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ANIMAL MODELS OF UROLOGIC CHRONIC PELVIC PAIN SYNDROMES (UCPPS): FINDINGS FROM THE MAPP RESEARCH NETWORK

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Abstract

Objectives—To describe the approach taken by MAPP (Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain) Research Network investigators to advance the utility of UCPPS (urologic chronic pelvic pain syndromes) animal models.

Methods—A multidisciplinary team of investigators representing basic science and clinical expertise defined key phenotypic criteria for rodent models of UCPPS. UCPPS symptoms were prioritized based on their clinical significance. Methods for quantifying animal correlates to patient symptoms were developed. The methods were implemented across proposed rodent models for evaluation and comparison of animals for phenotypic characteristics relevant to human symptomatology.

Results—Pelvic pain and urinary frequency were deemed primary features of human UCPPS and were prioritized for assessment in animals. Nociception was quantified using visceromotor response to bladder distention, and by applying von Frey filaments to the lower abdomen (referred tactile allodynia). Micturition activity was assessed as free voiding using micturition cages or blotting pad assays, and in response to bladder filling by cystometry. Models varied in both depth

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of characterization and degree of recapitulating pelvic pain and urinary frequency characteristics of UCPPS.

Conclusion—Rodent models that reflect multiple, key characteristics of human UCPPS may be identified and provide enhanced clinical significance to mechanistic studies. We have developed a strategy for evaluating current and future animal models of UCPPS based on human symptomatology. This approach provides a foundation for improved translation between mechanistic studies in animals and clinical research, and serves as a validation strategy for assessing validity of models for symptom-driven disorders of unknown etiology.

Keywords

interstitial cystitis; chronic prostatitis; animal models; pain models; translational research; pelvic pain

Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) are defined by chronic pain in the region of the pelvis, often accompanied by lower urinary tract symptoms. Based primarily on their similar symptom profiles, IC/BPS and CP/CPPS may be described under the umbrella term, urologic chronic pelvic pain syndromes (UCPPS). Despite previous research efforts our understanding of UCPPS remains poor, thus slowing the development of therapies.

Many underlying contributors to UCPPS have been postulated, including defective urothelial integrity and function, changes in sensitization and neuroplasticity, infectious etiologies, and inflammatory events. These have been explored in clinical studies of patients and *in vitro* approaches using cell culture systems and patient-derived biological samples. In addition, numerous research efforts have explored pathophysiology using various animal model systems. Although animal models have the potential to inform disease mechanisms at the molecular, cellular, organ and system levels, the value of animal models has been questioned due to the perceived lack of correlation between the models and human condition.^{1–3} To address this important caveat and enhance the translational relevance of animal studies, a standardized strategy to phenotype and validate animal models relative to UCPPS is needed.

In 2008, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) initiated the Multi-disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network to rigorously study UCPPS using an interdisciplinary approach. As the flagship NIDDK initiative for UCPPS research, the MAPP Research Network employs an integrated and multidisciplinary research design involving complementary clinical, epidemiologic and mechanistic studies. The MAPP Animal Models Working Group is engaged in animal model-based studies of potential UCPPS pathophysiological mechanisms. As part of this effort the Working Group has developed a strategy for validating models based on human symptom profiles, thus enhancing the significance of mechanistic findings by evaluating animal models in the context of clinical disease. Here, we describe our

approach to define key clinical criteria and phenotyping strategies for enhancing the translational relevance of UCPPS models.

This multidisciplinary approach comprises the collaborative efforts of MAPP Research Network leadership, urologists with a clinical focus on UCPPS diagnosis and management, and basic scientists with research expertise in mechanistic studies of UCPPS. Potential rodent models of interest were evaluated for key symptomatic commonalities to human UCPPS with an emphasis on IC/BPS. The hallmark symptoms of pelvic/bladder pain and urinary frequency^{4,5} drove the development of animal model phenotyping strategies. Technical methods for quantifying rodent correlates to clinical findings were developed on the basis of feasibility and physiologic relevance, and these methods have been implemented for ongoing MAPP Network studies.

Material and Methods

1. Assessing Nociception (Figure 1)

- a. ***Quantifying nociception with the abdominal visceromotor response (VMR).***
IC/BPS patients reported increasing pain with bladder filling.⁵ In rodents, this was examined with the bladder distention and VMR paradigm (described in detail in Figures 1A–B).⁶ VMR was used as a surrogate measure of nociception (pain) evoked by bladder distention. During phasic bladder distention, an increase of abdominal VMR or electromyographic signals corresponds to increased guarding and abdominal withdrawal when the animal experiences bladder pain from the distention. The VMR shows a dose-response relation with bladder distention pressure,⁶ which mimics the hypersensitivity (hyperalgesia) to bladder distention observed in IC/BPS patients.⁵ The VMR is facilitated by inflammation and inhibited by analgesics.⁷ Although changes in the VMR (electromyographic signals) of the body wall musculature are dependent upon somatic innervation of the referred body region, it nevertheless serves as a surrogate measure of bladder distention pain.
- b. ***Quantifying nociception and referred tactile allodynia with von Frey filaments.***
Pain originating from a visceral organ is typically referred to a corresponding “dermatome” on the skin that shares spinal innervation with the given visceral organ (viscerosomatic convergence).⁸ The most common site of referred pain in IC/BPS is the suprapubic area.⁹ IC/BPS patients also demonstrate hypersensitivity to mechanical stimulation in the suprapubic area during quantitative sensory testing.¹⁰ Therefore, to assess pelvic nociception in animals, we quantified the referred tactile allodynia of the lower abdominal region in response to applied force with a calibrated series of nylon von Frey filaments (described in details in Figures 1C–G).¹¹ The von Frey allodynia response is inhibited by analgesics.¹¹

