13. Yoshiyama M, Roppolo JR, Takeda M, et al. Effects of urethane on reflex activity of lower urinary tract in decerebrate unanesthetized rats. Am J Physiol Renal Physiol 2013;304:F390–6.
14. Andrade EL, Forner S, Bento AF, et al. TRPA1 receptor modulation attenuates bladder overactivity induced by spinal cord injury. Am J Physiol Renal Physiol 2011;300:F1223–34.
15. Munoz A, Somogyi GT, Boone TB, et al. Modulation of bladder afferent signals in normal and spinal cord-injured rats by purinergic P2 × 3 and P2 × 2/3 receptors. BJU Int 2012;110:E409–14.
16. Gang W, Hongjian T, Jasheng C, et al. The effect of the 5-HT7 serotonin receptor agonist, LP44, on micturition in rats with chronic spinal cord injury. Neurourol Urodyn 2013 July 16. doi: 10.1002/nau.22463. [Epub ahead of print]
17. Yoshimura N, Miyazato M, Kitta T, et al. Central nervous targets for the treatment of bladder dysfunction. Neurourol Urodyn 2014;33:59–66.
18. Chen J, Gu B, Wu G, et al. The effect of the 5-HT2A/2C receptor agonist on micturition in rats with chronic spinal cord injury. J Urol 2013;189:1982–8.
19. Craft RM, Carlisi VJ, Mattia A, et al. Behavioral characterization of the excitatory and desensitizing effects of intravesical capsaicin and resiniferatoxin in the rat. Pain 1993;55:205–215.
20. Birder LA, Nakamura Y, Kiss S, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 2002;5:856–60.
21. Zhang X, Igawa Y, Ishizuka O, et al. Effects of resiniferatoxin desensitization of capsaicin-sensitive afferents on detrusor over-activity induced by intravesical capsaicin, acetic acid or ATP in conscious rats. Naunyn Schmiedebergs Arch Pharmacol 2003;367:473–9.
22. Giannantoni A, Di Stasi SM, Stephen RL, et al. Intravesical capsaicin versus resiniferatoxin in patients with detrusor hyperreflexia: A prospective randomized study. J Urol 2002;167:1710–4.
23. Igawa Y, Satoh T, Mizusawa H, et al. The role of capsaicin-sensitive afferents in autonomic dysreflexia in patients with spinal cord injury. BJU Int 2003;91:637–41.

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Perspectives in Urology

Hypersensitive bladder: Towards clear taxonomy surrounding interstitial cystitis

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There is confusion surrounding the taxonomy of interstitial cystitis (IC) and its related symptom syndromes, painful bladder syndrome (PBS), bladder pain syndrome (BPS) and overactive bladder (OAB) syndrome.[1]

IC is a disease name long used in medical and consumer societies, but it lacks clear definition.[2] PBS is defined by the International Continence Society (ICS) as "suprapubic pain related to bladder filling, accompanied by other symptoms, such as increased daytime and night-time frequency, in the absence of proven urinary infection or other obvious pathology",[3] PBS[4,5] and BPS[6] definitions by other societies all share two requirements: symptoms including pain and lack of identifiable diseases explaining the symptoms. Chimeric terms combining IC and syndromes, IC/PBS (IC/BPS), apparently indicate conditions presenting the symptom complex in the absence of obvious diseases with the possible presence of IC. However, these terms are used interchangeably. In addition, PBS and BPS contain "pain", creating a misunderstanding that the patient must complain of pain, although a substantial proportion of patients do not. Complaints commonly heard are not directly linked to pain; that is, incessant need to void due to discomfort, loss of normal urinary sensation and an irritable sensation with only little urine in the bladder. These symptoms suggest increased sensation of the bladder or urothelium, a highly sensitive sensory tissue,[7] and an adequate term for such a condition is lacking.

