National Institute of Diabetes and Digestive and Kidney Diseases

Advancing Basic and Translational Research for Genitourinary Conditions: Female Urethral Function and Failure

Virtual

Executive Summary and Key Points

Description

The National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) convened a virtual workshop series composed of seven weekly sessions on "Advancing Basic and Translational Research for Genitourinary Conditions: Female Urethral Function and Failure" from September 30, 2020, to November 18, 2020. The workshop was open to the public, and more than 200 participants logged in from 71 institutions around the world, with more than 100 attendees at each session. Physicians and researchers registered in nearly equal numbers, and approximately 20 percent of the registrants were early-career researchers.

The goal of this iterative workshop series was to generate a systems model that demonstrates what is known about the urethra's role in continence and identifies knowledge gaps that impede progress toward better treatments. Participants received, in real time, information on developing peer-reviewed publications, an iterative systems diagram, and an infographic to stimulate research ideas. The workshop planning committee—composed of basic, clinical, and translational scientists from the NIDDK, academia, and industry—moderated the sessions. Topics of the sessions were as follow:

1. The Urethra: What We Know, What We Don't Know, and Why It Matters
2. The Urethra: Current Knowledge of Urethral Function in Health and Disease
3. The Urethra: The Mucosal, Submucosal, and Sensory Aspects
4. The Urethra: Neural Control and Integration
5. The Urethra: Technologies for Translation
6. The Urethra: Lessons from Animals, Cells, and Children
7. The Urethra: Current Therapies and Future Solutions

Background

Enormous knowledge gaps exist in understanding the function and dysfunction of the lower urinary tract (LUT). To fill these gaps, the NIDDK Division of Kidney, Urology and Hematology (KUH) pioneered a workshop series to seek out a multiperspective brain trust, including basic, translational, and clinical researchers at all stages of their careers; health professionals; patients; and industry representatives. The topic—female urethral function and failure—encouraged workshop participants to share knowledge, ask questions, and engage in real-time and asynchronous discussions regarding this understudied and not-well- understood area.

A key aim of the KUH is to increase its portfolio of fundable investigator-initiated research grants (e.g., R01s) grants on genitourinary conditions within NIDDK's mission. Traditionally, the NIDDK has sponsored meetings and workshops held at the NIH to foster cross-disciplinary conversations, often leading to a workshop summary or the development of a research agenda for the topic, but these conversations often stop short of the granularity of solid research questions that are informed by adequate clinical perspective.

Over time it has become increasingly challenging for clinicians to take time away from clinical care to attend meetings. This virtual webinar series was piloted to determine if this series of seven webinars organized around a single topic could stimulate and sustain cross-disciplinary conversations that extend beyond institutional boundaries and could lead to solid research questions (and approaches) that will result in a larger R01 portfolio.

Summary of Key Points

What We Know, What We Don't Know, And Why It Matters

Session 1 addressed the evidence detailing the importance of the urethra in all forms of urinary incontinence (UI) and other types of disorders. A renowned expert in the field identified two main issues that provided the motivation for the workshop: (1) The urethra has been underappreciated as both a contributing factor to UI and as a potential therapeutic target, and (2) the efficacy of current treatments has plateaued, so seeking new therapeutic targets is

critical to improving patient outcomes. The NIDDK incorporated the patient's perspective on experiencing UI and its impact on overall health and well-being, which helped to frame the purpose of this important and timely workshop. Highlighted as issues for the patient were knowledge gaps and misdiagnoses. Progress and improvement in symptoms (e.g., a larger volume of fluid loss) were demonstrated using physical therapy and a whole-body approach.