2. Assessing Urinary Frequency (Figure 2)

- a. ***Quantifying free voiding activities using micturition cages.*** Mice were placed in individual micturition cages that are available commercially (Columbus Instruments, Columbus, OH). Urinary frequency, voided volumes, total urinary

output, and general activity were recorded in real-time as described in details in Figures 2A–C. The use of micturition cages to quantify free voiding in mice has previously been described.¹²

- b. **Determining free voiding activities using urine blotting patterns.** Free voiding activity of rodents was determined semi-quantitatively through a blotting pad assay (described in details in Figure 2D). In this approach a rodent was placed into a cage with mesh floor (or an empty cage) with a blotting pad below, and voiding events were identified as urine spots visualized under UV illumination or stained with methylene blue and calibrated relative to known water volumes. Urinary frequency in 24 hours (numbers of urine spots), voided volume per void (sizes of urine spots) and total voided volume in 24 hours can be quantified as previously described.¹³ Important caveats with this assay are territorial marking behavior in males and the tendency of some inbred lines to repeatedly void at the corners of the cage.
- c. **Filling cystometry.** Bladder function and urinary frequency in response to bladder filling during urodynamics were also quantified using standardized methodology in rodents to complement the free voiding data.¹⁴ Filling cystometry permitted quantification of detrusor pressure, contractility, compliance, inter-contraction intervals, and the presence or absence of non-voiding bladder contractions.

3. Rodent models

The following proposed UCPPS models were phenotyped by MAPP Network investigators with respect to bladder/pelvic nociception (pain) and urinary frequency: (a) animals monitored during/following transient urinary tract infection (acute UTI mice, and mice with post-UTI pain),^{15–17} (b) animals infected with the pseudorabies virus (PRV mice),^{11,18,19} (c) animals exhibiting cyclophosphamide-induced cystitis (CYP mice),⁶ (d) animals expressing urothelial OVA (URO-OVA mice),²⁰ (e) animals expressing urothelial MCP-1 (URO-MCP-1 mice). This list does not represent an exhaustive list of potential UCPPS models or intends to exclude other models; rather this list represents the models used by MAPP Network investigators in their mechanistic studies of UCPPS. In addition, the following models (not under investigation in the MAPP Network) were selectively incorporated in this review for their UCPPS relevance: (f) Feline IC,^{2,21} (g) Stress models using either the water avoidance stress (WAS)^{22,23} or repeated variate stress (RVS)²⁴ paradigms.

Results

Rodent models were phenotyped during MAPP Research Network studies or prior work with respect to each of the following three key factors: (1) nociception to bladder distention, (2) pelvic nociception, and/or (3) urinary frequency, using the methods described previously. Figures 1 and 2 illustrate the methods used to assess nociception and voiding activities and select data from MAPP Network investigators. The overall results are summarized in Table 1. Table 2 describes other features of the models (sex bias, mast cell involvement, inflammation or urothelial lesions, spontaneous pain behaviors, pain outside the pelvis, and bowel-bladder cross-talk). These features are also relevant to clinical findings

in some UCPPS patients and may provide added value as secondary measures for validating animal models based on human symptoms.

The models phenotyped here recapitulate the key symptomatic features of IC/BPS in whole or in part. All rodents demonstrate pelvic nociception (referred tactile allodynia) with von Frey filament testing. However, some models are more extensively characterized than others. Nociception to bladder distention, pelvic nociception, and urinary frequency (all three key factors) are recapitulated in the CYP and the stress models (WAS or RVS). Pelvic nociception and urinary frequency (two key factors) are present in the URO-OVA and URO-MCP-1 models. The UTI, and PRV models demonstrate bladder hyperalgesia but there was no reported data on urinary frequency. Feline IC cats vocalize during urination suggesting painful behaviors, however, no data on VMR or von Frey testing with IC cats was found in the literature. The strengths and weaknesses of each model are presented in Table 1.

The magnitude of change in some of these animals closely resembled those observed in humans. For example, IC/BPS patients report pain ratings twice as high as those of controls during bladder filling experiments.⁵ UTI-treated mice and CYP-treated mice also show VMR magnitudes (a surrogate measure of bladder distention evoked pain) about twice as high compared to their respective controls.^{6,17} The percent changes in urinary frequency in the CYP model¹³ and the two stress models^{22,24} are also similar to those observed in IC/BPS patients.⁵

Comment

Animal models, by definition, cannot perfectly reflect human disease, yet medical history is replete with mechanistic insights gleaned from animal studies that were otherwise impossible, too invasive, or unethical to obtain from human patients. The shortcomings of animal models of pain, UCPPS in particular, are significant and can undermine the translational value of resulting mechanistic findings if not considered carefully.^{1–3} However, models that reflect multiple, key characteristics of human UCPPS can be identified to provide enhanced clinical significance to mechanistic studies. Here we have described the collaborative approach developed by the MAPP Research Network to define clinically relevant models to study UCPPS. This effort has yielded a unique strategy for validation of animals (specifically rodents) as models based on similarities with key symptoms in patients.

A general consensus among national societies with a mission in IC/BPS is that patients report unpleasant sensations (pain, pressure, discomfort) perceived to be related to the bladder, associated with lower urinary tract symptoms such as frequency, in the absence of other identifiable causes.⁴ Indeed, chronic pelvic/bladder pain is the cardinal symptom of IC/BPS.⁴ IC/BPS patients also demonstrate hypersensitivity to bladder distention.⁵ Patients reported increasing pain with bladder filling, increased urinary frequency, and decreased cystometric capacity compared to controls. As a result of these clinical findings, we deem bladder/pelvic pain and urinary frequency as the cardinal features that animal models should recapitulate. These features were given the highest priority in evaluating potential animal models in this study.

