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Meeting Report

Current research and future directions in non-malignant urologic research - proceedings of the annual CAIRIBU meeting

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Abstract: The Annual Collaborating for the Advancement of Interdisciplinary Research (CAIRIBU) Meeting in 2022 highlighted basic, translational, and clinical non-malignant urology research within five main areas affecting the urinary tract: urinary dysfunction due to prostate disease, microbes and infection, bladder function and physiology, neurology and neuromuscular influences and calculi and obstruction. In this paper, we summarize main findings and future directions outlined by CAIRIBU-affiliated scientists who presented as part of the scientific sessions.

Keywords: Benign urologic research, prostate, bladder, urogenital tract

Introduction

The annual meeting of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded group of U54 Urology O'Brien Centers, P20 Urology Centers, and K12 Urology Career Development Programs took place in December 2022. Known as CAIRIBU - Collaborating for the Advancement of Interdisciplinary Research in Benign Urology - the annual meeting is one of the primary mechanisms for exchanging knowledge about the non-malignant genitourinary research in which CAIRIBU investigators are involved. CAIRIBU was formed in 2018 by Program Officers with the NIDDK (see accompanying commentary paper in this issue for further details). A primary objective was to stimulate interactions between investigators leading to cross-disciplinary research that may be better able to address current knowledge gaps in the function and physiology of the genitourinary system and lower urinary tract. The meeting is organized each year by the CAIRIBU (U24) Interactions Core (PI, Kristina Penniston, PhD) in the Department of Urology at the University of Wisconsin School of Medicine and Public Health. This meeting brief provides a summary of the science presented by CAIRIBU-affiliated trainees and investigators at the 2022 CAIRIBU meeting.

Lower urinary tract symptoms/dysfunction (LUTS/D) and prostate

LUTS/D as a consequence of aging

LUTS/D related to non-malignant pathological changes in the prostate, also known as Benign Prostatic Hyperplasia (BPH), substantially deteriorate the quality of life of aging men. Most bothersome symptoms include nocturia, urgency and incomplete emptying, but BPH often leads to acute urinary retention with the inability to urinate and the need for long-term catheterization [1]. Age is the single most important risk factor in LUTS/D which was the focus of the research presented by Scott Bauer MD, ScM, Assistant Professor and former K12 Scholar at University of California San Francisco. Mitochondrial dysfunction has been associated with aging; patients with inherited mitochondrial diseases have significantly higher rate of urinary urgency, weak stream, incontinence and incomplete emptying [2, 3]. Dr. Bauer's research aims to elucidate whether mitochondrial content, protein expression and bioenergetic profile dif-

fer in the prostates of BPH patients and healthy individuals. He is also developing a prospective cohort of men and women to assess the association of symptom development and muscle function.

The role of resident and infiltrating cells in prostate disease

It is currently accepted that the prostate undergoes multiple pathological changes, including growth of epithelial and stromal nodules, smooth muscle dysfunction, inflammation and fibrosis [4, 5]. Research has been traditionally focusing on enlargement and smooth muscle physiology, whereas inflammation and fibrosis are understudied and pharmacologically untargeted. Fibrosis is the result of a developing imbalance between extracellular matrix (ECM) deposition and degradation with the former becoming progressively dominant over time [6]. Chronic inflammation has been recognized as the main driver of fibrosis in multiple diseases [7]. The dominant immune cell types in the prostate are T-cells and macrophages [8] and the mechanism attracting these cells to the prostate and their role in disease pathogenesis are important questions motivating the current goals of the non-malignant prostate research community.

Via sampling epithelial and stromal BPH nodules as well as normal prostate areas from the peripheral zone, the team of Jonathan Pollack MD, PhD at the Stanford University U54 Urology O'Brien Center (PI, James Brooks, MD) identified that chemokine (C-X-C motif) ligand 13 (CXCL13) expression was highly upregulated in BPH fibroblasts. CXCL13 expression was also associated with T- and B-cell infiltration [9]. Their future work will focus on B- and T-cell receptor repertoire to aid in identifying specific adaptive immune responses as well as to characterize T-cell subsets and the molecular drivers of CXCL13 expression.