Regarding what is known in the field, evidence suggests that the central dogma, a two-factor model of stress UI (SUI)—caused by poor urethral support from the pelvic floor connective tissue and muscles—and urge UI (UUI)—caused by detrusor overactivity—is oversimplified. A third common factor that has not been recognized; a "three-factor model" is an idea supported by the therapeutic ceiling effect seen for common UI treatments. Although current treatments targeting either the urinary bladder or urethral support are treating those parts of the problem, they fall short of addressing an underappreciated element of the disorder—namely, the failure of the urethral sphincter mechanism. In the absence of diagnostics and therapeutics targeting urethral failure, treatments using the two-factor model fail to achieve a higher level of success. Gynecologists and urologists were encouraged to model their clinical investigations based on the example set by cardiologists by first establishing a normal range, then identifying structural and functional abnormalities, and finally evaluating the results.

Workshop participants were informed that translation of pudendal neuromodulation and other therapies into the clinic could benefit from understanding who should get which therapy and why. An optimal patient-specific framework for therapy selection encompasses the identification of physiologic factors that indicate how well an individual patient will respond to a particular therapy. Clinicians treating patients with UI can use predictive modeling as a guide. Because UI is a multifactorial symptom, multiple physiologic variables should be examined simultaneously to improve a model's predictive performance.

Current Knowledge of Urethral Function in Health and Disease

Session 2 reviewed the current state of knowledge about the muscular wall of the urethra. Panelists described the biomechanics of striated muscle, noting the well-known force-length relationship and the fact that the traditional length-tension curve does not provide all necessary information about the dynamic force-length behavior of muscle. The force-velocity relationship of striated muscle is the approach used by many athletes for lengthening to achieve better performance, but can result in muscle injury. Such injuries are not a result of muscles working isometrically; therefore, women in the second stage of vaginal birth can avoid a stretch injury by not contracting pelvic floor muscles while attempting to push the baby out via the birth canal.

The idea of using a systems analysis approach was introduced to better understand not only how the central nervous system (CNS) controls the urethra, but also how the pelvic floor muscles provide support and all the other factors (CNS and others) influence urinary continence. A thorough systems analysis can help identify gaps in knowledge.

Isolated smooth muscle cells from animal models, specifically the rabbit urethra, revealed highly contractile smooth muscle cells and noncontractile interstitial cells similar in appearance to the gastrointestinal (GI) tract Cajal interstitial cells (pacemaker cells). Smooth muscle cells have been demonstrated to be electrically quiescent but excitable, whereas the interstitial cells were spontaneously active and developed rhythmic slow-wave events. This was similar to intact urethra, suggesting that these cells might be the source of the electrical activity that underpins urethral tone. Mechanistic studies suggest that TMEM16A calcium-activated chloride channel blockers are important regulators of urethral smooth muscle contraction and that the interstitial cells' inward currents were associated with the calcium waves, all modulated by neurotransmitters in the urethra. Species variation in the cellular distribution of TMEM16A channels in the urethral smooth muscle occurs, and the overall mechanisms in the human urethra remain unknown.

Regarding motor testing, a minimum evaluation of a patient with stress UI includes history, a physical, and urinalysis. Contribution of intrinsic muscle tone can be measured with urethral pressure profilometry and urethral tone plus support with leak point pressures. Several research tools are currently available, including urethral pressure reflectometry and the ManoScan catheter for high-resolution manometry. Envisioned is a dream technology that does not distend or stent the urethra and that will sense accurate pressures and resistance to flow circumferentially and along the length of the urethra, has a response time in the milliseconds, is comfortable enough to wear at home, and is easy to interpret for research and clinical use.

The Mucosal, Submucosal, and Sensory Aspects

Session 3 highlighted that afferent neurons in the urethra detect urine flow and the distension of the urethra, and the urethral epithelium likely plays a central role in monitoring distension and controlling urine flow. The urethral epithelium is likely central to many urethral functions. The proximal, middle, and distal portions of the urethral epithelium are different. The epithelium and subepithelial nerves send afferent information about urine flow and distension through the dorsal root ganglion, the sacral and lumbar spinal cord, the hindbrain, and, ultimately, the cortex.