The MAPP Research Network Animal Models Working Group determined that pelvic/bladder pain and voiding dysfunction (urinary frequency) are the hallmarks of IC/BPS and are thus essential elements of clinically-relevant mechanistic models. As a result, we make the recommendation that: (1) Animal models of IC/BPS must recapitulate, at minimum, pelvic/bladder nociception (pain) and/or voiding dysfunction, and (2) Models that recapitulate only histopathology or biochemical findings (e.g. urinary markers) in the absence of nociception or micturition correlates are inadequate for inferring mechanistic insights or as a basis for translational studies.

The research methodologies enumerated here have strong clinical correlates. One hallmark symptom of IC/BPS is bladder pain and hypersensitivity to bladder distention.⁵ In rodents, bladder distention evoked nociception was assessed using the VMR (Figure 1A). IC/BPS patients also demonstrated mechanical hypersensitivity to noxious stimulation in the suprapubic area during quantitative sensory testing.¹⁰ This clinical feature was assessed in rodents by applying von Frey filaments to the lower abdomen and quantifying the referred tactile allodynia (Figure 1C). Lower urinary tract symptoms are common among IC/BPS patients (e.g. urinary frequency, decreased voided volumes). These symptoms were assessed using cystometry, micturition cages, or blotting papers (Figure 2). Together, this core set of methods is amenable to quantifying UCPPS clinical correlates in rodent models.

MAPP Research Network investigators utilize a range of UCPPS models that reflect the diversity of postulated mechanisms. Historically, given the early understanding of IC/BPS as a chronic inflammatory condition of the bladder, inflammatory models have been highly represented in studies of UCPPS. Here, the clinical relevance of bladder inflammatory models was reexamined.¹⁻³ In the NIH-sponsored Interstitial Cystitis Data Base Project,²⁵ granulation tissue was found in 15% of biopsies. 51% of patients had mast cells in the lamina propria, and lamina propria mast cells and urothelial lesions were correlated with symptoms. In a bladder biopsy study that recruited 69 patients using the older NIDDK research criteria, 39% of patients had severe/moderate inflammation, 41% had mild inflammation, and 30% had no bladder inflammation.²⁶ Thus, inflammation is a variable finding in IC/BPS, so inflammatory models are relevant to a subset of patients.

Beyond the consequences of inflammation, an important consideration for animal models is how inflammation is induced. The CYP model is perhaps the most commonly used rodent model to study IC/BPS^{6,8,13,27}, yet exposure to CYP or its metabolite acrolein probably has little etiological basis in the clinical syndrome. There are some concerns that potential systemic side effects of CYP may reduce the clinical relevance of the model.¹³ Nevertheless, the model retains value in examining select biological processes of importance to bladder function and bladder pain: it recapitulates the key nociceptive and voiding phenotypes of IC/BPS (e.g. bladder distention pain, referred tactile allodynia, spontaneous painful behaviors, and urinary frequency, see Table 1), and other associated features (e.g. mast cells activation, bladder-bowel cross-talk, and elevated urinary nerve growth factor NGF levels, see Table 2).

Biologic inflammatory models utilize immune, viral or transgenic strategies and are actively studied within the MAPP Research Network. Sensitization to antigens recapitulates features

of IC/BPS, and the URO-OVA (cystitis) model may inform general bladder inflammatory mechanisms and provide further insights into visceral organ (bowel-bladder) crosstalk when used in conjunction with the Fabpl-OVA, (colitis) and URO/Fabpl-OVA (cystitis-colitis) models. Specific inflammatory mediators like MCP-1 have also been implicated in the pathophysiology of IC/BPS.²⁸ Data suggested that transgenic models over-expressing MCP-1 in the urothelium recapitulated pelvic pain (Figure 1G). Unlike models initiated by bladder insult that show peripheral sensitization and then progress to central sensitization (e.g., CYP, UTI),^{6,16} the PRV model involves neurogenic inflammation that recapitulates mast cell and urothelial pathologic findings associated with IC as well as pelvic specificity and organ crosstalk.¹¹

Active UTI is considered an exclusion criterion of IC/BPS. However, studies suggest that IC/BPS may be initiated by bladder infection, which then leads to post-infection bladder sensitization characterized by chronic bladder pain long after the bacterial load has cleared. Indeed, 18–36% of women presented with a documented UTI (positive cultures and/or nitrite) at the onset of IC/BPS symptoms.²⁹ Women with a history of documented recurrent UTI (≥ 3 per year) were also more likely to develop bladder hypersensitivity.³⁰ Therefore, the model using mutant *E. coli* strains lacking O-antigen expression to mimic post-UTI chronic pain may be relevant, given the preliminary studies suggesting irritative voiding behavior (Figures 1E).¹⁶

Feline IC is the only naturally occurring model of IC/BPS.^{2,21} While this chronic model offers obvious advantages over an induced rodent model, the utility of feline IC as a research model is hampered by the lack of genetic tools and difficulties of access for many researchers. Also, specific experiments to assess bladder distention pain (VMR) and pelvic tactile allodynia (von Frey filaments) have not been reported in the literature. Two rat models to study IC/BPS are the water avoidance stress (WAS) model and the repeated variate stress (RVS) models. Exposure of high-anxiety rats to WAS or RVS produced a phenotype characterized by bladder nociception (increased VMR),²³ referred tactile allodynia,²⁴ and urinary frequency,^{22,24} thus mimicking IC/BPS symptoms without inflicting bladder inflammation or noxious stimulation. The drawback of these stress models is their apparent limitation to specific rat strains. It has been difficult to use mice in these stress paradigms, thus genetic approaches in murine systems are not readily available here. Together these considerations indicate that rodents – particularly the mouse with its wealth of genetic tools – offer multiple approaches to effectively model key aspects of IC/BPS.

