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

Virtual Meeting
December 3–4, 2020

SUMMARY

THURSDAY, DECEMBER 3, 2020

WELCOME ADDRESS AND MEETING OVERVIEW

Christopher Mullins, Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Tamara Bavendam, M.D., M.S., NIDDK, NIH, and Kristina Penniston, Ph.D., UW–Madison

On behalf of the NIDDK and the meeting planners, Dr. Christopher Mullins, Program Official, NIDDK, welcomed attendees to the 2020 Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (CAIRIBU) meeting. CAIRIBU is a transdisciplinary network that supports collaborative research in benign urology (BU). Dr. Mullins explained that planning for the meeting was affected significantly by the coronavirus disease 2019 (COVID-19) pandemic, necessitating a virtual platform. The meeting organizers have worked to provide broad opportunities to foster networking and collaboration in accordance with previous meetings.

Dr. Mullins provided an overview of the NIDDK Benign Urology Research Centers Program, which contains four main components: the George M. O'Brien Urology Research Centers (O'Brien Centers) (U54 funding mechanism), Developmental Centers for Interdisciplinary Research in Benign Urology (P20 funding mechanism), Multidisciplinary K12 Urologic Research (KURe) and Urological Epidemiology (UroEpi) Career Development Programs (K12 funding mechanism), and the new Urology Centers Program Interactions Core (U24 funding mechanism). He also emphasized the importance of the research community's perspective. CAIRIBU leverages broad efforts to combine strengths to build a BU research base of established experts, trainees, and new investigators from other fields.

This year, the meeting registration was expanded to add broader expertise to the discussion. About one-third of the attendees represent areas outside the traditional CAIRIBU components. Dr. Mullins acknowledged the support of meeting organizers, which includes the meeting program committee, the abstracts and poster session committee, the trainee event organizing committee, community leaders and outside speakers, and NIDDK staff members. He also acknowledged Drs. Mark Nelson and Kristina Penniston for their leadership. Dr. Mullins noted that all meeting materials are posted on the CAIRIBU website.

Dr. Penniston thanked the meeting program committee for their efforts in the transition to a virtual platform. She provided an overview of the meeting agenda and encouraged the attendees to submit comments and questions to speakers through the Zoom chat box. Dr. Penniston also recognized the principal investigators of four outgoing P20 centers: Dr. Mark Zeidel (Beth Israel Deaconess Medical Center, Harvard Medical School), Dr. Simone Sanna-Cherchi (Columbia University), Dr. Indira Mysorekar (Washington University in St. Louis), and Drs. Simon Hayward and Timothy Ratliff

(NorthShore University HealthSystem and Purdue University, respectively). More information about the P20 centers can be found on the CAIRIBU website.

Dr. Tamara Bavendam, Project Scientist, NIDDK, presented a broad overview of CAIRIBU. The program's goals are to support the broader research community and expand its R01 research portfolio. Dr. Penniston has been tasked with building infrastructure to foster collaborations across the community. The CAIRIBU meeting provides opportunities for investigators to become acquainted, build trust, and develop leadership skills. Over the years, the NIDDK has initiated numerous efforts to establish its BU research portfolio. Support for the community remains an area of focus.

A learning community is critical to BU research, because a cohesive "story" does not exist for BU conditions. Rather, different medical specialties each hold a piece of the story. Most BU conditions are treated by surgeons who have limited opportunity to think scientifically. Additionally, screening for BU conditions is not routine, and an objective marker for diagnosis has not been established. Furthermore, most conditions are associated with a degree of stigma, which limits self-reporting and advocacy. Its perceived impact is related primarily to quality of life; many BU conditions thus are normalized.

Urinary stone disease (USD) (i.e., urolithiasis) is an outlier among BU conditions for several reasons: (1) USD is not considered a "quality of life" condition, (2) no stigma surrounds USD, (3) the patient presents with pain, (4) stones can be imaged, (5) stones can lead to renal failure, (6) USD is present in billing databases, and (7) nephrologists perform research on USD.

Dr. Bavendam emphasized that researchers at all stages of their careers must support one another and the new science; new researchers and their technologies should be captivated by the challenges, impact, and importance of these conditions. She reminded the attendees that the Interactions Core staff—Dr. Penniston and Dr. Betsy Rolland—are a first line of support for investigators.
Dr. Bavendam acknowledged NIDDK program staff, and she concluded by announcing her upcoming retirement from the NIDDK.

KEYNOTE LECTURE 1: URINARY INCONTINENCE—PERSPECTIVES ON NATURAL HISTORY AND CARE-SEEKING FROM THE NURSES' HEALTH STUDY
Francine Grodstein, Sc.D., Professor of Medicine, Harvard Medical School; Epidemiologist, Brigham and Women's Hospital; Director, Nurses' Health Study

Dr. Francine Grodstein discussed her research on the natural history, consequences, and care-seeking behaviors related to urinary incontinence (UI). Her data are based on cohorts from the Nurses' Health Study (NHS) and the Nurses' Health Study 2, which began in 1976 and 1989, respectively. The studies encompass 237,000 U.S. nurses ages 25–55 years at the baseline. Data were collected through biennial questionnaires. In the 1990s, questions regarding UI frequency, volume, and cause were added to the study in five repeated measures over 12 or more years. The researchers were interested in determining UI outcomes. Data were categorized by age (29–56 years, 56–81 years), severity (slight, moderate) and change in symptoms over time (persist/worsen, decreased symptoms, remission).

In 81 percent of women with slight UI, symptoms persisted or worsened during the study period. In 41 percent of women with moderate UI, symptoms persisted or worsened; 43 percent of women with moderate UI experienced decreased symptoms. Nearly 50 percent of women with severe UI experienced persistent or worsened symptoms; very few experienced complete remission.
Dr. Grodstein noted that body mass index (BMI) was considered a risk factor for UI progression.

The researchers were interested in the UI subtypes—stress, urgency, and mixed. Four repeated measures were performed over a 10-year period. Most respondents reported the same type of UI throughout the study period. Women who initially reported more severe UI, however, were more likely to progress to other subtypes. Dr. Grodstein noted also that racial differences were reported in the study. Black women reported less overall UI than white women but reported urgency UI more frequently; urgency UI often is associated with a worsened quality of life. Women from both groups were analyzed separately to determine the relationship between UI and depressive symptoms in both groups. In Black women, UI was more strongly associated with depression than in white women.

The researchers also were interested in determining health care–seeking behaviors in women who experience UI. Overall, 34 percent of women—from the older and younger cohorts—with prevalent UI reported talking with their health care provider. Dr. Grodstein pointed out the participants are employed as nurses and thus have access to health care services that many other women lack; the low degree of care-seeking thus represents the severity of these challenges across the U.S. population. She also noted that the determinants (e.g., frequency, severity, subtype, duration) for care-seeking did not differ significantly between the age groups. The greatest determinant for care-seeking in both groups was UI frequency. The researchers found, interestingly, that older women were less likely than younger women to seek care. Additionally, the relationship between UI and outpatient Medicare claims was examined. About 16 percent of women of women with an outpatient visit for UI submitted Medicare claims; women with severe, urgency, and mixed UI were more likely to submit claims.

Dr. Grodstein concluded her presentation by highlighting major conclusions: (1) Half of women with severe UI continue to experience severe symptoms over more than 10 years, and only a minority receive care; (2) most women even with mild UI symptoms experience persistent or worsening UI over more than decade of follow-up; and (3) care-seeking is remarkably infrequent, especially in women with mild symptoms, who likely could be successfully treated and reduce their likelihood of worsening symptoms. Dr. Grodstein emphasized that these correlations represent opportunities for researchers to better understand the dynamics of UI in women.

Discussion Points

- All women with UI were considered in the study, regardless of their treatment. Many women with UI never receive treatment.
- Women with comorbidities were more likely to seek care for UI. The driving factors behind this trend are not fully understood.
- Dr. Grodstein clarified that socioeconomic status was considered in this study. Because the study participants were employed as nurses, they often benefited from a higher socioeconomic status than other women in the general population.

SCIENTIFIC SESSION 1— EPIDEMIOLOGY AND POPULATION RESEARCH

Introduction and Overview

Moderators: Maryellen Kelly, D.Sc., M.S.N., Duke University (KURe Program), and David Bayne, M.D., M.P.H., University of California, San Francisco (USCF)–Kaiser Permanente Northern California (Kaiser) (UroEpi Program)

The moderators introduced the speakers and began the session. They encouraged the participants to submit questions through the Zoom chat.

Benign Urology in the Big Data World
Stephen Van Den Eeden, Ph.D., Kaiser Permanente Northern California (Co-Principal Investigator, K12)

Dr. Stephen Van Den Eeden presented on big data in the context of BU conditions. He explained that the realm of big data creates new opportunities for investigators, including greater funding, increased outcomes and covariates, increased geospatial area, more individuals and diversity, and longer follow-up periods. He suggested considering big data in at least two dimensions—methods (e.g., artificial intelligence/machine learning [AI/ML]) and applications. Data sources for AI/ML include electronic health records (EHRs), Google, omics (e.g., genomics, metabolomics), wearables (e.g., smart fitness watches) and mobile data sourcing, patient and caretaker reports, and provider patterns. The use of big data in AI/ML has addressed primarily urological cancer and radiomics.

