



## Review Article

# Pathogenesis evidence from human and animal models of detrusor underactivity

Jia-Fong Jhang<sup>a</sup>, Yuan-Hong Jiang<sup>a</sup>, Yung-Hsiang Hsu<sup>b</sup>, Han-Chen Ho<sup>c</sup>, Hann-Chorng Kuo<sup>a\*</sup>

<sup>a</sup>Department of Urology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation and Tzu Chi University, Hualien, Taiwan; <sup>b</sup>Department of Pathology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation and Tzu Chi University, Hualien, Taiwan; <sup>c</sup>Department of Anatomy, Tzu Chi University, Hualien, Taiwan

Submission : 26-Nov-2020  
Revision : 25-Dec-2020  
Acceptance : 02-Jan-2021  
Web Publication : 11-May-2021

### ABSTRACT

Detrusor underactivity (DU) is a common urodynamic diagnosis in patients with lower urinary tract symptoms and large post-voiding residual volume. Animal and human studies showed the possible etiologies of DU include central or peripheral nerve injury, bladder outlet obstruction, chronic ischemia, aging, diabetes mellitus, and sympathetic inhibition of micturition reflex. Evidence from animal and human DU studies with various etiologies revealed highly similar gross and histological characteristics in the bladders, including increased bladder weight, bladder wall thickening, inflammation, collagen deposition, and fibrosis. In electron microscopy, smooth muscle destruction, swollen mitochondria, decreased nerve innervation, caveolae, and umbrella cell fusiform vesicles were noted in the DU bladders. Most animal DU models demonstrate detrusor contractility changes from compensatory to the decompensatory stage, and the change was compatible with human DU observation. The cystometry in the DU animal studies is characterized by impaired contractility, prolong intercontraction interval, and hyposensation, while *in vitro* bladder muscle strips experiment may exhibit normal detrusor contractility. Decreased bladder blood flow and increased oxidative stress in bladders had been proved in different animal DU models, suggesting they should be important in the DU pathogenesis pathway. Sensory receptors mRNA and protein expression changes in DU bladders had been observed in both animal and human studies, including muscarinic receptors M2, M3, adrenergic receptor  $\beta$ 3, purinergic receptor P2X1, P2X3, and transient receptor potential vanilloid (TRPV) 1 and TRPV4. Although some of the sensory receptors changes remain controversial, it might be the target for further pharmacologic treatments.

**KEYWORDS:** *Detrusor underactivity, Immunochemical, Molecular, Protein*

### INTRODUCTION

Detrusor underactivity (DU) is a urodynamic diagnosis which was defined as “a contraction of reduced detrusor strength and/or duration, resulting in prolonged bladder emptying and/or a failure to achieve complete emptying within a normal period” [1]. Patients with DU usually suffer from a weak urinary stream, straining to void, hesitancy, with or without a feeling of incomplete bladder emptying, and sometimes with storage symptoms [1]. In patients with lower urinary tract symptoms (LUTS) and aged over 65 years without neurological or anatomic abnormality, the urodynamic study revealed that 40.2% of men and 13.3% of women were diagnosed as DU [2]. Although DU is a common etiology among elderly patients with LUTS, effective treatment for patients with DU is still limited. Current guidelines only suggest DU patients with large post-voiding residual volume

to receive clean intermittent catheterization rather than active medical or surgical treatment [3]. Medical or surgical treatments for patients with DU mainly focus on reducing bladder outlet resistance to facilitate voiding efficiency by using abdominal pressure [3]. Pharmacological treatment or surgical procedures to restore bladder contractility was still lacking [3].

Current insufficient understanding in the pathogenesis of DU leads to the lack of effective pharmacological treatments. The micturition reflex involved the coordination of persisting detrusor muscle contraction and simultaneous relaxation of the urethra sphincter [4]. The sacral

#### \*Address for correspondence:

Dr. Hann-Chorng Kuo,  
Department of Urology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.  
E-mail: hck@tzuchi.com.tw

#### Access this article online

##### Quick Response Code:



Website: [www.tcmjmed.com](http://www.tcmjmed.com)

DOI: 10.4103/tcmj.tcmj\_284\_20

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [WKHLRPMedknow\\_reprints@wolterskluwer.com](mailto:WKHLRPMedknow_reprints@wolterskluwer.com)

**How to cite this article:** Jhang JF, Jiang YH, Hsu YH, Ho HC, Kuo HC. Pathogenesis evidence from human and animal models of detrusor underactivity. *Tzu Chi Med J* 2022;34(3):287-96.

parasympathetic nucleus controls the bladder emptying reflex, and sacral spinal cord injury (SCI) could directly cause detrusor areflexia and a loss of normal bladder sensation [5]. Clinically, DU patients with a history of sacral SCI or pelvic nerve injuries, such as pelvic trauma or pelvic organ radical surgery, were classified into neurogenic DU [6]. A large proportion of DU patients are neurologically intact and were generally classified into myogenic DU in some studies [3]. However, some patients might develop DU without any causative episodes which may lead to detrusor muscle dysfunction, and the pathogenesis in these patients remains a mystery.

Several animal models have been developed to imitate human DU, including central nervous system (CNS) or peripheral nerve injury, diabetes mellitus (DM), aging, bladder outlet obstruction (BOO), chronic ischemia, and cryoinjury of bladders. The above-mentioned animal models could result an acontractile or underactive detrusor, and distinct molecular changes also have been identified in different animal models. Observation of clinical DU studies and laboratory data from human DU bladders also provided some indirect evidence for the possible pathogenesis. The aim of the current study is to review the techniques and findings in the animal DU models, and the pathogenesis evidence from human DU studies.

## THE ANIMAL DETRUSOR UNDERACTIVITY MODELS

According to the possible pathogenesis which was observed from human DU patients, several animal models have been successfully developed. The animal DU models could be generally classified into neural (central or peripheral) injury, bladder injury, mixed nerve and bladder injury, and transgenic animal models. Due to the heterogeneity of human DU etiologies, the animal DU models are only corresponded to a part of the etiologies in human DU, and some possible mechanism of human DU has not been reproduced in the animal study.

### NEURAL INJURY ASSOCIATED DETRUSOR

#### UNDERACTIVITY ANIMAL MODEL

##### Central nerve system injury detrusor underactivity model

Although the micturition reflex center locates in the sacral spinal cord, injury in a higher level of the spinal cord also may result in functional damage of central nerve descending modulatory pathways, leading to at least transient bladder contractility impairment. Spinal cord transection and contusion, which mainly destroy dorsal columns, had been widely used to create animal neurogenic bladder to investigate detrusor overactivity (DO), but it also could result in transient DU. Unlike human, the lumbosacral spinal cord (L1–S5) in rats located within the T11-L1 vertebra [7]. A study showed rat spinal contusion injury at T4 and T9 level could result in transient increased residual urine volume with 2 weeks, but not in the rat with spinal contusion at T1 level [8]. Animal SCI model for persisted DU also has been developed [9,10]. Lumbar canal stenosis

by inserting a silicon rubber into the L6 epidural space could compress the sacrum spine, and exhibits persisted DU in 1 month after the procedure [10]. Unilateral L5–S2 ventral root avulsion in rats mimicked an injury in the cauda equina, and the cystometry showed markedly reduced voiding efficiency and maximum detrusor contractility amplitude at 12 weeks after the procedure [9]. The bladder weight was significantly increased in the sacral SCI DU rat, and fibrosis was also noted [11]. In the molecular analysis, the bladders from sacral SCI DU rat exhibited lower expression of gap junction alpha-1 protein and higher expression of transforming growth factor-beta (TGF- $\beta$ ) in both protein and mRNA level [11].

