with BPH Progression
Simon Hayward, Ph.D., Northshore University Health System, and Timothy Ratcliff, Ph.D., Purdue University

Dr. Timothy Ratcliff outlined the areas of research by his and Dr. Simon Hayward's laboratories in the study of the relationship between autoimmunity and BPH. Defining the leukocyte profiles between the early and late stages of BPH and the pathways that are important in disease progression is an important facet of this research. It has been hypothesized that BPH has an autoimmune or inflammatory component that involves a transition from an M2 macrophage phenotype to an M1 phenotype. The Ratcliff laboratory has been addressing the questions of whether autoimmune disease and BPH are related to comorbidities that occur in BPH and whether the treatment of autoimmune disease affects the progression of BPH. To address these questions, the group examined a database of 101,000 men, of whom 11,000 also had an autoimmune disease or had autoimmunity. Approximately 31 percent of men with autoimmune disease also had BPH, whereas 20 percent of men who did not have autoimmune disease had BPH. The treatment of men with BPH and autoimmunity with methotrexate had no effect on the incidence of BPH, whereas the treatment of this group with antitumor necrosis factor-α (TNF-α) decreased the incidence of BPH by 50 percent. Treatment with a combination of the two agents gave results similar to treatment with anti-

TNF-α alone. Future studies will involve studying gene expression in early and late BPH surgery samples, profiling inflammatory cells associated with the transition from M2 to M1, and studying inflammatory cell communication with fibroblast cells in BPH.

Discussion

- Because the anti-TNF-α treatment showed a decrease of BPH incidence in men with autoimmunity disease by approximately 30 percent to 20 percent, researchers speculated that different types of BPH may exist. This idea may be valid; however, these results could also reflect that TNF-α is only one cytokine in this system and that other cytokines could play a role as well.
- It is unknown whether anti TNF-α treatment indicates an association of inflammation with BHP or whether inflammation plays a more causative role in BHP pathogenesis. In the autoimmunity mouse model, proliferation and initiation of prostatic enlargement can be driven through the autoinflammatory process. This could be associated with initiation or progression.

Molecular and Neuro-inflammatory Biology of Aging Bladders
Indira Mysorekar, Ph.D., Washington University in St. Louis

Aging comprises a number of chronic conditions. Many effects of aging can be traced to changes in the immune system of aging individuals. Dr. Indira Mysorekar discussed her research interest in the incidence UTIs in aging individuals. UTIs are some of the most common infections seen in older women and can have substantial economic consequences on those who have these infections. Dr. Mysorekar's group focuses on the causes of UTIs in women and on more effective ways to treat these infections. The immune response in the bladder has not been well characterized. Dr. Mysorekar described the use of aged (18–22 months) male and female mice as animal models for UTI pathogenesis in older individuals. When aged mice are given a UTI, there are more frequent spontaneous recurrences and hyperinflammatory responses. These findings are consistent with what were observed in older women with UTIs.
Dr. Mysorekar stated that the goals of the follicle research are to define the cellular composition of these follicles and to determine what activates the immune cells in these structures. There are a high number of T cells, B cells, and plasma cells in these follicles, suggesting a proinflammatory profile. The follicles also are innervated densely by nerve tissue; studying the nerve-immune cell communication pathways may be critical to understanding the inflammatory response in aged bladders.

Discussion

- Fibrosis may be examined in the follicles, but that type of testing has not been done yet.
- Male mice with inflammatory infiltration appear to have smaller nodules in the bladders, but they are not as pronounced as those of the aged female mice.
- There are $CJRP^{+}$ (calcitonin gene-related peptide) neurons that innervate and wrap around the immune cells in the follicles. These nerve cells send signals to the brain that attract an inflammatory response.

SCIENTIFIC SESSION 4—URINARY STONES

Introduction, Overview, and Pathways to Stone Formation, Stone Imaging, and Stone Epidemiology
John Lieske, M.D., Mayo Clinic (Principal Investigator, U54)

Dr. John Lieske combined his opening remarks with his presentation on urinary stone formation, imaging, and epidemiology. The study of the mechanisms of urinary stone disease involves multiple disciplines. Genetics and environment play important roles in determining urine supersaturation and nephrolithiasis. First-time urinary stone formers appear to have differences in calcium metabolism and vitamin D levels compared with controls. Urine supersaturation can lead to the formation of a stone by at least two different pathways, the formation of a plaque or a plug. In his studies, Dr. Lieske found that some stone formers have a high content of plaque in their stones and a low content of plug and that other stone formers have a high plug content and low plaque content. The growth of urinary stones in the kidney mimics geology in that growth occurs in layers. There are layers of minerals with layers of biomass as well. Microbial biomass metabolism may influence the way a urinary stone will grow.