Dr. Van Den Eeden highlighted BU-related conditions that have been treated using AI/ML approaches; these included stone composition, cardiovascular disease and stroke risk assessment in patients with chronic kidney disease, responses to anticholinergic medications for overactive bladder (OAB) syndrome, and impedance-based bladder state detection. Additional AI/ML efforts include frameworks for UI diagnosis and risk of pelvic floor disorders. Issues of concern include data bias, data evolution and continuity, and validity and replication. New data opportunities include predictive analytics, natural language processing, imaging processing, and phenotyping. Dr. Van Den Eeden also noted that AI/ML could be applied to the improvement of diagnostic processes and treatment decisions.

EHRs typically are a provider-facing system supported by a large database. AI/ML allows providers to detect patterns that inform treatment plans. Dr. Marvin Langston at Kaiser Permanente Northern California is using AI/ML for prediction of patients at high risk of post-operative urinary retention and for prediction of benign prostatic hyperplasia (BPH)/lower urinary tract symptoms (LUTS) progression following treatment. Dr. Van Den Eeden also noted that CAIRIBU supports big data efforts. He highlighted issues surrounding big data, including data security, repeated evaluations and validations, patient assistance, data privacy issues, monetization, and false positives.

Disaggregating Gender from Sex in Sexual Dysfunction Epidemiology
Juno Obedin-Maliver, M.D., M.P.H., M.A.S., Stanford University School of Medicine (Co-Principal Investigator, K12)

Dr. Juno Obedin-Maliver discussed results from The Population Research in Identity and Disparities for Equality (PRIDE) Study related to the sexual and gender minority (SGM) population. The SGM community encompasses people who are not heterosexual or cisgender and comprises at least 4.5 percent of the U.S. adult population. SGM status is not unique to a particular race or ethnicity and is present across all income brackets and educational levels. Dr. Obedin-Maliver introduced the concept of a "gender unicorn," which describes five dimensions of gender: gender identity, gender expression and presentation, sex assigned at birth (SAAB), sexual attraction, and romantic or emotional attraction. Not included in the "gender unicorn" but an additional important consideration is sexual behavior. She briefly outlined the distinction between sex and gender.

Researchers and clinicians often fail to appropriately account for the complexity of sex and gender when treating patients; clinical tools have been developed for cisgender and heterosexual people. Dr. Obedin-Maliver stated that the relative lack of population-based data presents the greatest challenge to describing the health status and needs of the SGM population. The PRIDE Study is a national, online, longitudinal dynamic cohort study designed to address physical, mental, and social health. The Phase 1 pilot study has been completed; the Phase 2 study was launched in May 2017.

Participants engage in annual health questionnaires, and detailed participant profiles are maintained. The team constructed a novel web platform for the study.

Multiple assessment tools for sexual dysfunction have been developed. These tools, however, typically are based on heterosexual sexual contact and assume that SAAB and gender are congruent. In The PRIDE Study, the researchers determined the effectiveness of these tools in SGM individuals. Comorbidities were high across the study population; more analysis is needed to determine whether the current data encompass the entire SGM community. Dr. Obedin-Maliver concluded by highlighting the following points: (1) The SGM community is understudied and vulnerable; (2) The PRIDE Study and PRIDEnet are using technology to engage researchers and SGM communities in partnership; and (3) some measures might work well for SGM individuals, but many concepts need to be reconsidered.

Urologic Complications of Diabetes: UroEDIC Experience
Aruna Sarma, Ph.D., University of Michigan (Co-Principal Investigator, K12)

Dr. Aruna Sarma presented on the impact of diabetes on BU conditions. Commonly recognized complications of diabetes are neuropathy and cardiovascular disease; Dr. Sarma's research focuses on urologic complications that affect both urinary and sexual function. She formed a multidisciplinary team to characterize the complications and burdens experienced by affected individuals. Previously, limited research had been conducted to quantify the functions of these physiological systems. The team developed risk factor models for specific BU complications and characterized the impact of these conditions on quality of life.

UroEDIC is derived from two previous studies, the Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study. DCCT was a landmark study that compared intensive and conventional diabetes therapy for early vascular and neurologic complications in a multicenter, randomized clinical trial from 1983 to 1993. EDIC is an ongoing observational study of the DCCT cohort that began in 1994 to examine the long-term impact of initial randomization and subsequent glycemic control. UroEDIC began in 2003 (reinitiated in 2010) to examine burden, onset, and risk factors of urologic complications in type 1 diabetes. The investigators found that urologic conditions are prevalent among diabetes patients. They reported a significant impact of early glycemic control on prevention of erectile dysfunction (ED). Additionally, sustained glycemic control is important for prevention of bladder dysfunction. Dr. Sarma emphasized the importance of considering early, sustained, and current glycemic control in urologic conditions. The effects of hypertension on ED also should be considered. Autonomic neuropathy also appears to be associated with UI in women, as well as ED and LUTS in men.

The team also characterized longitudinal ED and UI phenotypes. The effects of ED and UI on poor quality of life was greater than those observed for other complications (e.g., retinopathy, nephropathy, neuropathy), and participants were affected significantly by BU conditions. Proactive solutions to these complications include medication adherence and weight loss. Dr. Sarma emphasized the importance of identifying high-risk individuals and treating or preventing urologic conditions. These efforts will necessitate a comprehensive study of natural history, validation of subphenotypes, and improved understanding of disease mechanisms.

Interpersonal Trauma as a Marker of Risk for Urinary Tract Dysfunction in Women
Alison Huang, M.D., University of California, San Francisco (Co-Principal Investigator, K12)

Dr. Alison Huang discussed the relationship between interpersonal trauma and symptomatic lower urinary tract dysfunction in women. She provided an overview of the psychological sequelae of

trauma, explaining that traumatic experiences invoke sympathetic flight, fight, or freeze responses. Traumatic experiences can cause people to react to normal stimuli with excess fear or anxiety, and victims of trauma often cope by turning to alcohol, other drugs, or high-risk behaviors. Post-traumatic stress disorder (PTSD) represents an extreme manifestation of trauma.

Epidemiologic studies suggest a relationship between abuse and genitourinary (GU) dysfunction in girls and young women. Adverse childhood experiences are associated with an increased risk of sexually transmitted infections (STIs) in adulthood, and childhood and adolescent abuse are associated with increased urinary frequency, urgency, and nocturia in adulthood. The Reproductive Risks of Incontinence Study in Kaiser (RRISK) study characterized assessment of exposure to interpersonal violence in home-based study visits from 2008 to 2012. A self-administered questionnaire assessed lifetime exposure to physical intimate partner violence, emotional and verbal intimate partner violence, and sexual assault. Structured items focused on the most common symptoms in women: stress UI, urgency UI, nocturia, bladder pain, and clinician-diagnosed UTI.

In the study, incontinence, nocturia, bladder pain, and UTI were associated significantly with interpersonal violence and sexual assault. More than one-third of women in each interpersonal violence exposure group reported PTSD symptoms (e.g., incontinence, nocturia, bladder pain, UTI). Dr. Huang emphasized that interpersonal abuse could be an underrecognized marker of risk for UTI in midlife and older women. PTSD is most strongly associated with symptomatic urinary tract dysfunction.

Dr. Huang advocated trauma-informed care, which realizes the widespread impact of trauma; understands potential paths for recovery; recognizes signs and symptoms of trauma; integrates knowledge about trauma into policies, procedures, and practices; and seeks to actively resist re-traumatization. She outlined future research directions: (1) prospective evaluation of temporal associations and dose relationships between trauma exposures and urinary outcomes; (2) clinical evaluation of therapies directed at ameliorating trauma to assess effects on urinary outcomes; (3) mechanistic research to identify pathways linking trauma, psychological stress, and urinary tract dysfunction; and (4) evaluation of relationships between trauma and urinary tract dysfunction in men.

Inflammation and the Prostate
Marvin Langston, Ph.D., Kaiser Permanente Northern California (Co-Principal Investigator, K12)

Dr. Langston emphasized that although inflammation and infection are well correlated, indirect pathways for inflammation also exist. Bacterial and viral infections have long been described as triggers of acute inflammation. Dr. Langston's work suggests that these infections also create long-term effects on prostate health. Clinical population-based research uses several methods for analysis, including digital assessment of biological samples. However, biopsies are challenging and invasive. Thus, other proxies for analysis often are employed; these include prostate-specific antigen (PSA) testing and prostatitis diagnosis. Dr. Langston noted that he utilizes a variety of analytical approaches (e.g., analysis of semen and urine, prostate imaging).