Urine retention due to transient DU is a common phenomenon in patients with brain trauma or cerebrovascular accident (CVA), but the mechanism is not clear. A rat traumatic brain injury study revealed cystometric evidence of detrusor function changes from underactive to overactive in 1 month after the brain injury. The bladder weight and collagen deposition were also noted in rats' bladder with brain injury [12]. A recent study investigated the long-term voiding function in mice after traumatic brain injury study, and the cystometry showed DO with decreased maximal voiding pressures in chronic brain injury [13]. The voiding dysfunction and DU might be caused by neuroinflammation in the sensory input reflex pathways at rostral but not midbrain and hindbrain regions [13].

##### Peripheral nerve injury detrusor underactivity models

Lower urinary tract organs are innervated by 3 sets of peripheral nerve systems: Sacral parasympathetic pelvic plexus, sympathetic hypogastric plexus, and sacral somatic nerves (primarily the pudendal nerves) [4]. The pelvic nerve injury, including transection and crush injury, has been widely used in creating animal DU model [14-17]. For the pelvic nerve transection, the bilateral L6 and S1 nerve bundles were exposed near the transverse processes and then were transected with microscissors [16]. For the crush injury model, first, the major pelvis ganglion near the prostate and bladder should be identified, and a hemostat clamp was used to crush each pelvic nerve for 30s to 2 min [15,18]. Both bilateral pelvic nerve injury (BPNI) models could significantly increase the bladder capacity and intercontraction intervals in cystometry, and the residual urine volume. The bladder weight was significantly increased, and decreased smooth muscle distribution with increased collagen deposition was noted in the rat with BPNI [15]. In the molecular level, the protein gene product 9.5 (marker for sensory and autonomic nerve fibers) and choline acetyltransferase expression in the bladder were significantly reduced [14], while the transient receptor potential vanilloid 4 (TRPV4) expression level was significantly increased [15]. The bladder muscarinic receptors expression level changes in BPNI DU rats remain controversial. Hannan *et al.* reported the M3 receptor expression in the bladder was decreased and the M2 was unchanged [19]. However, the other study showed increased expression of the M2 receptor, but the M3 expression was not significantly different [15].

## **BLADDER INJURY ASSOCIATED DETRUSOR UNDERACTIVITY ANIMAL MODEL**

### **Bladder outlet obstruction associated detrusor underactivity animal model**

BOO is a common human disease, and detrusor DO or DU was often observed in the patients with chronic BOO. Partial BOO has been used to create lower urinary tract dysfunctions in numerous male and female animal models, including pig, dog, rabbit, guinea pig, rat, and mouse. After abdominal lower midline incision in rat and introduction of a urethral catheter, the partial BOO could be performed by tying a suture with rubber ring or 4-0 nylon silks in proximal urethral, with or without a small rod placed adjacent to the urethra to avoid complete urethral obstruction [20,21]. In the rat, transurethral ligation of the perineal urethra also has been used to create partial BOO successfully for both sexes. Prolong partial BOO could lead to DU in various animal models, and the duration to cause DU varied from 2 to 8 weeks in different studies [20-23]. Similar to the clinical observation in human BOO patients, animal BOO model elicits bladder structural and functional changes in three stages: hypertrophy with overdistention, compensation with DO and appropriate emptying, and decompensation with loss of functional emptying ability [22]. Since the hypertrophy phase, the BOO bladders rapidly increased both the total weight and total cellular content, and the growth continued until the decompensation phase [22]. Evidence showed significantly increased peri-bladder artery blood flow since the initial stage of rat and rabbit BOO (may increase within first 4 h), and may persist to 4 weeks after the procedure [24,25]. Immunohistochemical staining showed significantly increased hypoxyprobe-1 (a marker for tissue ischemia) in the BOO rat bladders from day 3 to day 14 [24]. The results suggested the increased bladder blood flow might be responded to bladder ischemia after BOO, and the ischemia persisted even with increased blood flow. The molecular mechanism in the BOO associated bladder decompensation was still unclear. Some cell signaling proteins such as AMP-activated protein kinase, extracellular signal-regulated protein kinase 1/2, actin regulation protein Rho A and L-type  $Ca^{2+}$  channels expression were significantly changed in the compensation phase BOO, but not in the decompensation phase [26,27]. The level of 8-hydroxy-2-deoxyguanosine was increased, and superoxide dismutase was decreased in the bladder of BOO associated DU; the result suggested increased oxidative stress in the DU bladder [20]. Increased M2 receptor and decreased M3 receptor also were noted in the bladder of BOO associated DU. Although BOO associated DU animal model had been well established, the molecular changes of animal BOO associated DU remained controversial in different studies, such as the bladder purinergic receptors expression [20,25-27]. The difference might be resulted from different outlet obstruction duration and pressure, further studies to comprehensively investigate the molecular changes in different timing of BOO were necessary.

### **Artery obstruction/ischemia associated detrusor underactivity animal model**

Chronic ischemia has long been suspected as the possible etiology of DU. The risk factors of cardiovascular

diseases (e.g. hypertension and hyperlipidemia) were associated with LUTS in both male and female patients, so atherosclerosis with reduction of bladder blood flow has been considered as a possible cause of DU. The early bladder ischemia animal model was performed by internal iliac artery ligation with 5-0 silk suture [28]. Both unilateral and bilateral internal iliac artery ligation could result in decreased voiding pressure in 7 days after the procedure. However, internal iliac artery ligation is acute rather than chronic ischemia animal model. Combination of vascular endothelial damage by iliac arteries balloon repeat inflation and high cholesterol diet (from 0.5% to 2%) was used to simulate chronic ischemia in rat and rabbit bladder [29,30]. Eight to 16 weeks after the procedure, significant artery occlusion was found by angiography, and the detrusor voiding pressure was decreased [29,30]. In the chronic ischemia model, tissue inflammation increased bladder weight, and significant bladder fibrosis with increased TGF- $\beta$  were noted [29,30]. In the view of ultrastructure, swollen mitochondria, impaired microvasculature with thickened intima, and muscle fascicles destruction in the rabbit chronic ischemia bladder were observed by electron microscopy [31]. The S-100 protein-positive neurons and purinergic receptor P2X1 were reduced in the ischemia bladder, but the calcitonin gene-related peptide (CGRP) positive neurons was increased [29,30,32]. Kim *et al.*, analyzed the genome-wide gene expression in the chronic ischemia rat DU bladder, and the pathway analysis showed genes related to interleukin-17 and hypoxia-inducible factors 1 signaling pathways were upregulated [29]. The chronic ischemia DU animal model seems to well imitate the human ischemic DU, especially in elderly patients. The detrusor function in this model also initially presented as DO, and then progressed to DU [29]. However, currently, most animal chronic bladder ischemia study investigated the bladder at the overactivity stage, the evidence about bladder changes in underactivity stage remains limited. The factors to cause detrusor function worsen from overactivity to underactivity is important and has the potential to develop to novel pharmacological treatment. Using  $\alpha$ 1A-adrenoceptor antagonist silodosin in bladder chronic ischemia rat with DO could increase bladder blood flow, ameliorate detrusor function from overactivity, and decrease the bladder oxidative stress [33]. Further studies focus on pharmacological treatment effects in chronic ischemia DU are necessary.