I should therefore like to propose hypersensitive bladder (HSB)[8] (although "hypersensitive" can be replaced by other words implying increased sensation). HSB syndrome is a condition with HSB symptoms with no obvious diseases. HSB symptoms are defined as "increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain". They are similar to OAB symptoms; urinary urgency, usually associated with urinary frequency and nocturia, with or without urgency incontinence.[3] Increased bladder sensation is described as "early and persistent desire to void".[3] IC is defined as a disease requiring: (i) HSB symptoms; (ii) no obvious diseases; and (iii) bladder pathology, such as Hunner's ulcer or mucosal bleeding after overdistension (Fig. 1a). Symptoms of OAB and HSB substantially overlap, whereas OAB with leakage and HSB with pain are readily separated. The compelling need to void in OAB is characterized by the sudden onset and/or fear of leakage, whereas that in HSB is of a persistent unpleasant sensation and/or fear of pain.[9] IC is a representative disease causing HSB symptoms, most typically with pain, but might be painless and indistinguishable from OAB (Fig. 1b).

With these definitions, the following make sense: (i) a woman complaining of HSB symptoms proved to have carcinoma *in situ* of the bladder; (ii) a woman with HSB symptoms was diagnosed as HSB because no obvious diseases were apparent, but now she is diagnosed with IC based on cystoscopic abnormality; and (iii) a man with HSB showed no definite findings for IC at hydrodistension, so he remained as HSB.

Introducing HSB, the counter concept of OAB, into bladder dysfunction taxonomy will clarify the current confusion surrounding IC.

Conflict of Interest

None declared.

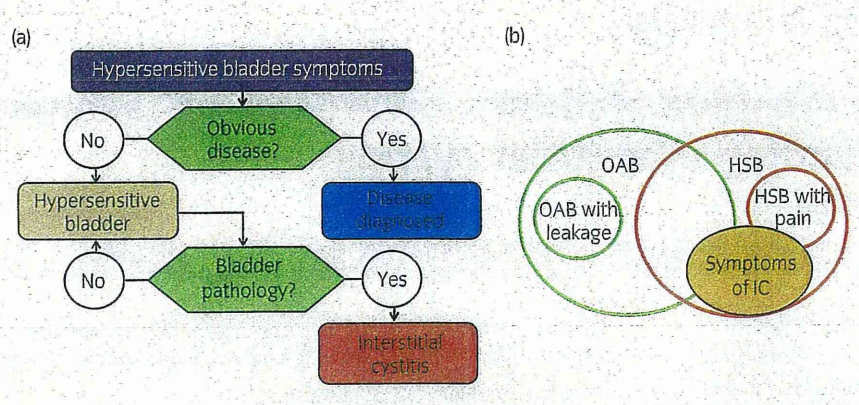

Fig. 1 Relationship of hypersensitive bladder (HSB) and its related terms. (a) Hypersensitive bladder symptoms (increased bladder sensation, usually associated with urinary frequency and nocturia, with or without bladder pain) are to be explored for the causes. When there is no obvious disease, the condition is called HSB. Furthermore, when bladder pathology (such as Hunner's ulcer or mucosal bleeding after overdistension) is identified in HSB, the diagnosis of interstitial cystitis (IC) is made. When there is no such bladder abnormality, the condition remains as HSB. (b) Symptoms of overactive bladder (OAB; urinary urgency, usually associated with urinary frequency and nocturia, with or without urgency incontinence) and HSB are substantially overlapping, although OAB with leakage and HSB with pain can be readily discriminated. Symptoms of IC mostly present as HSB symptoms (typically with pain), but occasionally can be indistinguishable from OAB.

References

1 Homma Y. Lower urinary tract symptomatology: its definition and confusion. *Int. J. Urol.* 2008; **15**: 35–43.
2 Teichman JM, Parsons CL. Contemporary clinical presentation of interstitial cystitis. *Urology* 2007; **69**: 41–7.
3 Abrams P, Cardozo L, Fall M *et al.* The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Neurourol. Urodyn.* 2002; **21**: 167–78.
4 Hanno P, Dmochowski R. Status of international consensus on interstitial cystitis/bladder pain syndrome/painful bladder syndrome: 2008 snapshot. *Neurourol. Urodyn.* 2009; **28**: 274–86.
5 Hanno PM, Burks DA, Clemens JQ *et al.* AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. *J. Urol.* 2011; **185**: 2162–70.
6 van de Merwe JP, Nordling J, Bouchelouche P *et al.* Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. *Eur. Urol.* 2008; **53**: 60–7.
7 Birder LA, Ruggieri M, Takeda M *et al.* How does the urothelium affect bladder function in health and disease? *Neurourol. Urodyn.* 2012; **31**: 293–9.
8 Homma Y, Ueda T, Tomoe H *et al.* Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome. *Int. J. Urol.* 2009; **16**: 597–615.
9 Diggs C, Meyer WA, Langenberg P, Greenberg P, Horne L, Warren JW. Assessing urgency in interstitial cystitis/painful bladder syndrome. *Urology* 2007; **69**: 210–14.