Dr. Langston provided the results of his study examining acute and long-term prostate infections (e.g., chlamydia, gonorrhea) on PSA in young military men, using samples collected from a sero-repository. To determine acute and long-term effects of infection, the researchers selected four timepoints for analysis. They found that men with chlamydia and gonorrhea were more likely to exhibit a large change in PSA. For acute infections, PSA likely represents prostate infection and associated inflammation. Dr. Langston noted, however, that a generalized response to infection might also play a role in these findings. Interestingly, similar results were mounted by more systemic infections that are not known to target the prostate directly (e.g., infectious mononucleosis, whooping cough,

Staphylococcus infection). Sustained rises in PSA might be predictive of future prostate disease risk and severity.

The researchers conducted a long-term study on the PSA trajectory. They found that men with STIs displayed an increase in PSA over the study period; this change was driven primarily by the effects of chlamydia, which presents as an asymptomatic infection and is likely to be undiagnosed for long periods of time. Additionally, systemic effects appear to impart long-term effects on PSA. Dr. Langston explained that this effect might result from increased vascular permeability, a factor that should be considered in future studies. Current work is examining the reported association between adult-onset mononucleosis and clinical prostatitis. Alternatively, general immune responses might account for the reported associations. He emphasized that other unknown factors might influence the reported results. Several experiments addressing this question currently are in progress.

Outcomes of Pregnancy and Delivery among Women with Physical Disabilities
Courtney Streur, M.D., University of Michigan (Co-Principal Investigator, K12)

Dr. Courtney Streur presented on the reproductive outcomes of women with spina bifida (SB), a neural tube defect that is associated with hydrocephalus, scoliosis, contractures, tethered core, and neurogenic bladder and bowel issues. Early interventions help with the management and prevention of SB symptoms. Many women with SB survive to adulthood and desire to become pregnant. The outcomes for pregnancy associated with SB, however, have not been characterized fully. Using the National Inpatient Sample, Dr. Streur's team compared data from women with SB hospitalized for delivery between 2003 and 2013. Compared with those without SB, women with SB were significantly younger and more likely to have a comorbid condition. They also were more likely to undergo a cesarean delivery and experience more complications in both vaginal and cesarean deliveries. Areas for further study include characterization of complications during and after pregnancy.

Dr. Streur questioned whether women with SB are becoming pregnant unintentionally and whether they understand their reproductive health. She conducted a qualitative exploratory study to address these questions. Semistructured interviews were conducted with women with SB. The following themes emerged from the interviews: women's poor understanding of their reproductive potential, their interest in having a family, providers' opposition to their reproductive goals, and their going into pregnancy and delivery unprepared. Currently, Dr. Streur is working on a project using IBM MarketScan Research Databases to compare the outcomes of pregnant women with a physical disability (e.g., neurogenic bladder) with those without. She and her colleagues are studying antenatal diagnoses, prescriptions, bloodwork, tests, surgeries, and hospital admissions. The next steps are to develop an online sexual and reproductive health toolkit for adolescents with SB, with plans to expand to all adolescents with physical disabilities and to all reproductive age groups.

Discussion Points

- High rates of both depression and sexual assault are present in the SGM population. Dr. Obedin-Maliver is interested in analyzing mental health, comorbidities, and underlying factors. She also is developing a paper exploring the effects of COVID-19 on mental health in the SGM population.
- Many PRIDE Study participants are involved in every step of the cohort study and provide feedback on study designs.

- In younger groups, prevalence of UI appears comparable between people with diabetes and the general population of the same age. Between older groups, however, a clear disparity emerges between people with diabetes and the general population.
- Glycemic control appears to be important for people without diabetes. Dr. Sarma plans to pursue additional studies on this topic. People with diabetes who have attained glycemic control likely retain hyperglycemic load memory. More longitudinal data are needed.
- Many patients with disabilities experience sexual abuse, and their urinary complications are complex. Dr. Huang emphasized that physicians should remain aware of sexual abuse; open conversation with patients is crucial.
- Dr. Langston has not tested levels of inflammatory cytokines, but other investigators have performed work in this area. The correlations are moderate in patients with non-malignant conditions.
- Dr. Langston stated that the effect of the urinary microbiome on PSA represents an interesting area for investigators; more studies on this effect are needed. He will consider the interplay between intracellular and extracellular microbiotic organisms.
- Planned and emergent cesarean deliveries in women with SB could not be considered separately within the data set. Dr. Streur is addressing this question in current studies. Dr. Penniston suggested measuring patient self-efficacy to determine whether this factor correlates with pregnancy outcomes.
- In the semistructured interviews, women with SB reported reliance on family members for sexual education, but not for pregnancy. Some women faced resistance from their families during pregnancy.

CAIRIBU POSTER SESSIONS

Meeting participants submitted poster abstracts, which were viewed and discussed in the main meeting room in three groups. Prior to the meeting, poster authors submitted 1-minute flash talks on their research. Poster presenters hosted discussions in individual Zoom breakout rooms, which were open for 30 minutes. Posters were viewable as PDFs during the entire meeting, and a link to full abstracts was available to all attendees for download.

AWARDS

Abstracts considered particularly meritorious were selected for best abstract awards by reviewers prior to the meeting. Posters were evaluated during the virtual session in nine topic categories; the attendees voted for posters in a separate category.

REVIEWER Abstract Awards

Connor Beebout, Graduate Student, Vanderbilt University Medical Center (P20): "Cytochrome BD Is Required for Uropathogenic *Escherichia coli* Pathogenesis and Biofilm Development" ***(First Place)***

Sarah Maxwell, Graduate Student, The University of Tennessee Health Sciences Center (P20): "Age-dependent Expression of TRPM4 Channel in Guinea Pig Detrusor Smooth Muscle Is Associated with Altered Contractility" ***(Second Place)***

Derek Ho, Ph.D., Postdoctoral Researcher, Duke University (KURe, P20): "The Major Contribution of Cavitation to Stone Damage in Dusting Mode during Laser Lithotripsy" ***(Third Place)***

REVIEWER Poster Awards

Teresa Liu, Ph.D., Senior Research Associate, University of Wisconsin–Madison (U54): "Mitochondrial Dysfunction Due to Aging Contributes to Prostatic Fibrosis" ***(Prostate and Aging)***

Livianna Myklebust, Graduate Student, University of Wisconsin–Madison (U54): "The Co-localization of COX-1 and COX-2 in Aged Mouse Prostate and Human Prostate" ***(Prostate and Aging)***

Renee Vickman, Ph.D., NorthShore University Health System (P20): "Macrophage-derived Tumor Necrosis Factor Contributes to Benign Prostatic Hyperplasia through Increased Fibroblast Proliferation" ***(Prostate/GU Tract Signaling and Morphology)***

Hannah Ruetten, Graduate Student, University of Wisconsin–Madison (U54): "A Phenome-based Approach for Characterizing Mouse Urinary Pathophysiologies" ***(Prostate and LUTS, Pathophysiology)***

Khue Tran, Research Specialist, Houston Methodist Hospital (U54): "Preliminary Results of Novel Noninvasive Cortical Modulation Using Transcranial Rotating Permanent Magnet Stimulator in Improving Voiding Dysfunction in Female Multiple Sclerosis Patients" ***(Therapeutics and Treatment)***

Logan Hubbard, M.D., Urology Resident, Houston Methodist Hospital: "Preliminary Results of Baseline Cortical Neural Activity in Men with Benign Prostatic Hyperplasia and Bladder Outlet Obstruction" ***(Neuroregulation and Voiding or LUTS)***

Bisiayo Fashemi, Graduate Student, Washington University in St. Louis (P20): "The Decision Maker: The Role of IFRD1 in Urothelial Plasticity and Regeneration" ***(Genetics—Kidney or Bladder)***

John Brannon, Ph.D., Postdoctoral Researcher, Vanderbilt University Medical Center (P20): "Invasion of Vaginal Epithelial Cells by Uropathogenic *Escherichia coli*" ***(Infection)***

Scott Bauer, M.D., Assistant Professor, University of California, San Francisco (UroEpi): "Association of Frailty with Clinical Benign Prostatic Hyperplasia Progression and Serious Adverse Drug Events: The MTOPS Study" ***(Population Health)***

Matthew Grimes, M.D., Assistant Professor, University of Wisconsin–Madison (KURe): "Decreased CD44 Expression Is Associated with Increased Hyaluronic Acid Abundance in Human Lichen Sclerosus" ***(Other Kidney, Bladder, Prostate)***

PEOPLE'S CHOICE Poster Awards

Khue Tran, Research Specialist, Houston Methodist Hospital (U54): "Preliminary Results of Novel Noninvasive Cortical Modulation Using Transcranial Rotating Permanent Magnet Stimulator in Improving Voiding Dysfunction in Female Multiple Sclerosis Patients"

Sarah Maxwell, Graduate Student, The University of Tennessee Health Sciences Center (P20): "Age-dependent Expression of TRPM4 Channel in Guinea Pig Detrusor Smooth Muscle Is Associated with Altered Contractility"

Jim Hokanson, Ph.D., Research Scientist, Duke University (KURe): "Sacral Neuromodulation in Rats: Parameters and Pathways"

Connor Beebout, Graduate Student, Vanderbilt University Medical Center (P20): "Cytochrome BD Is Required for Uropathogenic *Escherichia coli* Pathogenesis and Biofilm Development"

FRIDAY, DECEMBER 4, 2020

KEYNOTE LECTURE 2: CONTRIBUTIONS OF CLINICAL STUDIES IN BENIGN LUTD—WHAT DO WE STILL NEED?