### **Bladder cryoinjury detrusor underactivity animal model**

In contrast to the other bladder injury associated DU animal model, bladder cryoinjury might be the only model which is designed to directly and purely result in DU. Somogyi *et al.* used an 8-mm aluminum rod which was chilled on  $-40^{\circ}C$  dry ice to place against the serosal surface of the bladder wall for 30 s in rat [34]. Five days after the cryoinjury, the square wave and adenosine triphosphate (ATP) evoked bladder strips contractility were significantly lower [34]. Bladder cryoinjury could result in decreased cystometric detrusor voiding pressure without changing the intercontraction interval, and the effect could last for 4 weeks [35,36]. Significantly increased cyclooxygenase-2 and TGF- $\beta$  protein expression suggested inflammation and fibrosis in the cryoinjury bladder, while the

CGRP expression nerve was significantly decreased [35,36]. CGRP is a sensory neurotransmitter which is localized to both C and A $\delta$  sensory fibers [37], depletion of CGRP in the cryoinjury bladder suggested impairment of sensory function might be the key mechanism of DU. However, the other molecular and neural changes in the cryoinjury bladder has not been well investigated. Although the cryoinjury is a pure bladder injury DU model, the mechanism of this model is different from most human DU. Using the cryoinjury model to investigate DU treatment intervention were reasonable [35,36], but the feasibility in human is still questionable.

## MIXED NERVE AND BLADDER INJURY DETRUSOR UNDERACTIVITY MODEL

### Aging detrusor underactivity animal model

Since the prevalence of DU indeed increased with age, DU has been considered as the age-associated changes in the urinary bladder. Impaired detrusor contractility has been regarded as an important etiologic of DU, but neural sensation or activation also may play a role in the mechanism of age-associated DU. Aging models has been widely used in bladder function researches in different species animal, including mice (about 22–25 months old in C57BL6 mice), rats (about 18–24 months old in male SD rat), guinea pigs and dogs [38-41]. Increased bladder weight was observed in the male aged mice, but not in females [42]. From the view of histopathology, urothelial thinning, lower muscle mass, fibrosis, and increased suburothelial collagen deposition had been reported in aged rats and mice studies [39,42,43]. *In vivo* cystometry in aged bladder yielded variable results, both increased and decreased voiding pressure might be developed in aged animals [39,41,42]. Similar to cystometry studies, the *in vitro* bladder strips contractility in aged animals also showed highly variable results [41,42,44-46], the aged animal bladder strips response to electric field stimulation (EFS) or agonists for muscarinic receptors might demonstrate increased or decreased contractility. The difference might be due to various species animal and age used in the studies. Furthermore, recent aged animal studies showed decreased *in vivo* bladder contractility in cystometry but preserved similar *in vitro* bladder strips contractility response to potassium or ATP stimulation [42,47,48]. This result suggested *in vitro* DU might not only due to detrusor functional failure, neural activation dysfunction also involved in the mechanism of aged DU. DU is common in elderly patients with CNS disorders, such as dementia and CVA [5], which also might happen in the aged animal model. The impact of degenerative CNS deterioration on voiding dysfunction should be important but has not been well investigated. Evidence of molecular changes of the aged animal bladder was limited, especially in the aged DU animal model. Daly *et al.* reported increased P2X3 receptor in aged DU mice bladder mucosa, but the mRNA expression of all muscarinic and purinergic receptors was decreased [48]. Another study showed decreased M3 and P2X1 receptor mRNA in male aged mice with DU, but not in female aged mice with preserved bladder contractility [42]. Caveolae is a special type of lipid raft in the plasma membrane and plays a role in cell mechanoprotection and mechanosensation [49].

An electron microscopy study showed the caveolar area and depth were decreased in aged bladder smooth muscle cells, suggesting loss of caveolin protein might alter bladder contractility resulting in aged bladder dysfunctions [50]. Aged animals might be the most well-imitated model to human DU, but the aged effect in voiding dysfunction is complicated, resulting in conflicting physiologic and molecular results in different studies. Since both DU or DO might be observed in aged animals, studies to compare the physiologic and molecular differences in aged animals with DU or DO should be the key to understand the pathogenesis of DU.

### Diabetic animal detrusor underactivity model

DM is a common disease which could be contributed to multiple tissue pathologic change in organs, such as neuropathy and myopathy. Diabetic associated bladder dysfunction is characterized by decreased bladder sensation, and the urodynamic finding might include DO or DU. The commonly used DM animal models in voiding dysfunction include streptozotocin and alloxan-induced DM, which could produce pancreatic islet  $\beta$ -cell destruction and insulin-dependent DM [51]. Hyperglycemia could be detected in several days after streptozotocin or alloxan injection, and time-dependent bladder contractility changes from a compensated (DO) to a decompensated state (DU) were also observed [52]. The time to developed DU in the diabetic animal model highly varied in different studies; DU might be developed as early as 4 weeks later in rats [53], or sixteen weeks later in rabbits [53]. Similar to other DU animal models, grossly increased bladder weight, histologically inflammatory cells infiltration, collagen deposition, and even increased apoptotic cells in the bladders were observed in the diabetic DU model [52-54]. Decreased umbrella cells fusiform vesicle and broken cell-cell junctions between adjacent cells in the DU rat bladders were also noted in electron microscopy [55]. The bladder blood flow was also decreased in the rat DU model [56]. The cystometry results of the animal diabetic animal model were characterized by significantly increased intercontraction interval, nonvoiding contraction, voiding volume, and residual volume [53,56,57]. The peak voiding pressure was constant in most studies [53,56,57], but decreased voiding pressure also might be observed in the decompensated stage [52]. The cystometry characteristics of the DU animal model correspond with some human DU patients with urodynamic hyposensation, increased residual volume, but the voiding pressure was still within the normal range. *In vitro* bladder muscle strip contractility test in DU animal showed higher contractile responses to muscarinic receptor agonist carbachol, ATP, and EFS in the early compensated stage, but it might be reversed in the decompensated stage [58-60]. In transcriptome level, one study showed the level of M2, M3, TRPV1, and P2X3 mRNA expression was significantly increased in the diabetic DU rat bladders [55], but the other conflicted study showed the level of M2 and M3 mRNA expression was similar between diabetic and control rat bladders [61]. In the protein level, immunochemical studies showed decreased nerve growth factor [62] and sensory nerve C-fiber marker peripherin [56] in the diabetic animal DU bladders. The protein expression level of mitochondrial Bax, cytosolic cytochrome

c, nuclear factor erythroid 2-related factor 2, and Keap1 were increased in the diabetic DU bladder in a recent study, and the results suggested increase of bladder oxidative stress and mitochondria dysfunction in the bladders [63]. Although the diabetic animal model for voiding dysfunction has been well established, it should be noted most studies used the type 1 DM model, but most human patients with diabetic associated bladder dysfunction are the type 2. Since the DM animal model is characterized by bladder hyposensation with possibly preserved contractility, the molecular difference between DM animal and the other DU animal model bladders should be compared to understand the mechanism of DU.