The research aims of Petra Popovics, PhD (Assistant Professor at Eastern Virginia Medical School and formerly a K12 Scholar at UW-Madison) are to identify the prostate immune landscape that develops in response to steroid hormone imbalance in a mouse model replicating hormone levels in the aging human prostate [10]. Interestingly, she observed an increase in macrophages but not in T-cells, as well as the migration of macrophages to the prostate lumen differentiating into lipid-accumulating cells aka "foam cells". Foam cells were also observed in human BPH tissues in association with regional lipid accumulation sites. Future goals of the Popovics Lab will be to identify what cell types are the main sources of foam cells, what mechanisms regulate macrophage migration to the luminal space and which factors drive lipid accumulation in LUTS/D.

Studying the hallmarks of prostatic fibrosis in the human prostate and identifying which prostatic cell type is responsible for this pathological change have been the main goals of Chad Vezina, PhD (Co-Director of the UW-Madison U54 Urology O'Brien Center). His laboratory identified that collagen density in the prostate increases near the urethra only in BPH patients where it is most likely to drive urinary symptoms. Using lineage tracing with the Cre/lox technology, his team also showed that myeloid cells and fibroblasts are the major source of collagen deposition. Dr. Vezina's future work will focus on identifying molecules that attract pro-fibrotic myeloid cells to the prostate.

The work of Jill Macoska, PhD (Professor at the University of Massachusetts Boston and Project Leader in the UW-Madison U54 Urology O'Brien Center) has been focusing primarily on how fibroblasts become ECM-producing factories and promote prostatic fibrosis. By investigating cytokines that are upregulated in prostate fibroblasts in aging men, her group at the University of Massachusetts has identified a significant increase in CXCL12 expression [11] which drives conversion of fibroblasts to myofibroblasts, a transition that results in increased production of collagen I [12, 13]. Moreover, the use of an antagonist of the CXCL12 receptor (CXCR4) ameliorated high fat diet-induced periurethral collagen accumulation and urinary dysfunction [14]. In contrast, her most recent studies have shown that the interleukin (IL) 4/IL13/signal transducer and activator of transcription 6 (STAT6) axis is also pro-inflammatory in the prostate, but it does not transform fibroblasts to myofibroblasts. Rather, the stimulation of the pathway generates activated fibroblasts that preserve their proliferative potential [15]. Future directions of the Lab include studying fibroblast chemotaxis and characterization of fibroblast plasticity and resistance to apoptosis in response to IL4 and IL13.

Specimen and data resources are spare in non-malignant prostate research in part due to surgeries not prioritizing the preservation of tissue integrity and the lack of normal "healthy" tissue that could be utilized as controls. Douglas Strand, PhD, Associate Professor at the University of Texas Southwestern Medical Center and Project Leader in the UW-Madison U54 O'Brien Center has developed a prostate biorepository utilizing prostatectomy specimens and normal prostates from organ donors with associated patient data that now fuels his and his collaborators' research. In addition, using single-cell RNA sequencing (scRNAseq), his lab identified the large diversity across epithelial (basal, luminal, club, hillock cells) and stromal cells (peri-epithelial and interstitial) in the prostate [16-18]. Future directions in his lab include providing a comprehensive hierarchical definition of all human prostate cells, a human bladder cell reference atlas as well as to create a multi-omic analysis of the lower urinary tract.

Imaging fibrosis in the prostate

As the pathological diversity of BPH is becoming more and more apparent, the clinical implementation of novel diagnostic strategies identifying pathological features has become a crucial goal in non-malignant urology research. Shane Wells, MD, an Opportunity Pool Award recipient from the UW-Madison U54 Urology O'Brien Center, tackles how gross pathological abnormalities in the prostate can be visualized by imaging techniques. He uses elastography, which is an image-based, quantitative form of palpitation involving low-frequency sounds waves transmitted into the tissue followed by their detection. The stiffness map presented by Dr. Wells outlined how increased stiffness in the prostate transitional zone can be visualized. He also identified that the thickness of the fibrotic capsule increases with prostate volume. Dr. Wells' collaboration with Alejandro Roldán-Alzate, PhD (also of the UW-Madison U54 Urology O'Brien Center) aims to characterize bladder emptying during voiding using dynamic MRI. Preliminary results are pointing to a urethral opening dysfunction in BPH patients.