Quentin Clemens, M.D., Edward J. McGuire Professor of Urology; Director, Female Pelvic Medicine and Reconstructive Surgery Fellowship; Associate Chair for Research, University of Michigan

Dr. Quentin Clemens presented on chronic pain in benign lower urinary tract dysfunction (LUTD) and needs in the research community. Three categories of abnormalities are present with LUTD—afferent, efferent, and structural. Efferent and structural abnormalities can be quantified; afferent abnormalities are more difficult to characterize. Afferent abnormalities are characterized by pain and urgency; Dr. Clemens explained that the two symptoms exhibit overlapping characteristics. He shared patient perspectives to highlight the impact of afferent abnormalities on quality of life.

In the 1980s, the research community had not reached a consensus on epidemiology, terminology, diagnosis, treatment, or pathophysiology of benign LUTD. The NIDDK Consensus Definition Cohort Studies were performed to address these deficits. The early multisite cohort studies addressed the feasibility of identifying urological chronic pelvic pain syndrome (UCPPS) in patients, using standardized inclusion criteria and validated instrumentation over a longitudinal period. They assessed the severity of impact on quality of life and morbidity. In men, no association was recorded between prostate cancer and PSA. Dr. Clemens noted that these cohort studies were largely negative, but many of the results were published in high-impact journals. Additionally, some of the treatments investigated in the negative studies (e.g., beta blockers) still are used in treatment. The Urological Pelvic Pain Collaborative Research Network yielded data demonstrating the therapeutic effectiveness of myofascial physical therapy on treatment for physical tenderness. Dr. Clemens highlighted challenges in the UCPPS clinical trials, including inclusion criteria, concomitant treatments, and endpoints.

Dr. Clemens described the Clinical Pharmacogenetics Implementation Consortium study, which was a response to a request for applications (RFA) from the NIDDK. He coded BU diagnoses from electronic medical records to determine prevalence, costs, risks factors, and comorbidities. The researchers found that symptoms were more common than coded diagnoses. Interstitial cystitis (IC) was associated with endometriosis, fibromyalgia, irritable bowel syndrome, anxiety, depression, and headaches. A strikingly high incidence of nonbladder surgeries (e.g., hysterectomy) was reported before IC onset, suggesting susceptibility or central sensitization. The early IC cases tended to show greater improvement, although few experienced resolution of all symptoms. Lastly, the RAND Interstitial Cystitis Epidemiology (RICE) Study determined the prevalence and burden of IC and bladder pain syndrome (BPS) in U.S. women. Notable findings were as follows: similar symptom severity and impact, widespread care-seeking, persistent symptoms, sexual dysfunction, and short sleep duration.

Administrative data often are derided because of the high range of prevalence estimates. Dr. Clemens emphasized the importance of characterizing patients' symptoms from pathology reports. The clinical challenge for investigators is to develop treatments for symptom-based urologic disorders that are

personalized, evidence-based, and effective. Network studies are ongoing to address these challenges. The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network recognizes a lack of clinical advancement and interdisciplinary work in the field of UCPPS. New literature and clinical experience suggest that UCPPS likely represents a heterogeneous group of patients; improved phenotyping is likely to lead to better outcomes. The MAPP study involves six discovery sites, a data coordinating center, tissue and technology core facilities, additional sites, and the NIDDK. MAPP findings suggest two phenotypes: pain-predominant and urinary-predominant. The researchers recommended abandoning composite symptom scores for clinical and research use.

Symptom progression affected widespread pain and severity of bladder-focused symptoms. These results suggest that UCPPS dysfunction is not confined to the pelvis. Increased functional connectivity in the frontoparietal brain network is associated with reduced UCPPS symptoms. Pain testing indicated a centralized phenotype; Dr. Clemens proposed that patients with more widespread pain should be treated differently. Body maps have been developed for clinical use; categories include pelvic pain only, widespread pain, and intermediate pain. Neuropathic pain represents an area for further study. The Antiretroviral Therapy as Long-Acting Suppression (ATLAS) study can provide the basis for clinical trials that consider widespread pain.

Dr. Clemens concluded by providing his perspective on network studies. Funded RFAs often are preceded by a consensus conference and involve coordination across multiple study sites. U01 studies typically are funded for two cycles (i.e., 10 years) or more. Dr. Clemens pointed out that turnover of core investigators over this period is common. He emphasized the importance of communication, frequent in-person meetings, identification of common themes, budget considerations, division of responsibilities, and methods manuscripts. Research coordinators, frequent meetings, and identification of best practices are critical for recruitment and retention. Additionally, publications and messaging should occur throughout the duration of the network. Network opportunities include trainee research, young investigator involvement, and ancillary grants. He outlined current needs: new NIDDK definitions for IC and BPS, bladder biopsy tissue for LUTD, and new capabilities for network studies. Future visions include standardized clinical evaluation, increased focus on patient experience, novel pain agents for UCPPS, extension of neuroimaging studies, continued examination of the urinary microbiome, and increased utilization of the NIDDK repository.

SCIENTIFIC SESSION 2—CLINICAL AND TRANSLATIONAL RESEARCH

Introduction and Overview

Moderators: Renee Vickman, Ph.D., NorthShore University HealthSystem (P20 Center), and Bisiayo Fashemi, Graduate Student, Washington University in St. Louis (P20 Center)

TNF-Alpha Antagonism as a Potential Approach to Modify BPH Pathogenesis

Simon Hayward, Ph.D., NorthShore University HealthSystem (Co-Principal Investigator, P20)

Dr. Hayward presented on BPH pathogenesis and the development of treatment approaches. Prior work has suggested that BPH-associated inflammation contributes to fibrotic changes and growth in the prostate. Study goals were to (1) define the profile of leukocytes and their changes during prostate enlargement with single-cell RNA sequencing (RNA-seq), (2) identify cellular clusters that possess distinct pathway topologies in BPH progression, and (3) identify potential intervention strategies. The researchers sought to understand how extracellular signaling pathways are altered during BPH progression and to identify potential therapeutic targets.

Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) was used to determine simultaneous gene and cell surface protein expression at the single-cell level. CITE-seq enables

confirmation of immune cell categorization with widely accepted protein markers. These findings provide early insights on macrophage populations. The general dogma regarding macrophage action in injury involves three steps: inflammation, anti-inflammation, and resolution. The researchers found that the macrophages clustered in distinct expression groups; some demonstrated unexpected functions. These results suggest a persistent, non-resolving inflammatory response. In BPH pathogenesis, pro inflammatory and anti-inflammatory responses appear to occur simultaneously.

Dr. Hayward explained that in men, autoimmune inflammatory diseases are associated with a greater risk of BPH. Men who have undergone treatment for autoimmune inflammatory diseases, however, have a reduced risk of BPH. He is interested in determining the underlying factors by producing a clearer picture of immune cell populations present in BPH. Dr. Hayward's group is developing a pathway analysis to determine how intercellular communication changes with progression, including communication with stromal and epithelial cells. Additionally, they are working to quantify the effects of androgens on leukocyte populations in tissue and are exploring the potential of immune signaling modifiers in clinical BPH.

Crossing the Disciplinary Divide to Chase the Phantom of Bladder Permeability
Pradeep Tyagi, Ph.D., University of Pittsburgh (Principal Investigator, U54)

Dr. Pradeep Tyagi described interdisciplinary approaches to characterize bladder and prostate permeability. He conveyed the challenges associated with measuring the menace of bladder permeability in the affected individuals and the critical role played by the architecture of the bladder in erecting the barrier against the ingress of urine and instilled drugs and dyes. Measures of bladder permeability includes two components—urothelial permeability and vascular permeability, which can be easily measured in animal bladder by measuring the dye concentration in bladder harvested after instillation and injection of polar dyes (Evans blue dye, Trypan blue), respectively. Since human bladder removal (cystectomy) is not clinically viable for assaying bladder permeability, investigators must cross the disciplinary divide to measure the bladder and prostate permeability with technology borrowed from radiology, especially magnetic resonance imaging (MRI). By replacing intravenous injection of Evans blue dye with extracellular T_1 shortening agent, Gadolinium chelates, blood brain barrier permeability, vascular permeability and the expansion of extracellular matrix in prostate can be measured non-invasively by deriving the rate constants of contrast enhancement and contrast washout in brain or prostate (rate of Gadolinium influx and efflux). Unlike MRI based permeability assay of brain and prostate, measurement of vascular permeability in bladder is complicated by the rapid urinary excretion of the injected Gadolinium chelate, which fills the bladder lumen to compromise the image contrast of bladder wall in vascular permeability assay. The problem encountered with the injection of Gadolinium chelate for assaying vascular permeability reappears in the assay of urothelial permeability by the instillation of Gadolinium chelate instead of Evans blue dye.