### Transgenic detrusor underactivity animal model

With the recent progress in genetic editing technique, several transgenic DU animal model has been developed. Although detrusor contraction is mediated by cholinergic neurotransmitter and muscarinic receptors, *in vitro* cystometry in M2 and M3 knocked out mice only exhibited longer voiding intervals without changes of voiding contractility [64]. Previous physiology study showed prostaglandin E2 and its receptor EP family could modulate detrusor muscle contractility [65]. The EP3 knockout mice demonstrated higher voided urine volumes and higher infused volumes required to stimulate micturition [66]. However, the average and threshold filling pressures were not significantly different between the EP3 knockout and wild type mice [66]. ATP released from parasympathetic neurons or urothelium could contribute to both contraction and sensation via the purinergic signaling pathway in the bladder [67]. The purinergic receptor P2X3 is critical for peripheral pain responses, the P2X3 knockout mice exhibited decreased voiding frequency and increased bladder capacity, but normal detrusor contractility [68,69]. In contrast, both bladder hyposensation and decreased contractility were observed in the P2X2 knockout mice [70]. TRPV4 is a nonselective cation channel activated by mechanical pressure, osmolality, warmth, and chemical stimuli [71]. Urothelial TRPV4 channels act as a pressure sensor to enhance bladder activity, predominantly through activation of bladder afferent pathways by ATP [72]. In the TRPV4 knockout mice, cystometry revealed the lower frequency of voiding contractions but a higher frequency of non-voiding contraction [73]. Although the cystometric contractility in the TRPV4 knockout mice did not decrease, the bladder strips study revealed decreased amplitude of the spontaneous contractions [73]. Stretch-induced ATP release from the urothelium was also decreased in the TRPV4 knockout mice [73]. The results of transgenic DU animal model revealed *in vitro* bladder contractility is controlled by multiple signaling pathways, and single receptor knockout may not cause changes of cystometric maximal contractility. Transgenic DU animal model could improve our understanding of bladder physiology, but the mechanism remains totally different from human DU. Hence, studies aim to develop novel pharmacological treatment for DU should not use the transgenic animal model.

### PATHOGENESIS OF DETRUSOR UNDERACTIVITY FROM HUMAN EVIDENCE

In contrast to many DU animal model studies, studies provided human DU pathogenesis evidence is extremely

limited. Except the patients with totally acontractile bladder, making the urodynamic diagnosis for DU patients with residual detrusor voiding contractility is often controversial. Currently, no universal guideline to define the threshold detrusor voiding pressure for the diagnosis of DU [3]. Studies usually used bladder contractility index (BCI) <100 and/or detrusor pressure at maximum flow <20 cmH<sub>2</sub>O with maximal flow rate <15 mL/s to enrolled patients with DU [3]. However, patients with delay evoke or failure to sustain detrusor contractility during voiding also meets the urodynamic diagnosis of DU, but maximal detrusor contractility might remain in the normal range [1]. Bladder hyposensation is a common characteristic of human DU [74], but the cystometric volume to confirm hyposensation has not been defined yet. Human DU studies might enroll DU patients which were contributed to one kind of etiology, but in fact, the pathogenesis of human DU should be multifactorial. Nevertheless, some studies still provided pathogenesis evidence, including clinical findings and laboratory data, to explore the possible mechanisms of human DU. Our previous study enrolled mid-age (mean age of 56 years old) DU patients without BOO or neuropathy, and the bladder biopsy specimens showed the decreased urothelial E-cadherin, M2, M3,  $\beta$ 3, and P2X3 receptors expression in patients with DU in compared to urodynamic normal controls, while the level of tryptase and terminal deoxynucleotidyl transferase dUTP nick end labeling expression was increased [75]. Our results revealed urothelial barrier function defect, decreased sensory receptors, increased inflammation, and apoptosis in urothelium of DU patients. The following sections reviewed the clinical or laboratory pathogenesis evidence from DU patients with different etiologies.

### HUMAN PATHOGENESIS EVIDENCE FROM NEUROGENIC DETRUSOR UNDERACTIVITY

Evidence from human studies revealed about 50% of patients with CVA were suffered from transient urine retention due to DU. About 95% of patients with CVA and urine retention were regained urination within 2 months, but a small proportion of patients with CVA also might suffer from persisted DU [76]. The pathogenesis of DU in the CVA patients with long-term persisted urine retention is still unknown. In the acute phase of SCI, the urodynamic study revealed an acontractile detrusor in about 37% of patients despite the injury level [77]. Our early study showed DU in 95% of patients with sacral SCI in long-term follow-up, while also 31% and 45% of patients with cervical and thoracolumbar SCI also presented with DU in the urodynamic study [78]. Injury of sacral micturition reflex center might directly cause DU, but the mechanism of long-term DU in higher-level SCI is still not clear. The detrusor muscle strip from human revealed delay EFS in the SCI patients with DU. The sensitivity of the detrusor muscle strip to EFS did not change in the SCI patients with DU, but the maximum force generated by each milligram of bladder tissue was significantly reduced [79]. Immunohistochemical staining also exhibited decreased acetylcholinesterase and protein gene product 9.5 activity in the detrusor of SCI with DU, suggesting loss of detrusor innervation [79]. Several

studies focused on the bladder sensory receptors change in the SCI patients with DO [80,81], however, the bladder changes in human SCI patients with DU had not been investigated yet.

### **HUMAN PATHOGENESIS EVIDENCE FROM BLADDER OUTLET OBSTRUCTION ASSOCIATED DETRUSOR UNDERACTIVITY**

Since BOO was diagnosed by the pressure-flow study result of high voiding detrusor pressure and low maximal flow rate, it is a long-existed controversial issue to make the urodynamic diagnosis of BOO and DU simultaneously [3,6]. Currently, experts suggest that the diagnosis of simultaneous BOO and DU might be indirectly made based obviously anatomical BOO (such as large prostate, urethral stricture, etc.) and urodynamic low detrusor pressure [6]. Recent studies revealed some patients with DU could regain spontaneous urination and detrusor contractility after surgery for removing outlet resistance [82]. The results support BOO and DU could be simultaneously existed, and BOO should be an etiology of DU in humans. Molecular analysis for the human bladders with DU and BOO is rare. Our previous study showed a decreased level of urothelial expression of E-cadherin and increased  $\beta 3$  and M3 receptors in the patients with BOO and DU in comparison to the patients with BOO and preserved detrusor contractility [83]. In addition, the level of urothelial M2 receptor was positively correlated to the maximal detrusor contractility in the patients with BOO [83].

### **HUMAN PATHOGENESIS EVIDENCE FROM AGED ASSOCIATED DETRUSOR UNDERACTIVITY**

Although animal studies provided evidence to support age as an important etiology in DU, it is still highly debatable whether age has an independent impact on detrusor contractility. Early cross-sectional studies showed conflicting results in the association between age and detrusor contractility [84,85], and the other factors such as BOO also might affect the detrusor function change in elderly patients. Our recent study provided the only longitudinal long-term follow-up study to see the impact of age on detrusor contractility [86]. In a cohort which had been followed for >10 years, the maximal detrusor contractility and BCI were significantly decreased in both sexes [86]. In addition, the maximal detrusor contractility and BCI both significantly decreased in men with or without BOO, and the decline of contractility was no significant difference between the two groups [86]. The results supported age should be an independent factor for detrusor contractility decline. A human bladder strips study from patients who underwent radical cystectomy showed *in vitro* detrusor contractility in response to EFS did not change with age [87]. In the view of histology, age was associated with increased bladder collagen deposition and decreased smooth muscle in both male and female patients [88]. The reduction of the acetylcholinesterase-positive nerve was also observed in the aged bladders [89]. The ultrastructural features of aged bladder included dense band patterns in the muscle cells, cell junctions, sarcolemma [90], and decreased axon counts [89]. Widespread degeneration of smooth muscle was found in

the aged detrusor with DU, but not in aged detrusor without DU [90]. The level of mRNA expression of M2, M3, P2X1, and P2X3 receptors did not change with age [87], however, increased density of M2 receptors in the mucosa was noted in the aged bladder [91]. Researches of urothelial protein expression changes in aged human are rare, a recent study showed the level of TRPV4 protein expression in both bladder mucosa and detrusor was increased in aged patients with preserved detrusor contractility [92]. It should be noted that most aged human bladder laboratory studies obtained the specimens from the patients without DU, hence, the effect of aging on human DU bladder remains uncertain.