Microbes and infection in the genitourinary tract

Urobiome composition in children in health and disease

Recent innovations in collection techniques and the launch of next generation sequencing for the identification of microbial strains have allowed the detection of uncultured microbial strains and led to the confirmation of a urobiome in 2014 [19-21]. Interestingly, about 60% of urogenital microbiome strains are shared with the gut and 30% with the vagina in women while various unique elements are found only in the urobiome [22]. Although increasingly more data are becoming available deciphering the adult urobiome, research in children is far less developed. Characterization of the healthy urobiome in infants is the goal of Seth Reasoner, a graduate student at Vanderbilt University in the laboratory of Maria Hadjifrangiskou, (PI of the Vanderbilt P20 Exploratory Center). The team has developed a collection strategy by targeting infant males (4-6 month) undergoing circumcision and catheterization. They combined blood or brucella agar urine culturing with MALDI-TOF-based identification and 16S rRNA sequencing. Importantly, they included saline flushed through the catheter as a control. This strategy has identified *Actinotignum schaalii* as a predominant strain in the healthy infant urinary tract. Going forward, the group is preparing to make their investigation publicly available and searchable and hope to utilize their standardized method to track age-related changes in urobiome composition. Maryellen Kelly, DNP, MHSc, a former K12 Scholar from Duke University, assessed urobiome composition in children up to five years of age with sequencing achieving species-specific resolution. Her team concentrated samples with vacuum filtration to overcome low biomass in the samples. They found that diversity was associated with age and with female sex and identified the predominance of Prevotella, Schaalia and Fusobacterium genera. Their future goal is to investigate the stability of the pediatric urobiome over time and compare the microbiomes of children with or without recurrent urinary tract infection (UTI). A more disease-focused approach was utilized by Miguel Verbitsky, PhD, from the lab of Ali Gharavi, MD (one of the PIs of the Columbia University U54 Urology O'Brien Center), who compared children with or without vesicoureteral reflux (VUR) who had UTIs. Using 16S rRNA sequencing, they identified that urobiota diversity was higher in children who had UTI but no VUR and that microbial diversity correlated with age. They performed a metabolite genome-wide association study (mGWAS) to investigate the relationship between genetic factors and the

urine metabolome. This analysis highlighted potential involvement of host genes associated with immune surveillance, inflammation and genitourinary tract development and diseases with the abundance of certain bacterial taxa.

Mechanism of colonization during urinary tract infections UTI

Although our knowledge on the existence of uropathogenic microbes preceded the discovery of the urobiome, research still falls short of eradicating these infections. UTI is diagnosed in 150 million people worldwide and in 7 million in the U.S. annually, whereas 15% of all antibiotic prescriptions are prescribed for UTI treatment. The majority of UTI cases involve uropathogenic *E. coli* (UPEC) strains [23]. Maria Hadjifrangiskou, PhD at Vanderbilt University (P20 Center PI) focuses on how *E. coli* is internalized in urothelial cells, how it develops intracellular biofilms/populations and what stress this imposes on the host cell. She discovered that in the *E. coli* biofilm, bacterial proteins, including quinol oxidase complexes, are spatially organized and coordinated by oxygen gradient [24]. The team identified that bacteria utilize the host cell's oxygen which leads to increased HIF-1α level thereby suppressing apoptosis and shifting the cells to glycolysis. Dr. Hadjifrangiskou's future studies will focus on understanding the role of HIF-1α in the progression of UTI. Tatyana Sysoeva, PhD, an Assistant Professor at the University of Alabama in Huntsville (and a former Duke University K12 Scholar) is studying horizontal gene transfer and multidrug resistance genes carried on the bacterial F-plasmid [25]. Her team showed that the bivalent action of the TraT factor prevents conjugation-related plasmid transfer, but also protects from phages, some antibiotics, detergents and serum complement. Her lab also discovered that TraT forms oligomers and interacts with bacterial outer membrane proteins. The team's future studies will decipher the role of TraT in UPEC serum resistance.