UCPPS is particularly challenging to model because it is a symptom-driven disorder of unknown etiology and pathophysiology. Many of the models phenotyped here recapitulated the key symptomatic features of IC/BPS in whole or in part. Although this symptom-based validation strategy may not necessarily disclose the underlying pathophysiology of the human condition, it is a necessary first-step to ensure that the preclinical models proposed for mechanistic studies of UCPPS at least recapitulate the key symptomatology of the human condition. Due to patient heterogeneity and the multifactorial complexities of UCPPS, it is unlikely that any single model will completely and perfectly reflect the human syndrome. Thus, a selected panel of models will be utilized for mechanistic studies for the foreseeable future.

Ultimately, the development of improved animal models for UCPPS will require a mechanistic understanding of the clinical syndrome. Because there is no known mechanism for the induction or maintenance of UCPPS, current animal models rely on reproducing the primary symptoms of the disorder. The overarching goal of the MAPP Research Network is to develop new insights into UCPPS that may ultimately inform clinical trials and improve patient care. Findings from ongoing clinical studies can be used to inform the development of new mechanistically valid animal models. This translation of clinical insights into the development of models will facilitate physiological, molecular, and interventional studies in animals to identify causes and new treatments for UCPPS. With our limited understanding of UCPPS, the field should strive to employ animal models that best recapitulate key symptomatology in patients. Here, we have focused on a subset of animal models that fulfill these criteria and, therefore, demonstrate increased validity for studies of UCPPS. The MAPP Research Network offers a unique opportunity for validation of animal models through our integrated and multidisciplinary approach. Further refinement of these models based on findings from ongoing MAPP Research Network studies will identify potential targets for therapeutic development and enable future preclinical intervention studies. While current studies primarily focus on IC/BPS models, our approach is generalizable to UCPPS and other symptom-driven disorders of unknown etiology.

Conclusions

The MAPP Network has developed a strategy for evaluating current and future animal models of UCPPS based on human symptomatology. This approach provides a foundation for improved translation between mechanistic studies in animals and clinical research, and serves as a validation strategy for assessing validity of models for symptom-driven disorders of unknown etiology.

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Abbreviations

CP/CPPS	chronic prostatitis/chronic pelvic pain syndrome
IC/BPS	interstitial cystitis/bladder pain syndrome
MAPP	Multi-disciplinary Approach to the Study of Chronic Pelvic Pain
MCP-1	monocyte chemotactic protein-1
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
OVA	ovalbumin
PRV	pseudorabies virus
UCPPS	urologic chronic pelvic pain syndromes

UTI	urinary tract infection
VMR	visceromotor response

References

1. Bjorling DE, Wang ZY, Bushman W. Models of inflammation of the lower urinary tract. *Neurourol Urodyn*. 2011; 30:673–82. [PubMed: 21661012]
2. Westropp JL, Buffington CA. In vivo models of interstitial cystitis. *J Urol*. 2002; 167:694–702. [PubMed: 11792956]
3. Fry CH, Daneshgari F, Thor K, Drake M, Eccles R, Kanai AJ, Birder LA. Animal models and their use in understanding lower urinary tract dysfunction. *Neurourol Urodyn*. 2010; 29:603–8. [PubMed: 20432320]
4. Hanno PM, Burks DA, Clemens JQ, Dmochowski RR, Erickson D, Fitzgerald MP, Forrest JB, Gordon B, Gray M, Mayer RD, et al. AUA Guideline for the Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome. *J Urol*. 2011; 185:2162–70. [PubMed: 21497847]
5. Ness TJ, Powell-Boone T, Cannon R, Lloyd LK, Fillingim RB. Psychophysical evidence of hypersensitivity in subjects with interstitial cystitis. *J Urol*. 2005; 173:1983–7. [PubMed: 15879797]
6. Lai HH, Qiu CS, Crock LW, Morales ME, Ness TJ, Gereau RWt. Activation of spinal extracellular signal-regulated kinases (ERK) 1/2 is associated with the development of visceral hyperalgesia of the bladder. *Pain*. 2011; 152:2117–24. [PubMed: 21705143]
7. Ness TJ, Elhefni H. Reliable visceromotor responses are evoked by noxious bladder distention in mice. *J Urol*. 2004; 171:1704–8. [PubMed: 15017270]
8. Bielefeldt K, Lamb K, Gebhart GF. Convergence of sensory pathways in the development of somatic and visceral hypersensitivity. *Am J Physiol Gastrointest Liver Physiol*. 2006; 291:G658–65. [PubMed: 16500917]
9. Warren JW, Langenberg P, Greenberg P, Diggs C, Jacobs S, Wesselmann U. Sites of pain from interstitial cystitis/painful bladder syndrome. *J Urol*. 2008; 180:1373–7. [PubMed: 18707715]
10. Lai HH, Gardner V, Ness TJ, Gereau RWt. Segmental Hyperalgesia to Mechanical Stimulus in Interstitial Cystitis/Bladder Pain Syndrome – Evidence of Central Sensitization. *J Urol*. 2013
11. Rudick CN, Chen MC, Mongiu AK, Klumpp DJ. Organ cross talk modulates pelvic pain. *Am J Physiol Regul Integr Comp Physiol*. 2007; 293:R1191–8. [PubMed: 17626130]
12. Wood R, Eichel L, Messing EM, Schwarz E. Automated noninvasive measurement of cyclophosphamide-induced changes in murine micturition frequency and volume and demonstration of pharmacologic sensitivity. *Urology*. 2001; 57:115–6.
13. Boudes M, Uvin P, Kerselaers S, Vennekens R, Voets T, De Ridder D. Functional characterization of a chronic cyclophosphamide-induced overactive bladder model in mice. *Neurourol Urodyn*. 2011; 30:1659–65. [PubMed: 21717507]
14. Andersson KE, Soler R, Fullhase C. Rodent models for urodynamic investigation. *Neurourol Urodyn*. 2011; 30:636–46. [PubMed: 21661007]
15. Rudick CN, Billips BK, Pavlov VI, Yaggie RE, Schaeffer AJ, Klumpp DJ. Host-pathogen interactions mediating pain of urinary tract infection. *J Infect Dis*. 2010; 201:1240–9. [PubMed: 20225955]
16. Rudick CN, Jiang M, Yaggie RE, Pavlov VI, Done J, Heckman CJ, Whitfield C, Schaeffer AJ, Klumpp DJ. O-antigen modulates infection-induced pain states. *PLoS One*. 2012; 7:e41273. [PubMed: 22899994]
17. Stemler KM, Crock LW, Lai HH, Mills JC, Gereau RWt, Mysorekar IU. Protamine sulfate induced bladder injury protects from distention induced bladder pain. *J Urol*. 2013; 189:343–51. [PubMed: 23174261]
18. Rudick CN, Bryce PJ, Guichelaar LA, Berry RE, Klumpp DJ. Mast cell-derived histamine mediates cystitis pain. *PLoS One*. 2008; 3:e2096. [PubMed: 18461160]