To develop a clinically viable urothelial permeability assay, Dr. Tyagi proposed an innovative solution of instilling small molecular sized Gadolinium chelate mixed with a non-absorbable, negative contrast agent. After instillation, Gadolinium passively diffuses into urothelium via paracellular routes and the measurement of positive contrast enhancement in urothelium segmented from other layers of bladder wall by respective differences in T_1 water relaxation rates can serve as index of urothelial permeability. Dr. Tyagi reported that the instilled Gadolinium rapidly equilibrates to a ten-fold lower concentration in the urothelium of capillary perfused bladder wall with exponentially lower concentration in detrusor smooth muscle due to the loss of instilled Gadolinium into systemic circulation.

Dr. Tyagi explained that the measured T_1 water relaxation rates after instillation of contrast mixture in the chronic mouse model of radiation cystitis and the findings corroborated with the measured water

permeability coefficients in same model. He highlighted the following conclusions: (1) Crossing the disciplinary divide can predict histology and function of diseased bladder and prostate, (2) voxel-wise T_1 mapping can be a noninvasive alternative to biopsy in BPS/IC patients, and (3) application of multiparametric MRI (mpMRI) for prostate cancer can be tweaked for advancing the clinical understanding of BPH/LUTS.

The Impact Equation: Relative Reach and Efficacy of Continence Promotion Efforts
Heidi Wendell Brown, M.D., MAS, FACOG, University of Wisconsin School of Medicine and Public Health (Co-Principal Investigator, K12)

Dr. Heidi W Brown discussed the application of dissemination and implementation research principles to benign urologic research. Her research—which bridges study of clinical effectiveness, dissemination, implementation, and impact—addresses gaps between evidence and practice. The United States spends $130 billion annually on health research, but only 14 percent of original research is translated into benefits for the public within 17 years. Most proven interventions are never implemented in community and clinical settings. Challenges for implementation include readiness, awareness, formatting, resources, and infrastructure. The RE-AIM framework describes five domains that contribute to public health impact: Reach, Effectiveness, Adoption, Implementation, and Maintenance. Dr. Brown's research focuses on how to increase the impact of evidence based interventions to improve UI. Effective solutions for UI have been developed (e.g., pelvic floor exercises, dietary changes, physical therapy) but many women with UI are not aware of them. Dr. Brown's initial work on the K12 questioned whether this behavioral modification information could be packaged into a format already used by community agencies to deliver information about behavior changes to improve other chronic diseases.

In her K12 project, Dr. Brown assessed the feasibility of an evidence-based bladder continence prevention program that was adapted for bladder and bowel symptoms and for community implementation. She partnered with the University of Wisconsin–Madison Community Academic Aging Research Network to develop, translate, and implement this intervention in community agencies. The Mind Over Matter: Healthy Bowels, Healthy Bladder (MOM) program helps women build skills and confidence to change behaviors, support and learn from one another, and seek professional help if needed. Dr. Brown's team secured supplemental funding to conduct a hybrid effectiveness-implementation trial. This trial confirmed MOM's effectiveness and collected preliminary information about adoption and maintenance by community agencies, which are core to ensuring that MOM can be delivered outside research settings.

Dr. Brown concluded by suggesting researchers can maximize their impact by considering how their findings will be ultimately implemented and disseminated as they formulate their research questions, and encouraging those interested in learning more about these principles to contact her directly.

Cystitis Cystica and Recurrent UTIs in Postmenopausal Women
Indira Mysorekar, Ph.D., Washington University in St. Louis School of Medicine (Principal Investigator, P20)

Dr. Mysorekar presented on the development of recurring UTIs in women with age and underlying causes. Urinary tract conditions and diseases worsen with age; these include recurrent UTI, IC/BPS, OAB, and stress UI. UTIs are the most common infectious disease in women and impose a burden of $2 billion annually in the United States, of which approximately 20 to 50 percent become recurrent. Inflammation increases with age, imparting both protective and pathogenic effects. Using a mouse more of aging, Dr. Mysorekar found that aged mice have frequent UTI recurrences and enhanced quiescent intracellular reservoir formation. RNA-seq reveals that the population of immune cells in

the bladder changes with age; lymphocytes displace macrophages as the predominant immune cell type. Lymphocytes localize to distinct aggregates (i.e., tertiary lymphoid tissues [TLTs]) in bladders from aged mice. TLTs form ectopically in chronically inflamed tissues and play a role in local immune responses. Their pathogenic and protective influences are not understood fully.

Cystoscopy can be performed in patients with UTI to assess inflammation. These measures have detected cystitis cystica, which are small nodules that appear in response to chronic inflammation. Their epidemiology, pathogenesis, and significance are unknown. Dr. Mysorekar posited that the nodules might be comparable to the TLTs in mice, because biopsies reveal similarities between the structures. The nodules are associated with age and UTI recurrence, and they appear associated with pain in affected patients. These findings suggest that the nodules impart a pathogenic effect. Dr. Mysorekar emphasized that these associations should be considered in future therapeutics. She also stated that the mechanisms require further investigation.

Infection! What Are We Up Against?
Anne-Catrin Uhlemann, M.D., Ph.D., Columbia University (Co-Principal Investigator, U54)

Dr. Anne-Catrin Uhlemann discussed multidrug resistance (MDR) challenges in the context of the gut microbiome and mechanism of resistance. UTIs appear to serve as reservoirs of MDR, and a national rise of antimicrobial resistance in UTI has been recorded. Gram-negative bacteria possess the ability to take up plasmids, leading to horizontal gene transfer and the development of resistance.

In a prospective cohort study, Dr. Uhlemann's group performed a longitudinal collection of fecal samples in adult liver transplant patients, who are at high risk of MDR organism (MDRO) infection. They found that MDRO colonization is associated with infection. They then used RNA-seq to characterize how the microbiome adapts during colonization. Alpha-diversity (i.e., diversity within sample) was reduced in patients with UTI. Additionally, alpha-diversity was reduced during MDR colonization, and all samples from MDRO-colonized patients had lower alpha-diversity. In contrast, beta-diversity (i.e., community composition) differed between colonized versus uncolonized samples. These findings could be applied to early diagnostics and therapeutic interventions.

The group also is investigating the mechanism of MDR. The genomic determinants of polymyxin resistance (PR), for example, result from many two-component systems involving a set of resistance genes. They performed a comprehensive genomic analysis of all PR isolates and found that mutations in canonical genes do not fully correlate with PR in clinical isolates. Dr. Uhlemann highlighted barriers in the field, which include lack of functional evidence, unclear determinants, novel determinants, near-pan-resistance, and lack of efficient marker-less genetic manipulation systems. She adapted a single plasmid CRISPR-Cas9 system to identify single-nucleotide polymorphisms that confer high-level resistance. She highlighted the following overarching conclusions: (1) The gut microbiome serves as an important reservoir for MDRO and subsequent infections, (2) last-resort antibiotic resistance is a polygenic trait with different levels of resistance and virulence, and (3) the intersection of antibiotic resistance and virulence requires further study.

Surgical Management of Urolithiasis: Mechanism of Stone Damage in a New Era of Laser Lithotripsy
Pei Zhong, Ph.D., Duke University (Principal Investigator, P20)

Dr. Pei Zhong discussed the different modalities for surgical treatment of urolithiasis. Major technological advancements have been achieved in research and laser lithotripsy for USD, but the fundamental knowledge about laser lithotripsy has remained unchanged for the past 20 years. He emphasized the importance of establishing optimal strategies for the use of novel devices. Laser

lithotripsy represents a novel contribution in this area; the theory behind ablation, however, is incomplete and requires further investigation.

Dr. Zhong and his team are working to characterize the mechanism of stone damage using this approach. Conventional theory states that photothermal ablation dominates, and cavitation damage is negligible. Unexpectedly, his group found larger stone damage after laser treatment in water, compared with ablation in air. The crater depth was found to be shallower in water, but the crater cross-sectional area was large, presumably resulting from potential damage by cavitation during the treatment. Hydrophone measurements show weak shock wave emission upon bubble collapse. Photoelastic imaging demonstrates stress concentration at the bubble collapse site in the stone. Maximum damage was produced at 1 mm from the surface in the fragmenting mode when the laser fiber was placed in parallel to the stone surface, characteristic of cavitation-induced stone treatment. They also looked at stone damage at higher repetition frequency with lower pulse energy in dusting mode, an approach that is becoming more popular among urologists. Dusting may significantly reduce the overall procedure time compared to fragmenting stones. Adjusting energy and standoff distance between the fiber tip and stone surface could help physicians optimize their treatments for maximum efficiency.

High pressure is the primary mechanism for producing stone erosion by cavitation bubble collapse; this process was captured using high-speed imaging. Microjet impact likely represents the mechanism for damage. Dr. Zhong's group is developing numerical models to simulate laser-generated bubble dynamics and interaction with stone. Their preliminary results suggest that an optimal standoff distance of the probe tip to stone surface will maximize the extent of microjet damage. They also measured the laser energy transmission at various standoff distances. The results matche well with optical absorption in the fluid at low pulse energy used for dusting, while deviating significantly at high pulse energy used for fragmenting, indicating the contribution of the MOSES effort. Dr. Zhong emphasized that for the same total laser energy delivered to the stone, fragmenting mode will produce a well-defined, concentrated deep crater, characteristic of photothermal ablation. In contrast, dusting mode will produce multiple shallow and diffused damage craters, characteristic of cavitation erosion. The group is now leveraging a new ultrasonic technique developed from their research in shock wave lithotripsy to monitor cavitation activity and optimize treatments. These results will help meet a critical need for collaborations between urologists and engineers to advance knowledge and technology development in the field of laser lithotripsy for the treatment of USD.