During a UTI, it is vital for the bacteria to capture iron from the host, which is needed for DNA replication and metabolism. Sources are elemental iron that are taken up by siderophores or via the recovery of iron from heme, transferrin or lactoferrin from the environment [26]. Jonathan Barasch, MD, PhD, one of the PIs of the Columbia University U54 Urology O'Brien Center, studies bacterial iron uptake during UTI. He has identified that bacteria cause damage in the epithelium which releases iron that in turn acts as a bacterial growth factor. Dr. Barasch has also discovered that the urothelium expresses NGAL upon bacterial infection, which blocks the transit of iron to bacteria by inhibiting bacterial siderophores [27]. Hematuria is also a hallmark of UTI which activates Heme-associated proteins and a defense mechanism against iron in the urothelium. The heme transporter, Slc48a1, is induced in the urothelium in a temporally different manner in female and male mice whereas HMOX1 splits heme intracellularly. Knocking out the heme transporter is protective in UTI, according to Dr. Barasch, due to the toxic effect of iron-induced ferroptosis, necroptosis and apoptosis.

Macrophage diversity in the bladder

Describing the normal macrophage populations and their change during the UTI immune response has been the focus of Nicholas Steers, PhD, an Opportunity Pool Award recipient from the Columbia University U54 Urology O'Brien Center. For a long time, it has been accepted that tissue resident macrophages are self-maintained and independent of hematopoietic cells, however, this dogma has shifted most recently [28, 29]. Hematopoietic stem cells/monocytes arrive from the yolk sac, fetal liver and bone marrow, depending on the stage of development, to compose and replenish the tissue resident macrophage population [30], however, this process has been understudied in the bladder. Using a deep phenotyping flow cytometry panel for macrophages, Dr. Steers identified five subpopulations of macrophages. More interestingly, he detected substantial changes in two subpopulations in response to UTI. His future goals include identifying the origin of macrophages via lineage tracing, studying the proliferative potential of tissue resident macrophages and their replenishment from monocytes.

Bladder function and physiology

Mechanical modeling of the bladder

The urinary bladder's simple mechanistic role is to store and empty urine. These functions are coupled to a highly complex tissue architecture involving a longitudinal and a circular smooth

muscle layer (detrusor), a mucosa and a specialized epithelium known as the urothelium. Mechanical properties of bladder filling have been the major goal of Eli Broemer, a graduate student in the Lab of Sara Roccabianca, PhD at Michigan State University. He used an imaging technique developed in the laboratory of Nathan Tykocki, PhD (also at Michigan State University), which registers bladder filling simultaneously using mirrors from different directions, and extracted a 3D model using spatial carving. From this, he modeled the volumetric change over time and associated it with the change in pressure. Comparing male and female mouse bladders, the study determined that bladder wall stress increases rapidly when the bladder reaches capacity and that males had higher stress in the bladder wall than females. Also, he identified that the bladder stretches more in the circumferential than the longitudinal direction i.e. possesses an anisotropic behavior.