The goal of the Lieske laboratory is to develop an *ex vivo* low dose, dual-energy CT exam to quantitatively assess stone composition, volume, and morphology. This exam could represent a noninvasive characterization of urinary stone composition and fragility using multienergy CT and machine learning. A major question for doctors who are treating first-time stone formers is whether or not the patient is at risk for recurrence. To address this question Dr. Lieske and co-investigators developed a Recurrence of Kidney Stone (ROKS) tool in 2014 to predict the risk of a symptomatic recurrence. The ROKS tool was updated in 2018 based on new research. This updated tool has incorporated additional criteria to predict the risk of stone recurrence. The development of better animal models may help to better define the pathogenesis of urinary stone formation and recurrence. Although rodent and *Drosophila* models have offered experimental and financial advantages in the study of urinary stones, their pathogenesis does not fully resemble the human pathogenesis of urinary stone disease. Canine and feline animal models may be better choices, because they more fully resemble the human pathogenesis.

Disordered Vitamin D and Calcium Metabolism in First-Time Calcium Stone Formers
Rajiv Kumar, M.D., Mayo Clinic (Project Leader, U54)

Dr. Rajiv Kumar described how his research on vitamin D metabolism disorders, specifically reduced 24-hydroxylation of $1,25(OH)_2D$ (1,25-dihydroxyvitamin D), involves studying the role of the pathogenesis of calcium oxalate nephrolithiasis. Major metabolic factors regulating calcium homeostasis are vitamin D metabolism and parathyroid hormone (PTH). The study of patients with Cyp24A1 gene mutations has revealed information about the abnormalities in vitamin D metabolism in urinary stone formers.
Dr. Kumar's group found that *Cyp24A1* mutations are associated with hypercalcemia, hypercalciuria, urinary stones, elevated concentrations of $1,25(OH)_2D$, and low concentrations of $24,25(OH)_2D$ and PTH. The results of a 4-year study on first-time stone formers showed that this group has abnormalities in the 24-hydroxylation of vitamin D metabolites associated with increased concentrations of $1,25(OH)_2D$, serum calcium, and urinary calcium. Other calcium-regulating molecules may be involved, as well. Future areas of research may include studies of variations in the coding and the promoter sequences of the *Cyp24A1* and *CaSR* genes. Studies of the epigenetic control of these genes also may be useful.

Discussion

- There is a concern about the increasing widespread use of vitamin D for health-related concerns and whether vitamin D would contribute to the formation of urinary stones. This does not appear to be a big problem since people with *Cyp24A1* mutations are asymptomatic and would need a large dose of vitamin D to show symptoms of hypercalciuria.
- The issue of whether getting patients to use tolvaptin to drink more water would help with calcium deposition, especially because the phasic nature of deposition was discussed.
- The mechanism of parathyroid hormone mobilization of calcium, the role of vitamin D in pregnancy, and whether they might contribute to urinary stone formation were noted.

- Some patients will form calcium oxide stones instead of calcium phosphorous stones. The prevailing supersaturation may be an important factor in calcium oxide stone formation.

Novel Models of Stone Formation
Michael Romero, Ph.D., Mayo Clinic (Project Leader, U54)

Dr. Michael Romero discussed his research collaboration on a *Drosophila* bicarbonate transporter, which also transports oxalate in malpighian tubules. The dog Zip 10 transporter has similarities with the bicarbonate transporter in *Drosophila.* The Zip 10 protein is expressed more extensively in the human and dog than in the mouse. The *hip1* and *ano4* genes in the dog are involved in calcium and oxalate metabolism. There is only one copy of these genes in *Drosophila,* which allows genetic manipulations in this model. Studies are being conducted to examine the knockouts of these transporter systems to better characterize the physiology of stone formation.

Discussion

- It is unknown whether the zinc transporter protein in the collecting ducts excrete or reabsorb zinc. Hypothetically, the zinc transporter protein should be involved in excretion, although research studies have not resolved that question. Zinc-regulated transporters are involved in zinc uptake.

Student Pipeline for the Future
Michael Romero, Ph.D., Mayo Clinic (Project Leader, U54)

Undergraduate students, who were recruited through the Mayo Clinic O'Brien Center nuSURF (Nephrology and Urology Summer Undergraduate Research Fellowship) program, have done much of this research over the summer. This program has expanded since 2014 when the first summer supplement was awarded by NIDDK. Some students presented their work at the American Society of Nephrology Annual Conference in 2018. The NIDDK hopes that students will be more engaged in nephrology research and consider a future career in the field. At the end of every summer, the NIDDK organizes a summer symposium for summer research students that allows them to meet other students from different institutions. Surveys from former nuSURF students over the last 5 years have shown that the program had a positive impact on the students and that some of them have been motivated to pursue a career in kidney research.