Increased mRNA Expression of Genes Involved in Pronociceptive Inflammatory Reactions in Bladder Tissue of Interstitial Cystitis

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Purpose: We assayed mRNA expression of the TRP family of channels and ASIC1 in bladder tissue from patients with interstitial cystitis.

Materials and Methods: Bladder biopsies of 1) nonclassic interstitial cystitis, 2) nonulcerative portions of classic interstitial cystitis, 3) ulcerative portions of classic interstitial cystitis and 4) noncancerous portions of bladder cancer as the control were placed immediately in ice-cold RNAlater® and subjected to real-time reverse transcriptase-polymerase chain reaction. We compared the mRNA expression of TRP channels, ASIC1, NGF, CXCL9 and UPK3A with that of controls, and correlated expression with symptom severity.

Results: We analyzed specimens from 17 patients with nonclassic interstitial cystitis, 22 with classic interstitial cystitis and 11 controls. In nonclassic interstitial cystitis samples TRPV2 and NGF showed significantly increased expression. In classic interstitial cystitis samples nonulcerative portions demonstrated a significant increase in the expression of TRPA1, TRPM2 and 8, TRPV1 and 2, ASIC1, NGF and CXCL9, and a significant decrease in UPK3A and TRPV4. Ulcerative portions showed similar changes for TRPM2, TRPV1, 2 and 4, CXCL9 and UPK3A. Increased expression of TRPM2, first noted in interstitial cystitis tissue, was the most pronounced one of the TRP family. All symptom measures correlated with TRPM2 and TRPV2 expression, and partially with that of the other genes.

Conclusions: This study showed increased expression of the genes involved in pronociceptive inflammatory reactions in interstitial cystitis, including TRPV1, 2 and 4, ASIC1, NGF and CXCL9, and to our knowledge TRPM2 for the first time. The different expression patterns suggest distinct pathophysiologies for classic and nonclassic interstitial cystitis. The genes and their products are potential candidates for use as biomarkers or novel therapy targets.

Abbreviations and Acronyms

ASIC = acid sensing ion channel
CXCL9 = chemokine (C-X-C motif) ligand 9
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
IC = interstitial cystitis
NGF = nerve growth factor
OSPI = O'Leary-Sant problem score
OSSI = O'Leary-Sant symptom score
qRT-PCR = quantitative real-time RT-polymerase chain reaction
RT = reverse transcriptase
TRP = transient receptor potential
TRPA = TRP ankyrin
TRPM = TRP melastatin
TRPV = TRP vanilloid
UPK = uroplakin
VAS = visual analog pain scale

Key Words: urinary bladder; cystitis, interstitial; gene expression; transient receptor potential channels; uroplakins

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INTERSTITIAL cystitis is characterized by bothersome lower urinary tract symptoms, including increased urinary frequency, increased bladder sensation, bladder discomfort and pain, which often severely impair patient quality of life. Histopathology reveals a varying extent of mast cell proliferation and inflammatory infiltrates as well as scarring and fibrosis at the end stage of disease.[1] However, its exact etiology remains unclear, hindering the development of effective therapeutic interventions.