### **HUMAN PATHOGENESIS EVIDENCE FROM DIABETIC ASSOCIATED DETRUSOR UNDERACTIVITY**

Although animal model researches showed DM could cause DU, direct human evidence of DM as an independent etiology of DU is still rare. Patients with DM associated LUTS might be just resulted from polyuria rather than bladder dysfunction. Our previous study revealed that the level of the urothelial tryptase and M3 receptor was higher in the DU patients with DM than that in those without DM [75]. Although direct evidence remains lacking, our results suggested DM might be associated with more urothelial inflammation and sensory receptors change in the DU patients.

### **HUMAN SYMPATHETIC INHIBITORY DETRUSOR UNDERACTIVITY**

Although it was rarely demonstrated in literatures, some of the DU patients in clinical practice were young or mid-age, neurologically intact, without obviously anatomical obstruction, surgical or systemic medical disease history. Some researchers refer to these patients as idiopathic DU [3], and the video-urodynamic study of idiopathic DU may exhibit non-relaxation bladder neck or external urethral sphincter with low detrusor pressure [93,94]. A hypothesis suggests the mechanism of idiopathic DU might be resulted from high sympathetic activity inhibition in detrusor reflex contraction, which the inhibition had been well demonstrated in early animal studies [95]. Our previous study showed some indirect human evidence to support this hypothesis. Female patients with long-term idiopathic DU and urine retention regained spontaneous urination after transurethral incision of the bladder neck, and the follow-up urodynamic study proved detrusor contractility recovery in some of DU patients [96,97]. The detrusor function recovery after the procedure also had been observed in cervical SCI patients with long-term DU, which physiologically should preserve bladder contractility [98]. Sympathetic efferent and afferent pathways project to the bladder neck [99,100] and inhibit detrusor reflex during the storage phase. The transurethral incision of the bladder neck aimed to destroy the sympathetic nerve innervation and the autonomic inhibition in the bladder neck, and the patients with idiopathic DU indeed regain detrusor contractility after the procedure. Although still lack of direct evidence, our result indirectly suggested sympathetic inhibition is a possible mechanism in patients with DU, further direct

animal or human evidence is necessary to confirm this hypothesis.

It should be noted that some animal studies for DU only demonstrated increased bladder capacity, large PVR, and prolong intercontraction interval, however, a significant reduction of detrusor contractility in cystometry may not present [15,53,56,57]. Although human with DU also could present with increased PVR and bladder hyposensation only, the urodynamic study in most DU patients indeed revealed a significantly lower detrusor contractility. Using human bladder tissue to investigate the pathogenesis with DU is necessary and might be clinically valuable, but some pitfalls still should be noted: (1) Most human DU bladder studies lack good control. Some studies [86,87] used bladder tissue from aged patients with bladder cancer as control, but it is hard to say those were normal bladders. (2) The methods of human bladder tissue harvest during operation, such as electrocauterization or cold-cup biopsies, may have an impact on bladder protein expression of functional experiment results. (3) Although some human studies focused on one possible etiology of DU, most human DU patients still might be multifactorial. DU patients might be contributed to old age, BOO and ischemia simultaneously, it is extremely difficult to enroll human DU patients with only one etiology.

## CONCLUSION

Animal and human studies showed the possible etiologies of DU include central or peripheral nerve injury, BOO, ischemia, aging, DM, and sympathetic inhibition. The bladder changes in animal and human DU studies are summarized in Table 1. Evidence from DU studies with various etiologies revealed similar histological characteristics in the bladders, including bladder wall thickening and fibrosis. Although lack of direct evidence, we speculated that the increased bladder weight might be resulted from increased collagen deposition in bladder lamina propria and muscle layer. In electron microscopy, smooth muscle destruction, swollen mitochondria, and decreased nerve innervation were noted in the DU bladders. The cystometry in the DU studies demonstrated impaired contractility, prolong intercontraction interval, and hyposensation, while *in vitro* bladder muscle strips experiment may exhibit normal detrusor contractility. Decreased bladder blood flow and increased oxidative stress in bladders had been observed in different animal DU models, suggesting they should be important in the DU pathogenesis pathway. Sensory receptors mRNA and protein expression changes in DU bladders had been proved in both animal and human studies, including M2, M3,  $\beta$ 3, P2X3, and TRPV4 receptors. The changed sensory receptors might be the target for further pharmacologic treatments.

**Table 1: Summary of the bladder pathological changes in animal and human detrusor underactivity studies**

	Gross and histology	Electron microscopy	mRNA or protein expression
Central nerve system injury	↑Bladder weight, fibrosis [11]		↓Gap junction alpha-1 [11], acetylcholinesterase, protein gene product 9.5 [78] ↑TGF- $\beta$ [11]
Peripheral nerve injury	↑Bladder weight, collagen deposition [14] ↓Smooth muscle [14]		↓Protein gene product 9.5, choline acetyltransferase [13] ↑TRPV4[14] ↔M2, M3[14,18]
BOO	↑Bladder weight, total cell count, collagen deposition [21], associated bladder artery flow [23,24]		↓M3, superoxide dismutase [19], E-cadherin[81] ↑ $\beta$ 3, M3 [81], M2, hypoxypyrene-1 [23], 8-hydroxy-2 deoxyguanosine [19]
Chronic ischemia	↑Bladder weight, inflammation, fibrosis [28,29]	Swollen mitochondria, impaired microvasculature, thickened intima, muscle fascicles destruction [31]	↓S-100, P2X1 [29,30,32] ↑TGF- $\beta$ , CGRP [29,30,32]
Cryoinjury	↑Inflammation [34,35]		↑Cyclooxygenase-2, TGF- $\beta$ [34,35] ↓CGRP [34,35]
Aging	↑Bladder weight, collagen deposition [39,42,43] ↓Smooth muscle, urothelial thinning [39,42,43]	Decreased caveolae [48], axon counts[87] Dense band pattern in the muscle cells, cell junctions, sarcolemma [88]	↔All muscarinic and purinergic receptors [41,47] ↓Acetylcholinesterase [89]
Diabetic	↑Bladder weight, inflammation, collagen deposition, apoptotic cells [51-53] ↓Bladder blood flow [56]	Decreased umbrella cells fusiform vesicle and broken cell-cell junctions [54]	↓Erve growth factor [61], peripherin[55] ↑TRPV1, P2X3 [54], Bax, cytosolic cytochrome c, nuclear factor erythroid 2-related factor 2, Keap1 [62], tryptase[74] ↔M2 and M3 [60]

↑: Increased expression, ↓: Decreased expression, ↔: Conflicted results in different studies, BOO: Bladder outlet obstruction

## Financial support and sponsorship

This study was funded by TCMMP 109-02-03, Buddhist Tzu Chi Medical Foundation.