Urothelial changes in injury, diabetes and aging

The urothelium exhibits a crucial role in allowing repeated stretching of the bladder during filling, but also provides an important barrier function against pathogenic microbes, toxins and carcinogens [31, 32]. Superficial/umbrella cells have a long life-span, exist as polyploid cells and are replenished from basal cells [33]. The research of Cathy Mendelsohn, PhD (one of the PIs of the Columbia University U54 Urology O'Brien Center) showed that basal cells can undergo transformation/activation during exposure to chemical or infectious agents and acquire squamous markers, leading to the loss of superficial cells. This process can deteriorate the permeability barrier and predispose to carcinogenesis. The Mendelsohn Lab also tackles the role of *Pparg* in urothelial cell differentiation; *Pparg* differentiates basal cells to superficial cells, whereas *Pparg* deletion results in squamous metaplasia [34]. They revealed that *Pparg* is required to shut down NFκB signaling its deletion resulting in continuous immune cell infiltration. Interestingly, deletion of *Pparg* in superficial cells makes neutral lipids become trapped in the mitochondria intermembrane space due to defective fatty acid transport [34]. Future studies of the lab will assess the potential beneficial activities of *Pparg* agonists in repairing the urothelium after recurrent UTI. Apart from external stimuli, aging is an important internal factor that can lead to the deterioration of bladder tissue structure and function [35]. Indira Mysorekar, PhD at the Baylor College of Medicine studies the hallmarks of aging in the urothelium. Her earlier work identified an increase in tertiary lymphoid structures [36]. More recently, using transmission electronmicroscopy, her team showed the accumulation of lysosomes, mitochondria with reduced activity, a decline in autophagy, elevated senescence and cell damage pathways, DNA damage and increased cell cycle arrest in the aged mouse bladder. The aged bladder had constitutively high level of reactive oxygen species (ROS) and deficient antioxidant response and was more susceptible to intracellular biofilm formation of UPEC. The future work of the lab focuses on testing the beneficial effects of D-mannose on reducing the observed age-associated changes in the urothelium. Bladder dysfunction is a common complication in diabetes (diabetic cystopathy) that may persist even with optimal glucose management; therapeutic options are sparse [37]. Michael Odom, PhD, works as a K12 Scholar in the Purves Lab at Duke University. He focuses on detrusor underactivity in diabetes. It is believed that diabetic metabolites activate NLRP3 inflammasome-related pyroptosis in the urothelium providing direct contact of urine with the lamina propria and the smooth muscle layers [38, 39]. Using the Akita diabetes mouse model, the team showed that NLRP3 is required for the development of increased voiding volume and decreased frequency associated with the underactive voiding phenotype [40]. Dr. Odom's current work focuses on testing the utilization of the Prostaglandin F2α receptor (FP) in increasing the contractility of underactive detrusor, and his future work will focus on studying FP receptor populations and assess FP receptor smooth muscle signaling.

Genetic studies in non-malignant urology

Urological diseases, including bladder dysfunction, may have underlying association with an individual's genetic composition. According to Ali Gharavi, MD, one of the PIs of the Columbia University U54 Urology O'Brien Center, genetic variance may affect 5-10% of patients with congenital urinary tract disorders. Congenital defects associated with genetic alterations that were identified by the group include vesicoure-

teral reflux, duplicated ureter, multicystic dysplastic kidneys and unilateral renal hypoplasia and can be linked to microdeletion syndromes or copy number variations [35, 41, 42]. Interestingly, the examination of common alleles in vesicoureteral reflux identified the involvement of developmental genes (eg. WNT5a, BMP5) whereas defective bladder and ureter development was observed in WNT5a KO mice further confirming the association [41]. Going forward, the utilization of available biorepositories for genomics research, including the All of Us Research Hub that includes many benign urological conditions such as urinary incontinence, retention and UTI, will accelerate discoveries in genomics research of other benign urological diseases, including the bladder.

Utilizing machine learning in cystoscopy analysis

Bladder trabeculation has been recognized as a surrogate marker for bladder function which correlates with urodynamic findings [43]. Ramy Goueli, MD, and his team at UT-Southwestern aim to develop a machine learning strategy using convoluted neural network, which is a class of deep neural networks most commonly used to analyze visual imagery. Dr. Goueli's team performed a retrospective chart review on non-neurogenic male urology patients presenting with LUTS. Cystoscopy videos were graded by two urologists for trabeculation and still images were used to create an algorithm for the presence and grade (none, mild, moderate, severe) of bladder trabeculations [44]. Studying only 27 patients, they acquired 76% accuracy for determining trabeculation grade with this technique. Due to the heterogeneity of bladder trabeculations within individual patients, the methodology was changed to patch-based labeling. This method is currently in testing to improve the detection of severe trabeculations. When the group develops satisfactory automated detection and grading of bladder trabeculations they will use the method to correlate grades with clinical measures of bladder function and also employ similar techniques in the neurogenic bladder population.