Management of Urolithiasis in Obese Patients
Kyle Wood, M.D., The University of Alabama at Birmingham (Principal Investigator, P20)

Dr. Kyle Wood reviewed the medical, dietary, and surgical management of urolithiasis in patients with obesity. USD often is treated as an episodic event and has been underrecognized within the research community. USD is associated with BMI and is particularly prevalent in communities with high rates of obesity. Additionally, other obesity-related conditions (e.g., diabetes, hypertension) also appear associated with USD. Most researchers associate uric acid stones with obesity.

Obese individuals tend to excrete more calcium, and oxalate excretion correlates directly with BMI. Dr. Wood's group was interested in further characterizing this association. They created a mouse model of obesity and, controlling for diet, reported increased oxalate excretion with BMI. They found that these levels likely result from changes to an oxalate precursor and reflect changes to the endogenous pathway. These results have been confirmed in clinical studies. Additionally, their results suggest that the glyoxylate pathway is not the cause of the endogenous oxalate production. Ascorbic acid represents a factor for further investigation.

Dr. Wood emphasized the importance of conducting research to inform treatment options for USD. Physicians are provided guideline statements for increased fluids and limiting intake of sodium, dietary oxalate, and ascorbic acid. Dr. Wood noted that surgical management is an option for treatment, but these procedures can be risky. He emphasized the importance of implementing prevention strategies for USD.

Discussion Points

- Dr. Hayward has observed results in prostate tissue that are complementary with the inflammatory effects reported by Dr. Mysorekar. Overlap in specific molecular profiles might be present. Because the inflammatory effect is present even in germ-free mice, it likely reflects luminal antigens.
- Dr. Tyagi's permeability monitoring tool has been applied to functional processes in other tissues (e.g., drug delivery in the brain). He uses a two-compartment model; the contrast agent is injected intravenously, and MRI is used to measure water relaxation time.
- Long-term consequences of bladder permeability are unknown; Dr. Tyagi is interested in performing experiments on this topic.
- The potential effects of antigens and urinary products on B-cell activation in the bladder are under investigation. Elevated immunoglobulin A levels in aged mice might also play a role; this effect warrants further investigation. Dr. Mysorekar presently is investigating how metabolism is altered with aging.
- Avoiding pathogen dominance is key to reestablishing diversity and preventing MDR. Researchers and clinicians must develop a strategy for moving forward because many conditions share common pathways.
- Analyses of bacterial mutations are challenging to perform; the traits are highly polygenic, and known gene markers are not detected consistently. Bacterial function must be evaluated systematically.
- Dr. Zhong has considered using low-energy shock waves for certain treatments, but his group is focusing primarily on laser treatments.
- Dr. Wood plans to examine whether obese individuals are more sensitive to ascorbic acid. This effect is likely to play a major role in urinary oxalate levels, although the mechanism is not yet understood fully. The microbiome might play a role in oxalate production; this process requires further investigation. Microbiome studies are challenging to perform.
- Protein intake has been shown to relate to glycolate excretion, but the dietary control studies were performed in healthy individuals. USD patients are likely to exhibit different effects.

KEYNOTE LECTURE 3: BENIGN GU RESEARCH—PAST, PRESENT, AND FUTURE
Mark Nelson, Ph.D., Distinguished Professor and Chair, Department of Pharmacology, University of Vermont; Member, National Academy of Sciences

Dr. Nelson presented on the past, present, and future state of benign GU research, with an emphasis on bladder function. He began with an overview of bladder anatomy and function. The urinary bladder is composed of the interstitium, urothelial cells, and nerves. Urine is made by smooth muscle contraction in the urethral sphincter, and local contractions are controlled by action potentials. In the 1980s, researchers identified potassium channels for their role in the action potential. Preclinical studies

suggested that activators of ATP-sensitive potassium channels would overactivate the bladder and lead to incontinence. However, many drugs used to treat UI are hypotensive agents.

The ion channels that underlie action potentials are well characterized. Dr. Nelson presented a brief overview of the channels' roles in altering membrane potential. In voiding, parasympathetic nerves are activated in urinary smooth muscle. A balance exists for optimal voiding; bladder twitching should be minimized, but voiding should not be impeded. Dr. Nelson's group screened potential drugs that target ATP-sensitive ion channels; none were effective in treating UI. Lack of selectivity was observed, particularly in intact tissue. The drugs also led to decreased blood pressure and weakened reflex tachycardia.

As the bladder expands, it wiggles and twitches. Dr. Nelson was interested in determining how the fullness is detected. A greater understanding of sensory feedback would help inform behavioral modification. Additionally, a greater understanding of mechanosensory function is needed. Dr. Nelson emphasized that the bladder is complex; its structure and function are not understood fully. He presented a video of a mouse bladder filling *in vivo*. Transient contractions (i.e., non-voiding contractions) are a common feature of the bladder, causing changes in local pressure. Local excitation of smooth muscle in the bladder wall creates an increase in action potential, which causes a transient flux of calcium and subsequent contraction, deformation of the bladder wall, and sensory signaling to provide the sensation of fullness. The coordination of signaling, determinants of excitation, and determinants of direction and extent are not understood fully.

Dr. Nelson's laboratory developed an *ex vivo* model to monitor nerve activity and pressure during bladder filling. As the bladder fills, small increases in pressure are observed, reflecting increased afferent sensory nerve activity. The mechanism behind this relationship has not been determined fully but appears to reflect angular distortion of the bladder wall; this distortion effect occurs when the bladder fills and reverts when the bladder is full. Dr. Nelson and his colleagues performed experiments in which pressure was increased slowly; afferent and efferent activity reflected physiological pressure fluctuations. The characteristics of distortion are not understood fully.
Dr. Nathan Tykocki at Michigan State University devised a strategy to observe all sides of the bladder simultaneously. Pressure, angular distortion, calcium levels, and afferent nerve activity can be observed as a full system. Bladder overactivity can be the result of numerous factors; this project will provide valuable insight on the dynamics of bladder function.

Several potential receptors were screened; none appeared to be involved in bladder contraction. The PIEZO1 and PIEZO2 channels are promising target candidates, but recent studies indicate that additional factors are involved in urination. PIEZO1 is highly abundant in the vasculature of the bladder wall; interestingly, blood flow increases markedly during voiding. Dr. Nelson summarized the following points: (1) Local excitation plays an important role in bladder function, but its mechanism is not understood fully; (2) local excitation creates micromotions that induce pressure, but distortion and activation are not understood fully; and (3) mechanosensors and their coordination and function require further investigation. Dr. Nelson emphasized that these questions are crucial to a complete understanding of GU disease processes.

Discussion Points

- People with diabetes often experience compromises to bladder function. Dr. Nelson noted that changes in excitability resulting from channel inhibition might lead to bladder instability. People with diabetes are more likely to experience urinary retention and UI. The effects of drug interactions should be considered in patients with comorbidities.

- Calcium-sensing proteins are expressed in smooth muscle in the bladder. Contraction is a function of both calcium and dephosphorylation, which represents part of a larger pathway. Other drugs are designed to target these areas. The local contractions appear to stimulate sensory fibers, reflecting action potentials in smooth muscle. This network is not well-understood,
- Dr. Nelson expressed interest in using a three-dimensional model to compare normal and OAB states. Imaging and analytical tools first must be refined.
- Afferent activities remain low in response to global expansion. Preliminary results *in vivo* are consistent with those reported from the *ex vivo* model. Changes in pressure result in changes to the interval between voiding periods.

SCIENTIFIC SESSION 3—BASIC SCIENCE RESEARCH

Introduction and Overview

Moderators: Petra Popovics, Ph.D., University of Wisconsin–Madison (KURe Program), and Guillermo Villegas, Ph.D., Albert Einstein College of Medicine (P20 Center)

Applying Nanotechnology to the Treatment of Benign Urologic Disease

Kelvin Davies, Ph.D., Albert Einstein College of Medicine (Principal Investigator, P20)

Dr. Kelvin Davies described the application of a nanotechnological delivery system to treatments for BU disease. The system, named the Einstein nanoparticle, was developed by his colleague, Dr. Joel M. Friedman, at the Albert Einstein College of Medicine. Using this technology, researchers can alter the release kinetics and physical and chemical characteristics of nanoparticles. Dr. Davies focused his presentation on the use of nanoparticles for the application of treatments for ED, diabetes, and OAB. He emphasized that diabetes and ED are closely associated; this relationship is not understood fully. Curcumin molecules have been identified as potential therapeutics for ED, but their limited bioavailability limits their effectiveness. Dr. Davies' curcumin-loaded nanoparticles, however, can be applied topically to the abdomen in a rodent model. The researchers assessed the treatment by measuring changes in intercaecal and systemic pressure. The nanoparticles resulted in restoration of erectile function.