19. Rudick CN, Pavlov VI, Chen MC, Klumpp DJ. Gender specific pelvic pain severity in neurogenic cystitis. *J Urol.* 2012; 187:715–24. [PubMed: 22177208]
20. Liu W, Evanoff DP, Chen X, Luo Y. Urinary bladder epithelium antigen induces CD8+ T cell tolerance, activation, and autoimmune response. *J Immunol.* 2007; 178:539–46. [PubMed: 17182594]
21. Buffington CA, Chew DJ, Kendall MS, Scrivani PV, Thompson SB, Blaisdell JL, Woodworth BE. Clinical evaluation of cats with nonobstructive urinary tract diseases. *J Am Vet Med Assoc.* 1997; 210:46–50. [PubMed: 8977647]
22. Smith AL, Leung J, Kun S, Zhang R, Karagiannides I, Raz S, Lee U, Glovatscka V, Pothoulakis C, Bradesi S, et al. The effects of acute and chronic psychological stress on bladder function in a rodent model. *Urology.* 2011; 78:967 e1–7. [PubMed: 21868072]
23. Robbins M, DeBerry J, Ness T. Chronic psychological stress enhances nociceptive processing in the urinary bladder in high-anxiety rats. *Physiol Behav.* 2007; 91:544–50. [PubMed: 17521683]
24. Merrill L, Malley S, Vizzard MA. Repeated variate stress in male rats induces increased voiding frequency, somatic sensitivity, and urinary bladder nerve growth factor expression. *Am J Physiol Regul Integr Comp Physiol.* 2013; 305:R147–56. [PubMed: 23657640]
25. Tomaszewski JE, Landis JR, Russack V, Williams TM, Wang LP, Hardy C, Brensinger C, Matthews YL, Abele ST, Kusek JW, et al. Biopsy features are associated with primary symptoms in interstitial cystitis: results from the interstitial cystitis database study. *Urology.* 2001; 57:67–81. [PubMed: 11378053]
26. Denson MA, Griebing TL, Cohen MB, Kreder KJ. Comparison of cystoscopic and histological findings in patients with suspected interstitial cystitis. *J Urol.* 2000; 164:1908–11. [PubMed: 11061878]
27. Bon K, Lichtensteiger CA, Wilson SG, Mogil JS. Characterization of cyclophosphamide cystitis, a model of visceral and referred pain, in the mouse: species and strain differences. *J Urol.* 2003; 170:1008–12. [PubMed: 12913760]
28. Tyagi P, Nikolavsky D, Vodovotz Y, Barclay D, Tyagi V, Peters KM, Chancellor MB. Urine levels of selected chemokines positively correlate with lower bladder capacity and psychometric scores in IC/BPS patients. *J Urol.* 2009; 181:21.
29. Warren JW, Brown V, Jacobs S, Horne L, Langenberg P, Greenberg P. Urinary tract infection and inflammation at onset of interstitial cystitis/painful bladder syndrome. *Urology.* 2008; 71:1085–90. [PubMed: 18538691]
30. Arya LA, Northington GM, Asfaw T, Harvie H, Malykhina A. Evidence of bladder oversensitivity in the absence of an infection in premenopausal women with a history of recurrent urinary tract infections. *BJU Int.* 2012; 110:247–51. [PubMed: 22129305]

Figure 1.

A. Quantitation of nociception using the bladder distention and VMR paradigm in mice (adapted from Lai et al 2011).⁶ Silver wire electrodes were placed on the external oblique abdominal muscle to allow differential amplification of the abdominal EMG signals. An angiocatheter was inserted into the bladder. Phasic bladder distention (20 to 80 mmHg, for 20 s) was used to assess bladder distention evoked nociception. The VMR signals were subtracted from the baseline, rectified, and integrated (area under the curve).

B. Development of bladder hyperalgesia and allodynia in the CYP mouse model (ANOVA, $p < 0.0001$). There was a left-shift of the stimulus-response curve. Stimulus that was normally painful (e.g. >50 mmHg) became more painful (hyperalgesia) while stimulus that was not

painful (e.g. <30 mmHg) became noxious (allodynia). * $p<0.05$, ** $p<0.01$, $n=8$ mice in CYP group, $n=10$ mice in saline group (adapted from Lai et al 2011).⁶

C. Quantification of pelvic nociception and referred tactile allodynia using the calibrated von Frey filaments in mice (adapted from Rudick et al 2007).¹¹ Mice were tested in individual Plexiglas chambers with a stainless steel wire grid floor. Frequency of withdrawal responses to the application of von Frey filaments was tested using five individual fibers with forces of 0.04, 0.16, 0.4, 1, and 4 g. Each filament was applied for ~1 s with an inter-stimulus interval of 2–5 s for a total 10 times, and the filaments were tested in ascending order of force. Stimulation was confined to the lower abdominal area in the general vicinity of the bladder, and care was taken to stimulate different areas within this region to avoid desensitization or “wind up” effects. Three types of behaviors were considered as positive responses: 1) sharp retraction of the abdomen, 2) immediate licking or scratching of the area of filament stimulation, or 3) jumping. To assess pain outside the pelvis, von Frey filaments were applied to the hind paw or fore paw.