Neurology and neuromuscular influences on the urinary tract

Mast cells in neurogenic bladder

Neurogenic bladder can develop when there is a disruption between the CNS and the somatic nervous system [45]. Transient contractions and the rate of pressure increase in the bladder affect sensory outflow and contribute to how bladder fullness is sensed [46]. Organization and composition of the ECM also influences the rate of smooth muscle contractility and drives sensation. Pragya Saxena, a graduate student from the Tykocki Lab at Michigan State University, tackles the role of mast cells in smooth muscle contractility in the bladder based on previous studies showing increased mast cell numbers in interstitial cystitis [47]. Using the Pentaplanar Reflected Image Macroscopy System, she showed that the mast cell activator compound 48/80 increased the amplitude and the slope of contractions. This effect was inhibited by a cyclooxygenase inhibitor that decreases prostaglandin levels. Her future research will identify the re-arrangement of the ECM in the bladder after mast cell activation, and identify prostaglandins that play key roles in this model. B. Malique Jones, also a graduate student from the Tykocki lab, studies how inflammatory factors can induce detrusor overactivity, mimicking neurogenic bladder, independent of afferent and efferent nerve signaling. She determined that the presence of urothelium in the tissue bath is required for compound 48/80 to increase contractility and phasic activity in smooth muscle and to augment nerve-evoked (electrical field stimulation) contractions. Using specific inhibitors, she determined that this was due to ATP and prostaglandins released from the compound 48/80-treated urothelium.

Neuron cross-talk in pain sensation

The work of LaTasha Crawford, VMD, PhD, DACVP (K12 Scholar at UW-Madison) targeted two questions: what if sensory neurons from the bladder can communicate with sensory neurons from the skin? And can these neuron groups cause hyperactivity of others in the same or nearby ganglia? She studies changes in the dorsal root ganglia (DRG) where diverse sensory neurons from different organs may interact [48]. Interestingly, she showed that the sural dermatoma of the foot becomes hypersensitive in the intravesicular acrolein model of cystitis, implying a neuronal cross-talk in DRG. Moreover, while voiding spot frequency resolved, allodynia persisted even after 8 days. The group has also developed an *ex vivo* skin-ganglion-cord preparation for neurophyisiology

assays, an image deconvolution and digital clearing method to visualize DRG neurons and their calcium signaling, whole mount staining of the bladder and used genetic strategies to label afferent neurons in mice. The Crawford Lab is also working on building a comparative neuro biobank of dogs, cats, and horses with dorsal root ganglia preparations and innervated tissues.

Environmental toxicants in bladder function

Kimberly Keil Stietz, PhD, an Assistant Professor at the UW-Madison and Project Leader for the U54 Urology O'Brien Center studies the role of polychlorinated biphenyls (PCBs), manmade compounds that were previously produced for chemical stabilizers, heat dissipaters and paints but which now occur as biproducts of paint production. PCBs affect dendrite morphology, elevate calcium signaling in the brain and cause inflammation [49, 50]. Dr. Keil Stietz's team exposed mice to a mixture of PCBs starting with the mother prior to mating and terminated the exposure at the time of weaning. They observed increased voiding frequency in both females and males, decreased intervals between voids in females and increased maximal bladder pressure in males. She concluded that PCB-enhanced bladder contractility in response to cholinergic stimuli may be partly due to increasing bladder nerve density [51]. Future studies in her lab and in her research related to the Opportunity Pool Award she received from the Stanford University U54 Urology O'Brien Center will assess how sensory function is altered, how inflammation is involved (increase in mast cells, macrophages), and how PCBs change neural activity within the Pontine micturition center.

Physical training for urinary incontinence (UI)

General factors contributing to UI include obesity, autonomic instability, bladder muscle over- or underactivity and pelvic floor weakness. With the onset of aging, additional factors, such as physical function and cognitive decline as well as frailty will also provoke UI events [52, 53]. Allison Huang, MD, (co-PI of the UCSF-Kaiser Permanente K12 Urologic Epidemiology Institutional Career Development Program) is interested in whether behavioral interventions that improve overall skeletal muscle, autonomic and general physiological function can prevent or reverse UI. Yoga is an ideal candidate for the development of such intervention because it may incorporate mindful awareness and improved control of pelvic floor muscles as well as improved overall physical conditioning. Moreover, breathing exercises increase peripheral balance and decrease detrusor muscle instability. The team hypothesized that the practice of yoga would lead to an overall increase in continence-related functioning and the improvement of quality of life. To test this hypothesis, Dr. Huang's team developed a multicenter randomized trial to implement a therapeutic yoga program for UI in older women, the "Lessening Incontinence with Low-Impact Activity (LILA)" trial. It identified that the specialized yoga training significantly decreased UI events more than a regular physical conditioning program in just three months. Future studies of the team will investigate factors that mediate these beneficial effects, explore differences across age groups and in-person vs. virtual training as well as monitor the persistence of improvements.