## Conflicts of interest

Dr. Yuan-Hong Jiang, Yung-Hsiang Hsu, Han-Chen Ho and Hann-Chorng Kuo, the editorial board members at *Tzu Chi Med J*, had no roles in the peer review process of or decision to publish this article. The other author declared no conflict of interest in writing this paper.

## REFERENCES

- Drake MJ. Fundamentals of terminology in lower urinary tract function. *Neurourol Urodyn* 2018;37:S13-9.
- Jeong SJ, Kim HJ, Lee YJ, Lee JK, Lee BK, Choo YM, et al. Prevalence and clinical features of detrusor underactivity among elderly with lower urinary tract symptoms: A comparison between men and women. *Korean J Urol* 2012;53:342-8.
- Osman NI, Esperto F, Chapple CR. Detrusor underactivity and the underactive bladder: A systematic review of preclinical and clinical studies. *Eur Urol* 2018;74:633-43.
- de Groat WC, Griffiths D, Yoshimura N. Neural control of the lower urinary tract. *Compr Physiol* 2015;5:327-96.
- Kadow BT, Tyagi P, Chermansky CJ. Neurogenic Causes of Detrusor Underactivity. *Curr Bladder Dysfunct Rep* 2015;10:325-31.
- Groen J, Pannek J, Castro Diaz D, Del Popolo G, Gross T, Hamid R, et al. Summary of European Association of Urology (EAU) Guidelines on Neuro-Urology. *Eur Urol* 2016;69:324-33.
- Lavrov I, Courtine G, Dy CJ, van den Brand R, Fong AJ, Gerasimenko Y, et al. Facilitation of stepping with epidural stimulation in spinal rats: Role of sensory input. *J Neurosci* 2008;28:7774-80.
- David BT, Steward O. Deficits in bladder function following spinal cord injury vary depending on the level of the injury. *Exp Neurol* 2010;226:128-35.
- Chang HH, Havton LA. Serotonergic 5-HT<sub>1A</sub> receptor agonist (8-OH-DPAT) ameliorates impaired micturition reflexes in a chronic ventral root avulsion model of incomplete cauda equina/conus medullaris injury. *Exp Neurol* 2013;239:210-7.
- Sekido N, Jyoraku A, Okada H, Wakamatsu D, Matsuya H, Nishiyama H. A novel animal model of underactive bladder: Analysis of lower urinary tract function in a rat lumbar canal stenosis model. *Neurourol Urodyn* 2012;31:1190-6.
- Zhao J, Wu M, Chen S, Ji Z, Zheng X. TGF- $\beta$ 1 and connexin-43 expression in neurogenic bladder from rats with sacral spinal cord injury. *Neurourol Urodyn* 2018;37:2502-9.
- Jiang HH, Kokiko-Cochran ON, Li K, Balog B, Lin CY, Damaser MS, et al. Bladder dysfunction changes from underactive to overactive after experimental traumatic brain injury. *Exp Neurol* 2013;240:57-63.
- Albayram O, MacIver B, Mathai J, Verstegen A, Baxley S, Qiu C, et al. Traumatic Brain Injury-related voiding dysfunction in mice is caused by damage to rostral pathways, altering inputs to the reflex pathways. *Sci Rep* 2019;9:8646.
- Dewulf K, Weyne E, Gevaert T, Deruyver Y, Voets T, Ridder D, et al. Functional and molecular characterisation of the bilateral pelvic nerve crush injury rat model for neurogenic detrusor underactivity. *BJU Int* 2019;123:E86-96.
- Kim SJ, Lee DS, Bae WJ, Kim S, Hong SH, Lee JY, et al. Functional and molecular changes of the bladder in rats with crushing injury of nerve bundles from major pelvic ganglion to the bladder: Role of RhoA/Rho kinase pathway. *Int J Mol Sci* 2013;14:17511-24.
- Kontani H, Hayashi K. Urinary bladder response to hypogastric nerve stimulation after bilateral resection of the pelvic nerve or spinal cord injury in rats. *Int J Urol* 1997;4:394-400.
- Takaoka EI, Kurobe M, Okada H, Takai S, Suzuki T, Shimizu N, et al. Effect of TRPV4 activation in a rat model of detrusor underactivity induced by bilateral pelvic nerve crush injury. *Neurourol Urodyn* 2018;37:2527-34.
- Tyagi P, Smith PP, Kuchel GA, de Groat WC, Birder LA, Chermansky CJ, et al. Pathophysiology and animal modeling of underactive bladder. *Int Urol Nephrol* 2014;46(Suppl 1):S11-21.
- Hannan JL, Powers SA, Wang VM, Castiglione F, Hedlund P, Bivalacqua TJ. Impaired contraction and decreased detrusor innervation in a female rat model of pelvic neuropraxia. *Int Urogynecol J* 2017;28:1049-56.
- Jung JW, Jeon SH, Bae WJ, Kim SJ, Chung MS, Yoon BI, et al. Suppression of oxidative stress of modified Gongjin-Dan (WSY-1075) in detrusor underactivity rat model bladder outlet induced by obstruction. *Chin J Integr Med* 2018;24:670-5.
- Schröder A, Tajimi M, Matsumoto H, Schröder C, Brands M, Andersson KE. Protective effect of an oral endothelin converting enzyme inhibitor on rat detrusor function after outlet obstruction. *J Urol* 2004;172:1171-4.
- Buttayan R, Chen MW, Levin RM. Animal models of bladder outlet obstruction and molecular insights into the basis for the development of bladder dysfunction. *Eur Urol* 1997;32(Suppl 1):32-9.
- Feng J, Gao J, Zhou S, Liu Y, Zhong Y, Shu Y, et al. Role of stem cell factor in the regulation of ICC proliferation and detrusor contraction in rats with an underactive bladder. *Mol Med Rep* 2017;16:1516-22.
- Ghafar MA, Shabsigh A, Chichester P, Anastasiadis AG, Borow A, Levin RM, et al. Effects of chronic partial outlet obstruction on blood flow and oxygenation of the rat bladder. *J Urol* 2002;167:1508-12.
- Lieb JL, Chichester P, Kogan B, Das AK, Leggett RE, Schröder A, et al. Rabbit urinary bladder blood flow changes during the initial stage of partial outlet obstruction. *J Urol* 2000;164:1390-7.
- Burmeister D, AbouShwareb T, D'Agostino R Jr., Andersson KE, Christ GJ. Impact of partial urethral obstruction on bladder function: Time-dependent changes and functional correlates of altered expression of Ca<sup>2+</sup> signaling regulators. *Am J Physiol Renal Physiol* 2012;302:F1517-28.
- Choi BH, Jin LH, Kim KH, Kang SA, Kang JH, Yoon SM, et al. Cystometric parameters and the activity of signaling proteins in association with the compensation or decompensation of bladder function in an animal experimental model of partial bladder outlet obstruction. *Int J Mol Med* 2013;32:1435-41.
- Saito M, Yokoi K, Ohmura M, Kondo A. Effects of ligation of the internal iliac artery on blood flow to the bladder and detrusor function in rat. *Int Urol Nephrol* 1998;30:283-92.
- Kim M, Yu HY, Ju H, Shin JH, Kim A, Lee J, et al. Induction of detrusor underactivity by extensive vascular endothelial damages of iliac arteries in a rat model and its pathophysiology in the genetic levels. *Sci Rep* 2019;9:16328.
- Yoshida M, Masunaga K, Nagata T, Satoji Y, Shiomi M. The effects of chronic hyperlipidemia on bladder function in myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits. *Neurourol Urodyn* 2010;29:1350-4.
- Azadzoï KM, Chen BG, Radisavljevic ZM, Siroky MB. Molecular reactions and ultrastructural damage in the chronically ischemic bladder. *J Urol* 2011;186:2115-22.
- Azadzoï KM, Tarcan T, Siroky MB, Krane RJ. Atherosclerosis-induced chronic ischemia causes bladder fibrosis and non-compliance in the rabbit. *J Urol* 1999;161:1626-35.
- Goi Y, Tomiyama Y, Nomiya M, Sagawa K, Aikawa K, Yamaguchi O. Effects of silodosin, a selective  $\alpha$ 1A-adrenoceptor antagonist, on bladder blood flow and bladder function in a rat model of atherosclerosis induced chronic bladder ischemia without bladder outlet obstruction. *J Urol*