D. PRV induced pelvic pain in female B6 mice. Referred tactile allodynia was measured using von Frey filaments. Data were mean percentages of response frequency before (baseline) and at post-infection days (PID) 1, 2, 3, and 4. ANOVA indicated a significant increase in response frequency from baseline at all filaments tested at PID3 and PID4 ($p=0.04$, $n=11$) (adapted from Rudick et al 2007).¹¹

E. Infection with uropathogenic E coli (UPEC) NU14 induced acute pain that resolved after the UTI ($p<0.001$ Days 2–5, $p<0.01$ Days 1 and 6, $p<0.05$ Days 7–10), while infection with *waaL* (mutant UPEC defective in O-antigen expression) induced chronic post-UTI pain after the second urinary infection (adapted from Rudick et al 2012).¹⁶

F. URO-OVA mice exhibited pelvic pain after cystitis induction with intravenous OT-I splenocytes. Mice were stimulated at the suprapubic area with von Frey filaments before (baseline) and 7 or 14 days after cystitis induction. *indicated responses that were significantly different from baseline ($p<0.05$).

G. URO-MCP-1 mice showed pelvic pain in response to intravesical LPS (lipopolysaccharide). Mice were stimulated at the suprapubic area with von Frey filaments before (baseline) and 1 or 3 days after cystitis induction. *indicated responses that were significantly different from baseline ($p<0.05$).

Figure 2.

A. Quantification of free voiding activities in mice using micturition cages. Mice were placed in individual custom-built micturition cages (Columbus Instruments, Columbus, OH) for 24-hour real time recording of voiding habits with 12-hour light and 12-hour dark cycles. The cages consist of a fluid receptacle that funnels the captured urine droplets into a specimen freezer that serves to prevent urine evaporation and chemical changes. A balance beneath the specimen freezer measures the weight of the collected urine. In addition, the cages are fitted with photocell-based activity monitors to record animal locomotion (in terms of the number of blockage of infrared beams during an interval). The status of animal activity reflects animal general wellbeing and may indirectly be used to assess pain. Mice had free access to drinking water but were restrained from solid food to prevent feces from interfering with measurement of urine output. The entire system was computer interfaced for automated data acquisition using Oxymax software (Columbus Instruments). Data were

acquired in 2-minute intervals during the 24-hour period. Urinary frequency and voided volume per micturition were measured.

B. URO-OVA mice showed voiding dysfunction after cystitis induction with intravenous OT-I splenocytes. At day 7 after cystitis induction, mice were placed in micturition cages for 24-hour micturition recording. Compared to baseline, cystitis-induced URO-OVA mice developed urinary frequency (11.6 ± 1.5 vs 6.2 ± 1.6 times, $p=0.0004$), decreased voided volume per micturition (0.113 ± 0.03 vs 0.274 ± 0.07 grams, $p=0.0002$), decreased bladder capacity (0.312 ± 0.147 vs 0.581 ± 0.092 grams, $p=0.0007$), and increased urination at night (6.4 ± 2.3 vs 3.1 ± 1.3 times, $p=0.0374$). The cystitis-induced URO-OVA mice also exhibited increased locomotion in the micturition cages. Red lines: a dark period.

C. URO-MCP-1 mice showed voiding dysfunction in response to intravesical LPS (lipopolysaccharide). At day 1 after cystitis induction, mice were placed in micturition cages for 24-hour micturition recording. Urinary frequency and urine output per void were recorded. Red lines: a dark period. $p<0.05$ for both mean urinary frequency and mean urine volume per void when compared between the two groups. i.b.: Intravesical administration.

D. Using blotting paper patterns to assess free voiding activities in mice. Mice were injected with intravenous methylene blue (2.5 mg/kg) after cystitis induction (URO-OVA model). Individual mice were kept in separate metabolic cages with a filter paper underneath. The urinary frequency per 24 hours was recorded by numerating blue drops and areas at days 1, 4, 7, 10, and 13 after cystitis induction. The blue areas were converted into volumes (mL) based on the diffusion factor established for the filter paper. Hollow dots: control B6 mice. Filled dots: URO-OVA mice.