Studies in humans have shown that the vascular supply contributes 28 percent of maximal urethral closure pressure and demonstrated that changing vascular flow in the lower limbs can affect UI. The causes of impaired urethral blood flow—atherosclerosis, hypertension, diabetes, aging, obesity, smoking, pelvic surgery, pelvic radiation treatment, and difficult vaginal delivery—are also common risk factors for urinary bladder dysfunction. Although limited, animal models—specifically the female rat, male spontaneously hypertensive rat, and female canine—all have provided insight into the vasculature of the urethra. Full understanding of disease pathology requires examination of the interchange between pelvic organs.

Steroid hormones are key drivers of urinary behaviors. In fact, hormone receptors are found throughout the urogenital tract and play a critical role during development. Researchers have found sex hormone receptors in the bladder and urethra of female humans, rabbits, baboons, dogs, and pigs. The overall receptor expression is highly variable, but androgen receptors are consistently at high to moderate levels in the urethral epithelium, and estrogen receptors are consistently expressed in smooth muscle.

Numerous preclinical studies have demonstrated an effect of oophorectomy and ovariohysterectomy on bladder histology and function, revealing no difference in the expression of estrogen receptor alpha (ER-α) compared with control animals, but the ER-α differentially expressed in different sections of the urethra. Oophorectomized mice lost the expression of urethral progesterone receptors but regained normal expression, which could be reversed with estrogen supplements. Therefore, oophorectomy in animals is used to model loss of estrogen in women, such as at menopause. Estrogen therapy for postmenopausal female UI patients remains controversial, showing improvement in some studies but similar or worsening effects with other therapies. These data suggest that this treatment should not be applied universally.

Improved understanding of urethral sensory innervation is needed to better understand disease states, apply prevention strategies, and target afferent pathways for treatment of UI. The urethra has been implicated in the development of SUI, and several urodynamic measurements—including the Valsalva leak point pressure test and the maximum urethral closure pressure test—demonstrate poor urethral function and have informed treatment algorithm designs. Therapeutic techniques aimed at afferent nerve pathways, such as sacral neuromodulation, have been successful in women.

Urethra sensory testing, particularly current perception threshold (CPT), evaluates afferent nerve functions and, routinely, peripheral neuropathy. CPT is the only technique available to quantify the functional integrity of specific afferent nerve fibers from the periphery to the CNS. Use of the Neurometer® constant-current stimulator in CPT can maintain and deliver a constant current despite differences in tissues.

Neural Control and Integration

In Session 4, panelists reported that neuromodulatory cells are embedded in the epithelium of the urethra but not of the bladder, and the rapid transition between the two areas could provide some boundary for the bladder neck. Gene expression differences between the epithelium of the bladder and urethra as demonstrated in the mouse model provide one way to assess the boundaries microscopically, which can contribute to better macroscopic definitions. Researchers can consider how communication between the bladder and urethra occurs and whether the bladder neck is involved in this coordination or acts independently. Understanding external nerve tracts and determining how the bladder neck innervation communicates with the rest of the bladder and the urethra is critical. Additional remaining considerations include what scientists can learn from comparisons across species and sexes and whether cell targets of LUT nerves should be expanded.

A neurophysiological view of the urethra through the lens of reflexes is what scientists are missing in treating women with incontinence and other LUT dysfunction. Urethral reflexes are part of an overarching phenomenon known as the micturition cycle. Several types of urethral reflex activity, named for the afferent and efferent arms, occur during the micturition cycle.

From a urethral reflex perspective, SUI often is associated with birth trauma and involves the denervation of the efferent arm of the vesico urethral guarding reflexes. Autonomic signals to the smooth muscle of the bladder neck are inadequate, resulting in urine leak during stress. Inadequate somatomotor signals to the striated external urinary sphincter muscle lead to weak contractions and result in leakage. UUI, which is attributed to the loss of cortical inhibition, also can be explained by failure of the urethro urethral reflex, which is one of the guarding reflexes. Weak external sphincter contraction sends a weak afferent signal to the spinal cord, and the resulting efferent signal to continue contraction of the sphincter will be compromised, as well. The clinical evidence for this pathway includes the success of Kegel exercises in treating UUI by helping to recruit the external sphincter and pelvic floor muscles more effectively and suppress detrusor contraction.