- 2013;190:1116-22.
34. Somogyi GT, Yokoyama T, Szell EA, Smith CP, de Groat WC, Huard J, et al. Effect of cryoinjury on the contractile parameters of bladder strips: A model of impaired detrusor contractility. *Brain Res Bull* 2002;59:23-8.
  35. Chuang YC, Tyagi P, Luo HL, Lee WC, Wang HJ, Huang CC, et al. Long-term functional change of cryoinjury-induced detrusor underactivity and effects of extracorporeal shock wave therapy in a rat model. *Int Urol Nephrol* 2019;51:617-26.
  36. Chuang YC, Tyagi P, Wang HJ, Huang CC, Lin CC, Chancellor MB. Urodynamic and molecular characteristics of detrusor underactivity in a rat cryoinjury model and effects of low energy shock wave therapy. *Neurourol Urodyn* 2018;37:708-15.
  37. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: Physiology and pathophysiology. *Physiol Rev* 2014;94:1099-142.
  38. Finkbeiner A, Lapidus J. Effect of distension on blood flow in dog's urinary bladder. *Invest Urol* 1974;12:210-2.
  39. Gómez-Pinilla PJ, Pozo MJ, Camello PJ. Aging impairs neurogenic contraction in guinea pig urinary bladder: Role of oxidative stress and melatonin. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R793-803.
  40. Gomez-Pinilla PJ, Pozo MJ, Camello PJ. Aging differentially modifies agonist-evoked mouse detrusor contraction and calcium signals. *Age (Dordr)* 2011;33:81-8.
  41. Birder LA, Kullmann AF, Chapple CR. The aging bladder insights from animal models. *Asian J Urol* 2018;5:135-40.
  42. Kamei J, Ito H, Aizawa N, Hotta H, Kojima T, Fujita Y, et al. Age-related changes in function and gene expression of the male and female mouse bladder. *Sci Rep* 2018;8:2089.
  43. Zhao W, Aboushwareb T, Turner C, Mathis C, Bennett C, Sonntag WE, et al. Impaired bladder function in aging male rats. *J Urol* 2010;184:378-85.
  44. Suzuki Y, Moriyama N, Okaya Y, Nishimatsu H, Kawabe K, Aisaka K. Age-related change of the role of alpha1L-adrenoceptor in canine urethral smooth muscle. *Gen Pharmacol* 1999;33:347-54.
  45. Takahashi S, Moriyama N, Yamazaki R, Kawabe K. Urodynamic analysis of age-related changes of alpha 1-adrenoceptor responsiveness in female beagle dogs. *J Urol* 1996;156:1485-8.
  46. Triguero D, Lafuente-Sanchis A, Garcia-Pascual A. Changes in nerve-mediated contractility of the lower urinary tract in a mouse model of premature ageing. *Br J Pharmacol* 2014;171:1687-705.
  47. de Oliveira MG, Alexandre EC, Bonilla-Becerra SM, Bertolotto GM, Justo AF, Mónica FZ, et al. Autonomic dysregulation at multiple sites is implicated in age-associated underactive bladder in female mice. *Neurourol Urodyn* 2019;38:1212-21.
  48. Daly DM, Nocchi L, Liaskos M, McKay NG, Chapple C, Grundy D. Age-related changes in afferent pathways and urothelial function in the male mouse bladder. *J Physiol* 2014;592:537-49.
  49. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. *Nat Rev Mol Cell Biol* 2013;14:98-112.
  50. Lowalekar SK, Cristofaro V, Radisavljevic ZM, Yalla SV, Sullivan MP. Loss of bladder smooth muscle caveolae in the aging bladder. *Neurourol Urodyn* 2012;31:586-92.
  51. Furman BL. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc Pharmacol* 2015;70:5.47.1-5.47.20.
  52. Daneshgari F, Liu G, Imrey PB. Time dependent changes in diabetic cystopathy in rats include compensated and decompensated bladder function. *J Urol* 2006;176:380-6.
  53. Nirmal J, Tyagi P, Chuang YC, Lee WC, Yoshimura N, Huang CC, et al. Functional and molecular characterization of hyposensitive underactive bladder tissue and urine in streptozotocin-induced diabetic rat. *PLoS One* 2014;9:e102644.
  54. Daneshgari F, Leiter EH, Liu G, Reeder J. Animal models of diabetic uropathy. *J Urol* 2009;182:S8-13.
  55. Hanna-Mitchell AT, Ruiz GW, Daneshgari F, Liu G, Apodaca G, Birder LA. Impact of diabetes mellitus on bladder uroepithelial cells. *Am J Physiol Regul Integr Comp Physiol* 2013;304:R84-93.
  56. Yonekubo S, Tatemichi S, Maruyama K, Kobayashi M. Alpha1A-adrenoceptor antagonist improves underactive bladder associated with diabetic cystopathy via bladder blood flow in rats. *BMC Urol* 2017;17:64.
  57. Sekido N, Otsuki T, Kida J, Mashimo H, Wakamatsu D, Okada H, et al. EP2 and EP3 receptors as therapeutic targets for underactive bladder/detrusor underactivity due to diabetic cystopathy in a type 1 diabetic rat model. *Low Urin Tract Symptoms* 2020;12:285-91.
  58. Kendig DM, Ets HK, Moreland RS. Effect of type II diabetes on male rat bladder contractility. *Am J Physiol Renal Physiol* 2016;310:F909-22.
  59. Klee NS, Moreland RS, Kendig DM. Detrusor contractility to parasympathetic mediators is differentially altered in the compensated and decompensated states of diabetic bladder dysfunction. *Am J Physiol Renal Physiol* 2019;317:F388-98.
  60. Masuda K, Aizawa N, Watanabe D, Okegawa T, Kume H, Igawa Y, et al. Pathophysiological changes of the lower urinary tract behind voiding dysfunction in streptozotocin-induced long-term diabetic rats. *Sci Rep* 2020;10:4182.
  61. Imamura T, Ishizuka O, Ogawa T, Yamagishi T, Yokoyama H, Minagawa T, et al. Muscarinic receptors mediate cold stress-induced detrusor overactivity in type 2 diabetes mellitus rats. *Int J Urol* 2014;21:1051-8.
  62. Liang CC, Shaw SS, Huang YH, Lin YH, Lee TH. Improvement in bladder dysfunction after bladder transplantation of amniotic fluid stem cells in diabetic rats. *Sci Rep* 2018;8:2105.
  63. Lin CF, Chueh TH, Chung CH, Chung SD, Chang TC, Chien CT. Sulforaphane improves voiding function via the preserving mitochondrial function in diabetic rats. *J Formos Med Assoc* 2020;119:1422-30.
  64. Igawa Y, Zhang X, Nishizawa O, Umeda M, Iwata A, Taketo MM, et al. Cystometric findings in mice lacking muscarinic M2 or M3 receptors. *J Urol* 2004;172:2460-4.
  65. Stromberga Z, Chess-Williams R, Moro C. Prostaglandin E2 and F2alpha modulate urinary bladder urothelium, lamina propria and detrusor contractility via the FP receptor. *Front Physiol* 2020;11:705.
  66. McCafferty GP, Misajet BA, Laping NJ, Edwards RM, Thorneloe KS. Enhanced bladder capacity and reduced prostaglandin E2-mediated bladder hyperactivity in EP3 receptor knockout mice. *Am J Physiol Renal Physiol* 2008;295:F507-14.
  67. Andersson KE. Purinergic signalling in the urinary bladder. *Auton Neurosci* 2015;191:78-81.
  68. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 2000;407:1011-5.
  69. Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, et al. P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. *J Neurosci* 2001;21:5670-7.
  70. Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, et al. P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. *J Physiol* 2005;567:621-39.
  71. Shibasaki K. TRPV4 activation by thermal and mechanical stimuli in disease progression. *Lab Invest* 2020;100:218-23.
  72. Birder L, Kullmann FA, Lee H, Barrick S, de Groat W, Kanai A, et al. Activation of urothelial transient receptor potential vanilloid 4 by 4alpha-phorbol 12,13-didecanoate contributes to altered bladder reflexes in the rat. *J Pharmacol Exp Ther* 2007;323:227-35.
  73. Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K, et al. Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. *J Clin Invest* 2007;117:3453-62.
  74. Schreiber AK, Nones CF, Reis RC, Chichorro JG, Cunha JM. Diabetic