Calculi and obstruction in the urinary tract

Improving urolithiasis diagnostics with machine learning

The Center for Machine Learning in Urology is a CAIRIBU P20 Exploratory Center (PI, Greg Tasian, MD, MSc, MSCE, University of Pennsylvania Perelman School of Medicine and Children's Hospital of Philadelphia [CHOP]). The Center has been exploring machine learning techniques to predict urologic outcomes and to aid diagnosis [54-57]. In the first of two talks from the Center, Yuemeng Li, a graduate student in the Center for Biomedical Image Computing and Analytics at the Perelman School of Medicine, described the development of a transformer model of deep learning to automatically identify and measure kidney stones on CT scans, the result of which would mean more efficient, accurate, less burdensome, and standardized quantification of stones within the urinary tract. The first task was image segmentation for which urologists manually reviewed >100 CT urograms from patients of different sizes and ages and manually annotated areas of interest. Machine learning experts then designed a predictive stone mapping model based on an atlas derived from

the annotated dataset. After the model achieved acceptable performance (Mean Dice Score 0.96), investigators began training the model to generate spatial maps of kidney stones from non-contrast CT scans of >100 unique patients [58]. Ultimately, the automated stone identification algorithm detected individual kidney stones with 100% sensitivity; spatial mapping (3D reconstruction) efforts to quantify the frequency of stones across subjects are underway.

Building on this work, Perelman School of Medicine medical student Abhay Singh described a machine learning project aimed at predicting ureteral stone passage, the result of which could mean earlier identification of patients likely to successfully pass their stones vs. requiring surgical intervention. A retrospective cohort study was assembled from >250 children and adults who presented to CHOP and University of Pennsylvania with ureteral stones, 48% of whom spontaneously passed their stones (44 and 54% of adults and children, respectively). Axial, vertical, and horizontal stone lengths were available from CTs. Other variables - BMI, hematuria, nausea, fever, flank pain, stone location, and others - were assessed. Random forest modeling, a type of supervised machine learning algorithm, was employed in an attempt to predict stone passage. The adult and pediatric models achieved 63% and 70% accuracy, respectively. Patient age and the orthogonal measurement product (the product of axial vertical and horizontal stone length) were the most predictive variables in both.

Mechanisms and safety of laser ablation in lithotripsy

Two investigators from the Center for Urological Laser Technologies at Duke University (a new CAIRIBU FORWARD Urology P20 Center; PI, Pei Zhong, PhD) delivered presentations related to laser lithotripsy (LL) in surgical stone removal. Ureteroscopic LL using a holmium: YAG laser (YAG = yttrium-aluminum-garnet) is a common procedure for vaporizing ureteral and renal calculi. Fragments may then be either removed with a basket or, if small enough, excreted painlessly by patients after the intervention. A deeper understanding of the mechanisms of LL will inform the development of new and more effective technology. John Dolbow, PhD, Professor in the Department of Mechanical Engineering and Materials Science at Duke University, described efforts in collaboration with investigators from Virginia Tech to understand the relative importance of the photothermal effects vs. fluid-stone interactions of LL. During lithotripsy there is usually continuous irrigation. In this milieu, the holmium: YAG laser pulse generates an elongated cavitation bubble (a type of fluid-stone interaction) that generates a weak shock wave. While conventional wisdom is that cavitation is a primary mechanism for inducing damage to the stone [59], Dolbow described bench top experiments and mathematical modeling that demonstrate an effect of thermal ablation. Because the kidneys are susceptible to thermal injury during LL, his work supports concern about the energy released by the lasers as well as the frequency of pulses delivered [60].