Functional magnetic resonance imaging (fMRI) measures the changes in capillary activity in response to brain activity. Data-driven approaches assist researchers in finding areas that are modulated by associated triggers for neural control of the bladder. A recent fMRI study suggested that trigger images selectively activate the precuneus, which is involved in motor imagery and response inhibition, such as the attempt to hold the bladder. The other area activated, the ventromedial prefrontal cortex, is associated with decision making and emotional consequences, such as those that may occur during UI episodes.

Technologies for Translation

Session 5 highlighted the various technologies poised for translation and for evaluating and treating female urethra function and failure. Investigators engaged in an ongoing project to establish a urethral atlas are evaluating new approaches to characterize sex differences in the urethra that are leveraging the NIDDK-sponsored GenitoUrinary Development Molecular Anatomy Project (GUDMAP). The goal is to develop a map of gene expression along the anterior–posterior axis of the LUT in mouse models using state-of-the-art methodologies, including single-cell RNA sequencing and nanoscale computed tomography. The regionalization of urethral cell types has important clinical implications. Information about different cell types may reveal cellular and molecular differences that contribute to the sexually dimorphic prevalence of urinary tract infections.

Workshop participants were introduced to a urethral multiphysics model, a holistic computational model of urethral sphincter function that combines muscle layers, the vascular plexus, urodynamics, sensory function, and urethral support. This anatomically accurate, subject-specific, finite element analysis, three-dimensional (3-D) model already is addressing several knowledge gaps concerning the function of urethral anatomic components. The effects of age and injury on circular striated muscle are not fully understood, nor is the mechanism by which the urethral vasculature helps to maintain continence. This multiphysics modeling approach can combine simulations of different aspects of the urethra to better understand urethral function.

In this session, workshop participants also discussed establishing and maintaining research collaborations (Ph.D.-M.D.). Four common themes emerged. First, external collaborations can be established by intentionally meeting people at conferences or by volunteering for conference review panels or moderating roles. Second, external collaborations can be maintained by visiting sites, sharing equipment, and remaining patient with conflicting schedules and demands. Third, collaborations are essential to success and can be formed at scientific conferences and seminars. Fourth, researchers should be open to serendipitous meetings that can lead to long-term relationships.

Lessons from Animals, Cells, and Children

In Session 6, it was noted that animal models have been used extensively for studies of female continence and incontinence; rodents (e.g., mice and rats) are the most frequently used model species for studies of the urethra. Studies in the female rat model have revealed several similarities to humans, particularly (1) smooth and striated muscle distribution; (2) innervation; (3) striated muscle as key to normal function of both the urethra and the bladder; and (4) degeneration of urethral striated muscle with age.

Recent studies using genetic and genomic approaches to characterize the structure–function relationship between genes and neurological–urological interactions have provided insight to the field. The GFL–RET (oncogene) signaling pathway is used for physiological studies in mice because the pathway regulates the progenitor proliferation and biology (e.g., migration, differentiation, regeneration, and survival) of different types of neurons. The functions of RET signaling—Trp channel activation, Na+ and K+ channel activation, neuropeptide production, improved neuropathic pain, and axonal survival and growth—are well known. Additionally, GFLs play a role in peptidergic, non-peptidergic, cholinergic, and noradrenergic functions of the bladder.

Panelists reported that children with kidney malformations continue to experience bladder dysfunction after kidney transplant. These patients have been known to have inherited a set of mutations in genes involved in the RET pathway, leading to abnormalities in glial cell line–derived neurotrophic growth factor (GDNF)–RET–MAPK activity. Researchers evaluated mutant mice to model the patient's voiding abnormalities. Subsequent research has characterized the expression of RET in the adult pelvic ganglia, bladder, and urethra. Using a GDNF treatment in mice with genetically labeled RET-expressing neurons suggests that the pelvic ganglia and nerves require the GFL–RET pathways for fiber growth. Other data have revealed abnormal bladder innervation in the bladder walls and mucosa of RET-null mice, emphasizing that these defects arise early in development.