Basically, IC diagnosis is based on symptomatology, cystoscopy and the exclusion of other bladder diseases.[2] On cystoscopy IC can be divided into 2 subtypes, including classic IC with Hunner ulcers (ulcerative lesions) and nonclassic IC without ulcers. Classic IC is associated with older patient age, more severe symptoms and more evident inflammatory histological reactions.[3] Recent studies focused on biomarkers to diagnose IC and/or discriminate between the 2 types of IC.[4] Candidate biomarkers include NGF,[5] CXCL9[4] and UPK III-δ4, a splicing variant of UPK III.[6]

Emerging evidence suggests that the TRP family of channels is likely to have important roles in regulating urothelial sensory perception and overall bladder function. TRPA1, TRPM7 and 8, and TRPV1, 2 and 4 are expressed in the urothelium of different regions of the urogenital tract.[7,8] TRPV1$^{-/-}$ mice feature abnormal bladder function, characterized by a high frequency of low amplitude and nonvoiding bladder contractions,[9] while TRPV4 stimulation facilitates the micturition reflex by activating mechanosensitive, capsaicin insensitive C fibers in rats.[10] In addition, functional ASICs, which are members of the amiloride sensitive epithelial Na^+ channel family, are expressed in the bladder of rats and mice.[11,12] An alteration in ASIC receptor expression was reported in a rat cystitis model.[13]

However, there is little or no clinical information on the comprehensive expression patterns and possible functional roles of these TRP channels and ASICs in IC pathophysiology. To explore IC pathophysiology, we comprehensively analyzed the mRNA expression of molecules that may be related to inflammation and/or mechanosensing/thermosensing, such as the TRP family, ASIC1 receptor, NGF, CXCL9 and UPK3A, using qRT-PCR.

MATERIALS AND METHODS

Subjects

Patients with IC scheduled for hydrodistention or those with noninvasive bladder cancer undergoing transurethral resection were enrolled as controls. The IC diagnosis was based on clinical guidelines for IC and hypersensitive bladder syndrome.[2] Patients with symptomatic bladder cancer or carcinoma in situ were excluded from analysis.

At study enrollment symptom severity was assessed by the OSSI, OSPI and VAS (score 0 to 10). The study protocol was approved by our institutional review board and fully explained to patients before written informed consent was obtained.

Using a rigid 18Fr cystoscope with the patient under spinal anesthesia, bladder specimens were obtained before hydrodistention or tumor resection with cold cup biopsy forceps from 1) retrotrigonal portions in nonclassic IC, 2) nonulcerative retrotrigonal portions in classic IC, 3) ulcerative portions in classic IC and 4) retrotrigonal, noncancerous, apparently normal portions in bladder cancer. The nonIC bladder served as the control. Samples were placed immediately in ice-cold RNAlater and stored at −80C. Total RNA was extracted from bladder samples and reverse transcribed into cDNA with RT. mRNA expression levels of several TRP channels (TRPA1, TRPM2, 7 and 8, and TRPV1, 2 and 4), ASIC1, NGF, CXCL9 and UPK3A were compared among the 3 groups. mRNA levels are shown as the fold change in the average control tissue value.

Total RNA Isolation

Total RNA was extracted from bladder samples by homogenization in Sepasol®-RNA I isolation solution according to the manufacturer protocol. RNA integrity and purity were assessed by capillary electrophoresis on a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California) with an RNA 6000 Nano LabChip® Kit and by measuring absorbance at 260/280 nm (A_{260}/A_{280} ratio). Only RNA samples with RNA integrity numbers of 6.5 or greater were considered for further analysis.

RT and qRT-PCR for mRNA Expression

Total RNA was reverse transcribed using the PrimeScript™ 1st Strand cDNA Synthesis Kit with 1 μg RNA and 6 mer random primers according to manufacturer instructions. qRT-PCR was performed with SYBR® Premix ExTaq™ II on a LightCycler®. The reaction volume was 20 μl, which contained 2 μl of a ten-fold dilution of cDNA, 10 μl SYBR Premix ExTaq II, 0.8 μl forward and reverse primers (10 μM each), and 6.4 μl water. qRT-PCR conditions were initial denaturation at 95C for 30 seconds, 40 to 45 amplification cycles with denaturation at 95C for 5 seconds, and annealing and extension at 60C for 20 seconds. Each experiment was done 3 times and average values were used for analysis. The primers used for qRT-PCR were selected using the Perfect Real Time Primer Support System (Takara Bio, Shiga, Japan) (see supplementary Appendix, http://jurology.com/).