- neuropathic pain: Physiopathology and treatment. *World J Diabetes* 2015;6:432-44.
75. Jiang YH, Kuo HC. Urothelial barrier deficits, suburothelial inflammation and altered sensory protein expression in detrusor underactivity. *J Urol* 2017;197:197-203.
  76. Kong KH, Young S. Incidence and outcome of poststroke urinary retention: A prospective study. *Arch Phys Med Rehabil* 2000;81:1464-7.
  77. Bywater M, Tornic J, Mehnert U, Kessler TM. Detrusor acontractility after acute spinal cord injury-myth or reality? *J Urol* 2018;199:1565-70.
  78. Kuo HC. Quality of life after active urological management of chronic spinal cord injury in eastern Taiwan. *Eur Urol* 1998;34:37-46.
  79. Drake MJ, Hedlund P, Mills IW, McCoy R, McMurray G, Gardner BP, et al. Structural and functional denervation of human detrusor after spinal cord injury. *Lab Invest* 2000;80:1491-9.
  80. Pannek J, Janek S, Sommerer F, Tannapfel A. Expression of purinergic P2X2-receptors in neurogenic bladder dysfunction due to spinal cord injury: A preliminary immunohistochemical study. *Spinal Cord* 2009;47:561-4.
  81. Zeng FS, Zhang L, Cui BJ, Huang LG, Zhang Q, Sun M, et al. Expression of autophagy in different stages of neurogenic bladder after spinal cord injury in rats. *Spinal Cord* 2017;55:834-9.
  82. Lee KH, Kuo HC. Recovery of voiding efficiency and bladder function in male patients with non-neurogenic detrusor underactivity after transurethral bladder outlet surgery. *Urology* 2019;123:235-41.
  83. Jiang YH, Lee CL, Kuo HC. Urothelial dysfunction, suburothelial inflammation and altered sensory protein expression in men with bladder outlet obstruction and various bladder dysfunctions: Correlation with urodynamics. *J Urol* 2016;196:831-7.
  84. Malone-Lee J, Wahedna I. Characterisation of detrusor contractile function in relation to old age. *Br J Urol* 1993;72:873-80.
  85. Pfisterer MH, Griffiths DJ, Schaefer W, Resnick NM. The effect of age on lower urinary tract function: A study in women. *J Am Geriatr Soc* 2006;54:405-12.
  86. Chen SF, Lee CL, Kuo HC. Change of detrusor contractility in patients with and without bladder outlet obstruction at ten or more years of follow-up. *Sci Rep* 2019;9:18887.
  87. Wuest M, Morgenstern K, Graf EM, Braeter M, Hakenberg OW, Wirth MP, et al. Cholinergic and purinergic responses in isolated human detrusor in relation to age. *J Urol* 2005;173:2182-9.
  88. Lepor H, Sunaryadi I, Hartanto V, Shapiro E. Quantitative morphometry of the adult human bladder. *J Urol* 1992;148:414-7.
  89. Gilpin SA, Gilpin CJ, Dixon JS, Gosling JA, Kirby RS. The effect of age on the autonomic innervation of the urinary bladder. *Br J Urol* 1986;58:378-81.
  90. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. II. Aging detrusor: Normal versus impaired contractility. *J Urol* 1993;150:1657-67.
  91. Mansfield KJ, Liu L, Mitchelson FJ, Moore KH, Millard RJ, Burcher E. Muscarinic receptor subtypes in human bladder detrusor and mucosa, studied by radioligand binding and quantitative competitive RT-PCR: Changes in ageing. *Br J Pharmacol* 2005;144:1089-99.
  92. Roberts MW, Sui G, Wu R, Rong W, Wildman S, Montgomery B, et al. TRPV4 receptor as a functional sensory molecule in bladder urothelium: Stretch-independent, tissue-specific actions and pathological implications. *FASEB J* 2020;34:263-86.
  93. Kalil J, D Ancona CAL. Detrusor underactivity versus bladder outlet obstruction clinical and urodynamic factors. *Int Braz J Urol* 2020;46:419-24.
  94. Yang TH, Chuang FC, Kuo HC. Urodynamic characteristics of detrusor underactivity in women with voiding dysfunction. *PLoS One* 2018;13:e0198764.
  95. Carlo Alberto Maggi PS, Meli A, Downie JW. Sympathetic inhibition of reflex activation of bladder motility during filling at a physiological-like rate in urethane anaesthetized rats. *Neurourol Urodyn* 1985;4:37-45.
  96. Jhang JF, Jiang YH, Kuo HC. Transurethral incision of the bladder neck improves voiding efficiency in female patients with detrusor underactivity. *Int Urogynecol J* 2014;25:671-6.
  97. Jhang JF, Jiang YH, Lee CL, Kuo HC. Long-term follow up and predictive factors for successful outcome of transurethral incision of the bladder neck in women with detrusor underactivity. *J Formos Med Assoc* 2016;115:807-13.
  98. Ke QS, Kuo HC. Transurethral incision of the bladder neck to treat bladder neck dysfunction and voiding dysfunction in patients with high-level spinal cord injuries. *Neurourol Urodyn* 2010;29:748-52.
  99. Kihara K, de Groat WC. Sympathetic efferent pathways projecting to the bladder neck and proximal urethra in the rat. *J Auton Nerv Syst* 1997;62:134-42.
  100. Reitz A, Schmid DM, Curt A, Knapp PA, Schurch B. Afferent fibers of the pudendal nerve modulate sympathetic neurons controlling the bladder neck. *Neurourol Urodyn* 2003;22:597-601.