Table 1

Assessing of key IC/BPS features in the animal models

Human IC/BPS					
	Bladder nociception	Pelvic nociception	Voiding (urinary frequency)	Strength of the model	Weakness of the model
Human IC/BPS	Yes, chronic bladder pain, pressure or discomfort in the bladder/pelvis is the cardinal symptoms of IC/BPS. ⁴ Most patients have increasing pain with bladder distention (doubling of pain rating compared to controls). ⁵	Yes, chronic bladder pain, pressure or discomfort in the bladder/pelvis is the cardinal symptoms of IC/BPS. ⁴ The most common sites of referred pain are the suprapubic area, urethra, genitals and lower back. ⁹ Patients also have hypersensitivity to mechanical stimulation in the suprapubic area during quantitative sensory testing (25–40% increase in pain rating compared to controls). ¹⁰	Yes, lower urinary tract symptoms like frequency, urgency are common. The cystometric capacity and volume of first desire to void were significantly reduced during urodynamics (>50% reduction of capacity, >50% increase in urinary frequency). ⁵		
Mouse models used by MAPP Research Network investigators					
Model System	Bladder nociception	Pelvic nociception	Voiding (urinary frequency)	Strength of the model	Weakness of the model
UTI (acute bacterial cystitis)	Yes, increased visceromotor response to bladder distention in mice infected with UPEC isolate UTI89 (doubling of VMR). ¹⁷	Yes (von Frey), infection with UPEC isolate NU14 induced referred tactile allodynia that peaked at 2 days and subsided when the UTI resolved, see Figure 1E. ¹⁵ Asymptomatic bacteriuria strain ASB 83972 did not induce allodynia. ¹⁵	Not reported	Both bladder and pelvic nociception were demonstrated.	Urinary frequency has not been evaluated. No active UTI in IC/BPS.
Post-UTI pain	Persistent	UPEC mutant defective for O-antigen developed chronic allodynia that persisted after clearance of UTI. ¹⁶ See Figure 1E (see the tracing of <i>walL</i>).	Increased (unpublished results)	UPEC model defective in O-antigen expression may be used to study chronic post-UTI pain; consistent with prior UTI history among some IC/BPS patients.	
PRV (neurogenic cystitis)	Not reported	Yes (von Frey), increased referred tactile allodynia was noted on post-infection days 2, 3 and 4 (e.g. doubling of withdrawal response on post-infection day 4 compared to prior to infection). See Figure 1D. ¹¹	Not reported	Pain is specific to the pelvis (no systemic pain).	Bladder nociception to distention and urinary frequency were not evaluated. No active PRV infection in IC/BPS.

Human IC/BPS					
	Bladder nociception	Pelvic nociception	Voiding (urinary frequency)	Strength of the model	Weakness of the model
CYP (chemical cystitis)	Yes, increased visceromotor response to bladder distention (doubling of VMR). See Figure 1B. ⁶	Yes (von Frey), dose-dependent referred tactile allodynia was observed. ^{13,27}	Yes (cystometry, blotting paper pattern), number of urine spots was increased (by >50%), voided volume per void was decreased (by >50%), intercontractile intervals was decreased (by ~35%), ^{8,13}	Urinary frequency, bladder nociception and pelvic nociception were all demonstrated by multiple studies.	No active chemical cystitis in IC/PBS.
URO-OVA (autoimmune cystitis)	Not reported	Yes (von Frey), dose-dependent referred tactile allodynia was observed, see Figure 1F.	Yes (micturition cages, blotting paper pattern), increased frequency, increased nocturia, decreased voided volume, decreased bladder capacity, see Figures 2B and 2D.	Voiding dysfunction (increased frequency, increased nocturia, decreased voided volume, decreased bladder capacity) and pelvic nociception were demonstrated.	Bladder nociception was not evaluated. No strong evidence of bladder autoimmunity in IC/BPS.
URO-MCP-1 (bladder MCP-1 expression)	Not reported	Yes (von Frey), dose-dependent referred tactile allodynia was observed, see Figure 1G.	Yes (micturition cages), increased frequency, increased nocturia, decreased voided volume, decreased bladder capacity, see Figure 2C.	Voiding dysfunction (increased frequency, increased nocturia, decreased voided volume, decreased bladder capacity) and pelvic nociception were demonstrated. The bladders are hypersensitive to a low dose of stimuli such as LPS.	Bladder nociception was not evaluated.
Additional proposed models					
Model System	Bladder nociception	Pelvic nociception	Voiding (urinary frequency)	Strength of the model	Weakness of the model
Feline IC	±, enhanced firing of Aδ afferents during bladder distention compared to controls, but there were no reported visceromotor response experiments. Increased firing may or may not be related to pain versus fullness.	±, antedoctal report of vocalization with abdominal palpation, but this was not quantified.	Yes (cystometry), increased frequency, reduced voided volume, reduced capacity, no detrusor overactivity. ²	The only naturally occurring chronic model, not involving noxious stimulation of the bladder. Urinary frequency was demonstrated.	Nociception has not been fully evaluated (e.g. VMR, tactile allodynia). In cats only – no genetic tools or knockout animals. Access difficulties for many researchers, and expensive.
Stress models in rats (WAS = water avoidance stress, RVS = repeated variate stress)	Yes, increased visceromotor response to bladder distention in WAS rats compared to sham (doubling of VMR). ²³	Yes (von Frey), increased referred tactile allodynia was noted in the RVS model (doubling of withdrawal response). ²⁴	Yes (micturition cage, cystometry), increased frequency (by >80%), reduced voided volume in both WAS and RVS models. ^{22,24}	Urinary frequency, bladder and pelvic nociception were demonstrated. More naturalistic model, not involving noxious stimulation of the bladder.	In rats but not in mice – no genetic tools or knockout animals. While stress may exacerbate existing symptoms, it is unlikely that stress

Human IC/BPS				
Bladder nociception	Pelvic nociception	Voiding (urinary frequency)	Strength of the model	Weakness of the model
				is the sole etiological factor of IC/BPS; is the sole etiological factor of IC/BPS.