Automated Registries in Nephrolithiasis
Tom Chi, M.D., University of California, San Francisco (PI, P20)

Dr. Tom Chi described the similarities between the *Drosophila* research model and humans. Malpighian tubules in Drosophila are similar to kidneys in humans; *Drosophila* also can develop urinary stones. This model has been observed, but never manipulated experimentally to study urinary stone pathogenesis. The study of the *Drosophila* model has elucidated the role of zinc metabolism in kidney stone formation. Dr. Chi's group ran a bioscreen analysis in their *Drosophila,* in which they identified a candidate compound that prevented urinary stone formation. This compound also prevented stone formation in a mouse model. Bringing a compound that prevents urinary stone formation in mouse and *Drosophila* models, such as alpha-lipoic acid, to clinical trials is much more of a challenge, because getting patients is not an easy task. A prospective urinary stone registry would be invaluable in assisting researchers access data that could be used to obtain patients for clinical trials. A stone registry could capture etiology of stone formation, urology practice outcomes, and treatment outcomes. Ideally, a stone registry would retain patient clinical data, be easy to populate, be economical and could integrate with electronic medical records and store patient health information in a secure, HIPAA (Health Insurance Accountability and Portability Act)-compliant web environment. The stone registry was constructed by collaborative

interviews with endourologists from the United States, Canada, China, and Japan, referencing management guidelines by the European Association of Urology and the American Urological Association and using a condensed set of variables for inclusion into the ReSKU™ (Registry for Stones of the Kidney and Ureter) database. Future specific aims for this P20 grant include implementing the ReSKU™ data-capture templates, training providers in their use, and validating ReSKU™ by performing a nonblinded, crossover clinical trial of ReSKU™ versus a manual entry registry to assess accurate data collection.

Influence of Obesity on Urinary Oxalate Excretion (P20)
Dean G. Assimos, M.D., The University of Alabama at Birmingham

Urinary stones are an increasing problem, with increasing obesity and diabetes, in the United States. There is a strong association between having a metabolic disorder and being at increased risk for kidney stones. The risk of stone formation rises with increasing waist circumference, body weight, and a BMI greater than 30 and from having diabetes. A positive correlation with higher oxalate excretion occurs with body weight, surface area, and BMI. Dr. Dean Assimos' group has conducted research into the precursors of urinary oxalate, including diet, endogenous oxalate synthesis, and vitamin C. The current work funded by the P20 grant is investigating whether increased oxidative stress in an obese individual can drive increased endogenous synthesis of oxalate, possibly from vitamin C. Both human and animal studies are being conducted to investigate this possibility. The conclusion that can be drawn from this work is that obesity is associated with increased urinary oxalate excretion; increased endogenous oxalate excretion plays a role as well. The mechanisms of oxalate production must be defined further.

Discussion

- No discussion points were made.

NIDDK INTERACTIONS—SESSION 3

Developing O'Brien Center Hubs to Support Benign Urology Research
Kristina Penniston, Ph.D., University of Wisconsin–Madison

Dr. Penniston mentioned that participants would be meeting in groups to discuss what kind of cores and research resources would be needed. Three participants—Drs. John Lieske (Mayo Clinic), Rajiv Dhir (PITT), and Dale Bjorling (UW–Madison)—were invited to describe the core centers at their institutions before the breakout discussions occurred. Dr. Lieske described three distinct cores at the Mayo Clinic: a radiology core for methods development for postimaging data analysis; a urine-phenotyping core for biobanking and processing; and a biostatistics core for handling all statistical needs of investigator research. Dr. Dhir described the tissue resources and morphology core facilities at PITT, which focus on biobanking of tissue specimens, such as blood, urine, and prostate tissue. Pathology and imaging analysis services also are provided. Dr. Bjorling explained that the biomedical core at UW–Madison is divided into three components. The first component performs growth and function testing; the second component specializes in *in vivo* energy technology; and the third component provides imaging technologies.

Dr. Penniston then asked the breakout groups to assemble and discuss what a research core means and how to maximize investigator access to the cores. Participants suggested several ideas for O'Brien Center cores and identified unmet needs, including—

- Establish a standardized institutional review board for research approval of multicenter projects and a centralized facility for different protocols and provide investigator training.

- Maintain an updated list of cores and resources, especially biospecimens that might be available.
- Incorporate core costs into grants to facilitate core sustainability.
- Assess core demand by investigator use and consider a mechanism to prioritize access to investigators.
- Develop core coordination between centers.
- Consider establishing biostatistics and data management cores, and leveraging existing infrastructure for cores.
- Consider ways to minimize costs for collaboration.
- Consider establishing a core to serve as a broad resource for transgenic mice and an organized central repository for core data.

Conclusion
Tamara Bavendam, M.D., NIDDK, and Mark Nelson, Ph.D., The University of Vermont

Dr. Bavendam praised the quality of the student presentations and hoped that the presentations helped to strengthen investigator relationships. She also hoped that the field of kidney stone research would be advanced by investigator relationships and having research done at innovative centers.

Dr. Nelson thought it was very important that young investigators get acquainted with one another and have contact with other professionals providing clinical care. Meetings such as this one allow these investigators to interact and share knowledge and experiences. NIDDK conference organizers should be recognized for their help in planning this meeting, as well as for their assistance with funding.

After the concluding remarks finished, the meeting was adjourned.