From a pediatric perspective, UI in girls often results from neurological (spinal cord abnormalities), anatomic (ectopic ureter or ureterocele), and functional (dysfunctional voiding or acquired conditions) factors. Many acquired conditions are related to excessive stretching that results from high-impact sports and underlying potential issues in girls. Studies of SUI in young female athletes revealed that a high percentage of high school and college female athletes report having SUI, and many indicated that it had negatively affected their social lives. However, most generally do not discuss it with their primary care physicians or seek treatment.

Evidence has shown that female urethral failure results from repetitive, frequent, and excessive Valsalva action, which drives the pelvic organs through the levator hiatus. This leads to stretching and tearing of connective tissues, leading to pelvic floor weakness and, consequently, prolapse of the pelvic floor organs. High-impact sports lead to stretching and widening of the levator ani muscle. A cohort of healthy, athletic, nulliparous, and peri- or fully adolescent females presenting with complaints of daytime UI and/or SUI, without enuresis or urinary infection, was evaluated using biofeedback training between 2000 and 2016. For study participants whose bladder neck descent was less than 2 cm, biofeedback therapy was effective in resolving incontinence. For participants whose bladder neck descent was more than 2 cm, biofeedback therapy was ineffective, requiring surgery.

Current Therapies and Future Solutions

Session 7 panelists described current and future therapies for treating female urethra disorders. Electrical nerve stimulation has potential applications in the treatment of overactive bladder syndrome and urinary retention disorders, such as Fowler's syndrome and detrusor sphincter dyssynergia. Leveraging a history of these types of therapies, researchers are actively submitting grant applications for the development of an electrical stimulation–based treatment of neurogenic bladder and bowel dysfunction toward commercialization.

Exoskeletons (i.e., neuroprosthetics) and near-infrared spectroscopy (NIRS) have therapeutic applications for pelvic floor muscle function and the female urethra. Transvaginal NIRS uses interfaces placed into a speculum to monitor pelvic floor muscle function, quantify muscle oxygen kinetics, and evaluate changes after rehabilitation therapy. Functional NIRS now is used to demonstrate neuroexcitation in the cerebral cortex, allowing the study of LUT cortical control. Technology originally developed for other applications is increasingly relevant to patients with neurogenic bladder. Translation of current clinical research indicates that weight-bearing activity can reveal unexpected residual pelvic floor muscle function, and NIRS can be used to study pelvic floor muscles and the cerebral cortex during bladder filling and emptying.

Regarding tissue regeneration, none of the current treatments (bulking materials or the surgical insertion of mesh or autologous fascia) attempt to improve urethral function. Stem cell therapies, including bone marrow–derived and/or adipose-derived stem cells, could be used to improve urethral function. These therapies also could be directed at the specific area of dysfunction to replace or enhance muscles, restore innervation, improve vascular supply, or bulk up the extracellular matrix.

Behavioral management of UI and the role of the urethra has been and remains a research focus. Current behavioral interventions include a paper towel test to measure urine leakage performed in a clinical setting or at home and the Knack method, which involves Kegel exercises.

Next Steps

The NIDDK appreciates all who participated in the workshop. Detailed summaries of each session can be accessed from the NIDDK-sponsored Collaborating for the Advancement of Interdisciplinary Research in Benign Urology (commonly known as CAIRIBU) public website.

The NIDDK anticipates that the generation of a systems model incorporating interdisciplinary perspectives will facilitate the development of meaningful research questions that can lead to novel, impactful grant applications with the important goal of improving women's health. Research questions that are informed by cross-disciplinary discussions may lead to additional fundable R01s. The Stimulating Urology Interdisciplinary Team Opportunity Research (SUITOR) R01 (PAS-19-241) has been established as one funding opportunity available for research questions that evolve from this series of meetings. SUITOR has set aside funds to support R01 grants that fall outside of the NIDDK payline.

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