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Table 2

Other secondary features of the animal models

Human IC/BPS						
	Sex bias?	Mast cell involvement ?	Inflammation or urothelial lesions?	Spontaneous pain behaviors?	Pain outside the pelvis?	Bowel-bladder interaction?
Human IC/BPS	Historically IC/BPS is believed to be more common in females, but a recent RAND study revealed that male IC/BPS might be more common than previously thought, and there might be overlap between male IC/BPS and CP/CPPS.	Yes, in a subset of patients, 51% of patients have mast cells in lamina propria. ²⁵	Yes, in a subset of patients, 39%, 41% and 30% have severe/moderate, mild, and no inflammation respectively. ²⁶	Yes, patients reported ongoing pain without stimulation. In animals, spontaneous (stimulus-independent) behaviors consistent with pain may also be quantified (e.g. rounded back posture, licking of the abdomen, stretching, abdominal retraction, sudden tail hyperextension, and decreased locomotion and activities).	Yes, a subset of patients has fibromyalgia or chronic fatigue syndrome. Mechanical hypersensitivity to extra-pelvic sites was also noted during quantitative sensory testing. ⁵	Yes, bowel and bladder functions influence each other. A subset of IC/BPS patients have irritable bowel syndrome.
Mouse models used by MAPP Research Network investigators						
Model system	Sex bias?	Mast cell involvement ?	Inflammation or urothelial lesions?	Spontaneous pain behaviors?	Pain outside the pelvis?	Bowel-bladder interaction?
UTI (acute bacterial cystitis)	Not reported	No, mast cell deficient mice demonstrated the same magnitude of referred tactile allodynia as wild-type mice infected with NU14. ¹⁵	Yes, increased urine and tissue inflammatory scores (cytology, histology), major urothelial disruption was noted. ¹⁷	None observed	No (von Frey), no hyperalgesia in hind paw. ¹⁵	Yes, instillation of lidocaine into colon reduced referred tactile allodynia by ~66% in NU14 infected mice, suggesting bladder-colon cross-talk. ¹⁵
Post-UTI pain	Not reported	No, allodynia was unaffected in mast cell-deficient mice (unpublished results).	Transient inflammation that resolves.	None observed	Not observed, no hind paw allodynia.	Not reported
PRV (neurogenic cystitis)	Yes (von Frey), female mice infected with PRV have increased referred tactile allodynia compared to	Yes, mast cells mediated referred tactile allodynia in PRV infected mice. ¹⁸	Yes, areas of edema, leukocyte infiltrations, and dilated blood vessels were identified. Increased Evans	No (behaviors), no change in grooming, cage crossing, rearing. ¹⁵	No (von Frey), no hyperalgesia in hind paw. ¹⁵	Yes, positive and negative modulation. ¹⁵ Colonic lidocaine reduced referred tactile allodynia by ~63% in PRV infected mice, suggesting bladder-colon cross-talk. Sub-threshold colonic capsaicin increased allodynia by 57%.

Human IC/BPS						
	Sex bias?	Mast cell involvement ?	Inflammation or urothelial lesions?	Spontaneous pain behaviors?	Pain outside the pelvis?	Bowel-bladder interaction?
	infected males (e.g. doubling of response on post-infection day #). infected males (e.g. doubling of response on post-infection day #). infected males (e.g. doubling of response on post-infection day #). infected males (e.g. doubling of response on post-infection day #).	doubling of response on post-infection day #. doubling of response on post-infection day #. doubling of response on post-infection day #. doubling of response on post-infection day #).	hyperalgesia on post-infection day #. hyperalgesia on post-infection day #. hyperalgesia on post-infection day #. hyperalgesia on post-infection day #).	Yes (behaviors), dose-dependent reduction in locomotion and pain behavioral scores (e.g. licking of the abdomen, round-back posture) were observed. ¹³		
CYP (chemical cystitis)	No difference between male and female mice. ¹³	Yes in acute model, no in chronic model.	Yes, dose-dependent increase in inflammation and urothelial hyperalgesia (histology). ^{13,27}		Inconsistent results (von Frey). One study reported no hyperalgesia in hind paw. ¹³ However, one study demonstrated mechanical hypersensitivity in the hind-paw but the fore-paw was not affected. ⁸	Yes, CYP treatment to the bladder increased visceromotor response to colorectal distention (~3-fold). The magnitude of colon hypersensitivity was similar to that produced by colon inflammation. ⁸
URO-OVA (autoimmune cystitis)	No difference between male and female mice. ²⁰	Yes, increased mast cells in bladder histology. ²⁰	Yes, edema, leukocyte infiltrations, and dilated blood vessels were identified. ²⁰	Yes (micturition cage), increased locomotion, see Figure 2B.	No (von Frey), no hyperalgesia in hind paw (unpublished results).	Not reported
URO-MCP-1 (bladder MCP-1 expression)	Not reported	Yes, increased mast cells in chronic model (unpublished results).	Yes, edema, leukocyte infiltrations, and dilated blood vessels were identified (unpublished results).	Not reported	No (von Frey), no hyperalgesia in hind paw (unpublished results).	Not reported
Additional proposed models						
Model system	Sex bias?	Mast cell involvement ?	Inflammation or urothelial lesions?	Spontaneous pain behaviors?	Pain outside the pelvis?	Bowel-bladder interaction?
Feline IC	No, males and females are affected equally. ²	Yes, increased mast cells in ~20% of biopsy.	Yes, ulceration and inflammatory infiltrates were occasionally observed, overall more resembles the non-ulcerative form of IC/BPS. Increased bladder permeability, most notably after stress. ²	Yes, cats were identified by owners based on abnormal lower urinary tract signs (e.g. hematuria, stragguria, inappropriate voiding), however only a small percent (32% of females, 53% of males) exhibited behaviors to suggest pain (vocalization during urination). ²¹	Not reported	Not reported

Human IC/BPS						
	Sex bias?	Mast cell involvement ?	Inflammation or urothelial lesions?	Spontaneous pain behaviors?	Pain outside the pelvis?	Bowel-bladder interaction?
Stress models in rats (WAS = water avoidance stress, RVS = repeated variate stress)	Not reported	Yes, increased total and activated mast cells. ²³	Yes, increased inflammatory markers. ²⁵	Not reported	Yes (von Frey), hyperalgesia in hind=paw was noted in the RVS model (doubling of withdrawal response). ²⁵	Not reported