Nanoparticles also can be loaded with gaseous nitric oxide (NO) to treat ED with radical prostatectomy. The researchers used a similar physiological setup and monitored intercaecal and systemic pressure over the treatment period. NO-loaded nanoparticles were applied topically, and an erection was stimulated. A synergy was reported between NO and orally administered inhibitors. Dr. Davis' group now is pursuing this treatment in clinical translation. NO also can be applied for OAB associated with sickle cell disease.

In conclusion, Dr. Davis noted that Einstein nanoparticles are malleable and can be adapted as research tools for many BU conditions for clinical translation. Curcumin-loaded nanoparticles provide a dermal route of administration for improvement of ED. The NO-loaded nanoparticles act synergistically with inhibitors, and NO helps with the improvement of ED in sickle cell disease.

Mitochondria Dysfunction in Benign Prostatic Hyperplasia

Donald DeFranco, Ph.D., University of Pittsburgh School of Medicine (Principal Investigator, U54)

Dr. Donald DeFranco discussed his work understanding the role of inflammation, mitochondrial function, and BPH. Regulation of COX2 causes changes in COX1, leading to different gene

expression profiles. Additionally, the expression of COX1 versus COX2 affects the expression of metabolites in serum. The interplay of COX1 and COX2 is shifted in BPH pathogenesis. Dr. DeFranco explained that this effect likely reflects risk factors for BPH that are related to mitochondrial function (e.g., aging). The effect of mitochondrial dysfunction in prostate tissue has not been investigated extensively. The researchers studied respiratory complex 1, which represents a common drug target for human disease.

Dr. DeFranco collaborated with Dr. Tim Greenamyre at the University of Pittsburgh School of Medicine to examine the effects of rotenone on mitochondrial pathogenesis *in vitro* and *in vivo*. A significant decrease in mitochondrial mass was observed, suggesting a compensatory mechanism for the cell to overcome weakened mitochondrial biogenesis. In a collaboration with Dr. Teresa Hastings at the University of Pittsburgh School of Medicine, Dr. DeFranco observed a decrease in mitochondrial mass in the prostate tissue of a mouse model for Parkinson's disease. Rotenone exposure appears to result in the destruction of complex 1 in the prostate. Additionally, an increase in both COX1 and COX2 was observed. By Western blotting, the group determined that both autophagy (e.g., mitophagy) and mitochondrial assembly are promoted following rotenone exposure. Additionally, tight junction proteins show decreased expression, and epithelial cell permeability is compromised. Dr. DeFranco encouraged the participants to look outside their own systems to explore new knowledge and mechanisms on their disease of interest. He emphasized that a clearer understanding of mitochondrial function and BPH will provide new opportunities for therapeutic development.

Effects of Aging in the Prostate
Teresa Liu, Ph.D., University of Wisconsin–Madison (Co-Principal Investigator, K12/K01, U54)

Dr. Teresa Liu discussed her work on fibrosis in BPH/LUTS. Age is a major risk for factor for BPH; this association is widely recognized among researchers, but the determinants are not well understood. Dr. Liu's research addresses mitochondrial dysfunction and cellular senescence with aging. Senescent cells accumulate with age because they can no longer be cleared by the immune system. Accumulation of a senescent marker, B16, is present with BPH in prostate cells. Her group also observed increased senescence in a BPH mouse model; these results were consistent with measurements in aged mice.

The mitophagy pathway can provide insights into mitochondrial dysfunction. With aging, damaged mitochondria can overwhelm the system and lead to the accumulation of Pnk1, a marker for mitophagy. Dr. Liu's group hypothesized that respiratory complex 1 might play a role in the observed mitochondrial dysfunction. In normal prostate samples, they recorded decreased expression in a marker for complex 1 function, with aging. These results were consistent in samples of BPH pathogenesis, suggesting that BPH pathogenesis in mice mirrors that in humans.

The group hypothesized that the accumulation of senescent cells leads to fibrosis in the prostate. Fibrosis is also associated with normal aging. A better understanding of this process could be applied for alleviation of BPH fibrosis. Dr. Liu is testing the use of pirfenidone to decrease collagen accumulation, remove senescent cells, and restore mitochondrial function. Future directions include further characterization of pirfenidone and mitochondrial function and identification of drugs to bypass complex 1 to restore mitochondrial function.

Segregating Urinary Phenome Responses to Microbes and Other Factors—New Opportunities
Chad Vezina, Ph.D., University of Wisconsin–Madison (Principal Investigator, U54)

Dr. Chad Vezina highlighted new opportunities related to the influences of microbes and other factors in urinary function. A wealth of data on inflammation and bacterial populations in the prostate has

been reported, but the association has not been characterized fully. Dr. Vezina noted that study of these dynamics in the prostate is challenging. Prostate bacteria likely require a unique approach.

Inflammation is associated strongly with progressive voiding dysfunction, and bacterial inoculation can lead to increased voiding frequency and pain development. Dr. Vezina emphasized that these linkages are challenging to model in the laboratory. For example, physiological endpoints are well developed in humans but are often not standardized in mice. Thus, drawing comparisons across studies often is not feasible. The void spot assay, however, allows investigators to quantify voiding behaviors in mice. Results are measured using analytical software. Dr. Vezina is working with other institutions to standardize and optimize endpoint assays. He explained that phenotype assays are useful for differentiating between experimental groups but often are ineffective as diagnostic tools.

Dr. Vezina's group has developed and validated a mouse model for prostate infection using pathogenic *E. coli*. Various factors—such as concentration and injection volume—were tested to optimize the model. The infected mice displayed increased spot voiding. Different types of *E. coli* and inflammation induce different patterns of voiding (e.g., obstructive, irritative). He emphasized the importance of capturing phenotypic heterogeneity in data analysis. Models of voiding dysfunction can be differentiated in the assay; a map of these patterns would help investigators pose interesting questions on this topic. Phenomics represents a valuable opportunity for urologic researchers to better understand the influences of underlying causes when analyzing outcomes.

Interactions of Commensal Urinary Lactobacilli with Urinary Pathogens
Tatyana Sysoeva, Ph.D., The University of Alabama in Huntsville (Co-Principal Investigator, K12)

Dr. Tatyana Sysoeva presented on interactions between commensal bacteria and urinary pathogens. She began by emphasizing the prevalence of UTIs and MDR in the United States. UTIs are caused by enteric bacteria (e.g., uropathogenic *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*). The bladder microbiome contains a variety of bacteria, including lactobacilli, and exhibits temporal and individual variation. UTI are associated with BU conditions and cancers. UTIs might depend on the urinary microbiome.

Dr. Sysoeva's group collected a repository of urinary bacteria using the expanded quantitative urinary culture method, including 27 lactobacilli strains from seven species. Lactobacilli acidify the environment, form hydrogen peroxide, and produce various bacteriocins, thus inhibiting the growth of other microbes. Using an inhibition assay, Dr. Sysoeva collected data suggesting that urinary lactobacilli maintain a wealth of interactions between urinary commensal bacteria and uropathogens, including multidrug resistant pathogens. Dr. Sysoeva highlighted future project outcomes: (1) establishment of functional interactions within the urinary microbiome and assessment of the potential impact of these interactions on UTI development, (2) analysis of the mechanisms of urinary bacterial interactions, and (3) possible detection of predisposition to recurrent UTIs and identification of novel approaches to prevent and treat UTIs via microbiome modulation.

Vaginal Intracellular Reservoirs in Recurrent Urinary Tract Infection
John Brannon, Ph.D., Vanderbilt University Medical Center (Postdoctoral Research Fellow, P20)

Dr. John Brannon presented on his research with Dr. Maria Hadjifrangiskou to explore the colonization of uropathogenic *E. coli* in the vagina. The researchers are interested in how *E. coli* colonize the vagina and migrate to the bladder. After reaching the bladder, *E. coli* establish a mature bacterial colony and exhibit cluster dispersal, creating intracellular reservoirs. Dr. Brannon tested the hypothesis that the vaginal colony invades vaginal epithelial cells, similar to the process observed in

the bladder. He characterized the invasion of epithelial cells, examined mechanistic factors, and gathered clinically relevant data in a pilot study.

In the study, bacteria were incubated at the epithelial cell layer of interest (e.g., bladder, vagina). Non-cell-associated bacteria were washed with phosphate-buffered saline and eliminated with an antibiotic. Dr. Hadjifrangiskou collected deidentified patient data with *E. coli* isolates, representing a wide range of disease severities. Invasion phenotypes were confirmed by confocal-scanning laser microscopy and transmission electron microscopy. Staining of vaginal cells has revealed colony rearrangement, reflecting the early stages of epithelial invasion. Drs. Brannon and Hadjifrangiskou also inoculated mice through bladder and kidney titers, creating infection that transfers to the vagina. During a 4-week period, they observed bacteria residing in the bladder, kidney, and vagina. Microscopic analyses indicate clusters of bacteria in vaginal epithelial cells. Next, Dr. Brannon analyzed clinical vaginal swabs from women with UTIs. Bacteria associated with epithelial cells were analyzed in the generation of a three-dimensional model. Topics for future study include mechanisms of invasion, cellular signaling, and long-term impact of bacteria on the cellular community.