Michael Lipkin, MD described a new type of laser being used during ureteroscopic LL that vaporizes stones to dust, the thulium laser [61]. Stone dusting takes advantage of the much higher pulse frequency that is achievable with new lasers [62], especially the thulium. However, its mechanism of stone ablation is thought to differ from the traditional holmium: YAG laser. Because it is relatively new, the optimal thulium fiber settings for energy and frequency during stone dusting - i.e., those that appropriately balance efficiency and safety - have not yet been identified [63]. Lipkin presented data from porcine studies in which macroscopic tissue damage was observed in kidneys as a result of the heat from thulium laser energy [64]. With this knowledge, the group conducted bench top studies on both artificial and human stones. Results thus far suggest that, as compared to the holmium: YAG laser, the thulium laser should be set on higher energy but lower pulse frequency in order to avoid thermal-related injury. Questions remain about how close the laser tip should be to the stone during LL; this and other knowledge gaps will be addressed by the Duke P20 Center.

Oxalate metabolism in calcium oxalate urolithiasis

Kidney stones affect 1 in 11 Americans; there is increasing prevalence among women [65]. The most common type of kidney stone has some component of calcium oxalate. While multifactorial in etiology, urinary oxalate excre-

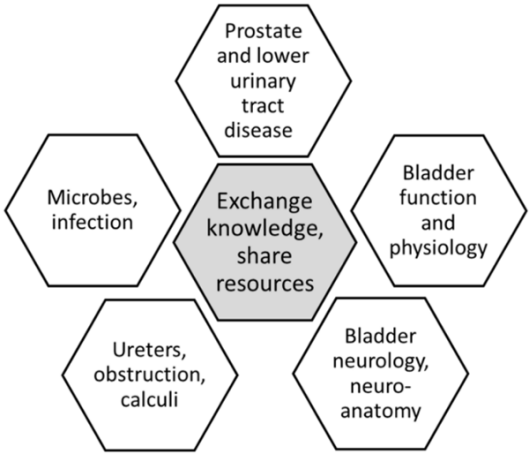

Figure 1. Main research areas discussed at the 2022 CAIRIBU Annual Meeting.

tion is a primary risk factor. Urine oxalate represents the sum total of exogenous oxalate (from the diet) as well as endogenous (i.e., that which is synthesized *in vivo* from precursors in both enzymatic and non-enzymatic pathways) [66]. Sonia Fargue, PhD, from the P20 Exploratory Center at the University of Alabama at Birmingham (PI, Dean Assimos, MD), said that endogenous oxalate pathways, depending on the individual, may account for a significant amount of urine oxalate [67]. She presented results from a clinical study in which intravenous infusions of carbon^{13} oxalate were administered to both stone formers and non-stone formers in order to measure oxalate synthesis. Urinary oxalate excretion was then examined. Compared to non-stone formers, stone formers with higher body mass excreted more oxalate in urine, even while on diets controlled for oxalate. Interestingly, lean mass but not fat mass was most associated with oxaluria in both patients and controls. Fargue thus suggested a larger than previously-thought role for muscle and bone (both are components of lean body mass) in oxalate synthesis. Weight-bearing muscles and bone are both rich in collagen. Fargue explained that the hydroxyproline produced by post-translational hydroxylation of proline residues in collagen is a glyoxylate precursor [68]. Glyoxylate is then converted to oxalate in a pathway catalyzed by lactate dehydrogenase. These data support the idea that endogenous oxalate synthesis has been previously underestimated and may account for a significant amount of the variability in the urinary oxalate excretion of patients who form calcium oxalate stones [67].

Conclusion

This year, CAIRIBU investigators reported progress in five major areas of non-malignant urologic research summarized in **Figure 1**. Recent clinical advances included the use of improved techniques in imaging, surgical management and implementing machine learning in analysis. Basic and translational tools have allowed the expansion of our knowledge on cellular composition and behavior as well as disease-driven molecular signaling in the lower urinary tract mainly in the aspects of inflammation, fibrosis and aging. CAIRIBU investigators are collaborating to provide resources (biospecimens, expressional and genetic databases, microbiome composition and cellular atlases) for the larger urology research community.

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Disclosure of conflict of interest

None.

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