Sensory Signal to Motor Action—from Bladder Fullness to Micturition Event
Anne (Hanneke) Verstegen, Ph.D., Beth Israel Deaconess Medical Center/Harvard Medical School (Principal Investigator, P20)

Dr. Anne Verstegen presented an overview of how bladder fullness signaling may lead to a micturition event. Neurons in the pontine micturition center (PMC) send direct excitatory axonal projections to the intermediolateral cell column (IML) and to interneurons in the dorsal gray commissure (DGC) in the spinal cord, hereby activating bladder and sphincter motor neurons. This reaction allows voiding to occur.

Dr. Verstegen's group uses single-cell transcriptome profiling to identify cell types and neurons involved in the neural control of bladder function. Ten distinct PMC neuron subpopulations were characterized in the analysis. The expression profiles of promising gene marker groups with respect to their localization near the PMC was determined, axonal projections of these neurons to the spinal cord were traced, and it was assessed whether the subpopulations play functional roles in regulating micturition behavior. Five subgroups remained that likely fit into the micturition network.

The activity of specific subpopulations of neurons can be manipulated by chemogenetic activation. Dr. Verstegen applied this approach while monitoring voiding behavior in mice with a heat-sensitive camera. Activation increased the frequency of voiding. Next, in vivo activity of select neuron groups correlates with bladder filling or voiding phases. Voiding events were identified from the bladder pressure trace. Her results show that specific excitatory neurons promote micturition. The information exchange between the bladder and brain was also measured; spinal cord neurons send axonal projections to periaqueductal gray matter (PAG) in the midbrain. Neuronal activity in PAG neurons correlates with bladder pressure; neurons are activated upon bladder filling, and activity decreases upon bladder voiding. In contrast, no changes in activity were reported during non voiding contractions.

Using electrophysiological recordings, they demonstrated that sensory-signal-receiving PAG neurons interface with the pontine micturition center (PMC). Inhibitory and excitatory PAG neurons innervate post-synaptic PMC neurons, hereby regulating activity in the latter neurons and preventing or allowing (respectively) voiding to occur. Future directions include transcriptome profiling of the PAG region, and further functional characterization and mapping of subpopulations, to better understand sensory signaling in the brain.

Never in the Urinary Tract—Causing Urinary Tract Malformations: The Case of TBX6
Greg Whittemore, Columbia University (Medical School Student, P20)

Mr. Greg Whittemore presented congenital anomalies of the kidney and urinary tract (CAKUT), which display diverse phenotypes (e.g., kidney malformation). Some CAKUT phenotypes are more common than others, and patients can experience multiple phenotypes simultaneously. Genetic drivers of CAKUT are categorized as point mutations or structural variants. A classical three-step paradigm has been developed for gene identification in humans: (1) unbiased, hypothesis-free genetic study to localize a gene or region of the genome associated with the phenotype; (2) candidate gene selection and prioritization; and (3) generation of a vertebrate model that recapitulates the human phenotype.

Using a modification of this approach, the group identified the chromosome 16p11.2 microdeletion syndrome as a cause of CAKUT. They performed deletion mapping to identify potential phenotype drivers; many genes appear to be involved. They identified *Tbx6* for further study; this gene is involved in early development, and truncating mutations are associated with congenital scoliosis. They examined mutations in an existing *Tbx6* mouse model. Severe reduction in *Tbx6* gene dosage causes CAKUT with complete penetrance. Additionally, milder mutations recapitulate CAKUT phenotypes in 16p11.2 deletion, including obstructive uropathy and duplicated ureters. Analysis of the lower urinary tract for CAKUT phenotypes identifies anorectal malformations. The mechanism of mutation, however, is not fully understood. *Tbx6* appears to be critical for insertion into the cloaca. *Tbx6* dose reduction results in ectopic neural tubes in place of posterior somites.

Mr. Whittemore highlighted major conclusions: (1) *Tbx6* gene dose reduction causes all categories of human CAKUT observed in the microdeletion syndrome, (2) *Tbx6* is expressed in peri-cloacal mesenchyme but never in the developing ureter or kidney, and (3) different mechanisms can explain the causal role and pleiotropy (e.g., failure of nephric duct insertion, ectopic neural tubes, failed or incomplete cloacal septation). He plans to conduct studies aimed at understanding the transcriptional dysregulation resulting from *Tbx6* mutations and identification of potential intervention targets.

Specification of Urothelial Cell Types During Homeostasis and After Injury
Cathy Mendelsohn, Ph.D., Columbia University (Co-Principal Investigator, U54)

Dr. Cathy Mendelsohn discussed ongoing work on urothelial cell type specification under different physiological conditions. The urothelium includes several populations of cell types with distinct functional roles. The K14 cell population is capable of *de novo* regeneration. Dr. Mendelsohn explained that atypical phenotypes arise following UTI, including upregulation of biomarkers typically associated with other organ systems. She hypothesized that the basal cells are being reprogrammed during this period. The immune response to UTI also might contribute to this effect. Mitochondria express large occlusions following UTI, which might suggest carnitine downregulation.

Dr. Mendelsohn found that the basal cells could be reprogrammed for differentiation. She emphasized that this pathway could be applied to the repair of the urothelial epithelium. Activation of the basal compartment occurs following repeated or severe injury. She is designing an experiment to examine the effects of gamma agonists on different cancer models. In this study, her group found that normal basal populations can be used for epithelial repair. The basal cells could be used as progenitors and reprogrammed as needed to restore urothelial defects. The group also is working to characterize involved pathways (e.g., retinoid signaling). Dr. Mendelsohn noted that all the animal models developed for these studies are freely available. She expressed her interest in assisting the community through section staining, antibody use, phenotype analysis, and testing.

Discussion Points

- Blood pressure is monitored continuously during nanoparticle treatment. Because NO has a short half-life (i.e., minutes) and the nanoparticles are applied topically, a systemic effect on blood pressure is unlikely. Large doses applied to the abdomen, however, might impart an effect.
- The nanoparticles contain nitrite. In early designs, NO was released enzymatically upon exposure to an aqueous environment. In more recent generations, NO has been stored in nitrosothiols and released spontaneously.
- Dr. DeFranco explained that the pathologists perform molecular analyses of BPH samples. He expressed interest in examining mitochondrial DNA damage.
- Nicotinamide is an inhibitor of sirtuins and thus will affect the lysine acetylation status of histones and other proteins. Dr. Liu expressed interest in further addressing this topic.
- Dr. Vezina commented that numerous factors that influence voiding function should be considered; the use of multiple platforms and approaches would be valuable.
- Bacterial invasion occurs through the vagina and the cervix in mice. Dr. Brannon stated that he plans to perform an extensive analysis comparing vaginal cells of women with UTIs (i.e., with varying degrees of severity) and those without UTIs in a future study.
- Dr. Brannon stated that he is unsure how *E. coli* enter vaginal epithelial cells. Pharmacological analyses provide preliminary insights, but the interactions between cells are not fully understood. He currently is pursuing work in this area.
- Dr. Verstegen speculated that the sensory control of bladder function is weakened with aging; mechanistic studies are needed to address this topic.
- The GU tract in *Tbx6* mice resembles the imperforate anus, with the rectourethral fistula in the expected location and an absence of the anterior urethra.
- Dr. Mendelsohn stated that a peroxisome proliferator-activated receptor gamma (PPARG) agonist could be used to promote repair of barrier function in bladders with disease or aging. PPARG is controversial but is approved by the U.S. Food and Drug Administration and likely would not require continuous dosage.
- Dr. Mendelsohn stated that she has not examined autophagy but noted that this would be a useful approach.

CONCLUDING COMMENTS AND ADJOURNMENT

Christopher Mullins, Ph.D., NIDDK, NIH; Tamara Bavendam, M.D., M.S., NIDDK, NIH; and Kristina Penniston, Ph.D., UW–Madison

Dr. Penniston expressed appreciation to the speakers and noted that presentation slides will be posted on the meeting website. She reminded the attendees that CAIRIBU is an umbrella organization composed of multiple centers and programs funded by the NIDDK. She remarked that the meeting was highly successful and highlighted the breadth and depth of discussion during the poster session.

Dr. Penniston thanked the meeting program committee, the abstracts and poster session committee, and the trainee event organizing committee. She recognized Drs. Mullins and Bavendam for their efforts. She expressed hope that an in-person meeting will be possible next year, which would allow interactive sessions and activities.

Dr. Bavendam expressed appreciation to Dr. Penniston for her efforts in planning and conducting the meeting and thanked the attendees for their engagement. Dr. Mullins commented that CAIRIBU reflects a team effort. He expressed appreciation for the attendees' input, particularly from young investigators and trainees. The next CAIRIBU Annual Meeting is planned for December 1–3, 2021, at a location to be determined. The meeting was adjourned at 5:31 p.m. EST.