Statistical and Data Analysis

For each sample gene expression levels were normalized to GAPDH and calculated as the fold expression relative to the median control value. The gene expression level was analyzed with the Wilcoxon rank sum test. Its relation to symptom severity was determined using the Pearson product moment correlation coefficient r with $p < 0.05$ considered statistically significant. R, version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analysis.

RESULTS

Enrolled in the study were 11 controls, 17 patients with nonclassic IC and 22 with classic IC (table 1). All bladder cancers were noninvasive and not associated with carcinoma in situ. Bladder cancer grade and stage was G1Ta in 6 cases, and G1T1, G1T2, G2Ta, G2T1 and G3T1 in 1 each. Biopsy sites were histologically confirmed to be free of cancer. All IC cases were compatible with National Institute of Diabetes and Digestive and Kidney Diseases criteria. Patients with classic IC were older than those with nonclassic IC (mean ± SD age 70.6 ± 10.6 vs 56.1 ± 18.5 years, $p = 0.003$) and they appeared to be more symptomatic. Mean OSSI and OSPI were 14.1 ± 4.1 and 12.4 ± 3.2 in classic IC cases, and 13.1 ± 3.2 and 11.5 ± 3.4, respectively, in nonclassic IC cases. The mean VAS was 7.55 ± 1.85 and 5.25 ± 2.77 for classic and nonclassic IC, respectively. Patients with IC had had no hydrodistention, received no botulinum toxin injection therapy and used no steroids within the last year. All patients had been on nonsteroidal anti-inflammatory drugs on demand for bladder pain or discomfort.

qRT-PCR results indicated a significant increase in TRPV2 and NGF expression in nonclassic IC tissue, while there were major changes in classic IC tissue (table 2, and figs. 1 and 2). Of the TRP channels TRPA1, TRPM2 and 8, and TRPV1 and 2 showed significantly increased mRNA expression in nonulcerative portions of classic IC compared with controls. In the same portions we noted a significant increase in ASIC1, NGF and CXCL9 mRNA expression, and a significant decrease in TRPV4 and UPK3A mRNA expression. Also, in the ulcerative portions of classic IC we found a significant increase in TRPM2, TRPV1 and 2, and CXCL9, and a significant decrease in TRPV4 and UPK3A.

As assessed by the OSSI and OSPI or VAS, symptom severity was significantly associated with the TRPM2 and TRPV2 expression level (coefficient 0.266 to 0.516). TRPV1, ASIC1, NGF and CXCL9 levels significantly correlated positively with 1 or 2 symptom measures, and the TRPV4 level significantly correlated inversely (table 3).

Table 2. *mRNA expression in interstitial cystitis bladder tissue*

	Nonclassic IC		Nonulcerative Classic IC		Ulcerative Classic IC	
Gene Symbol	Fold Change	p Value	Fold Change	p Value	Fold Change	p Value
TRPA1	1.18	0.611	2.20	0.014*	1.45	0.143
TRPM2	1.26	0.578	3.71	0.007†	3.74	0.012*
TRPM7	0.85	0.225	0.92	0.560	0.83	0.166
TRPM8	0.64	0.487	1.51	0.021*	1.57	0.053
TRPV1	1.40	0.059	2.03	0.000†	1.15	0.040*
TRPV2	1.39	0.037*	2.07	0.000†	2.22	0.000†
TRPV4	1.04	0.547	0.68	0.036*	0.68	0.029*
ASIC1	1.34	0.134	2.34	0.000†	1.22	0.097
NGF	3.41	0.002†	2.81	0.014*	1.52	0.375
CXCL9	1.33	0.711	7.55	0.000†	6.04	0.006†
UPK3A	1.04	1.000	0.04	0.000†	0.07	0.000†

*p <0.05 vs control.
†p <0.01 vs control.

DISCUSSION

We compared mRNA expression levels of 7 TRP channels and ASIC1 as well as NGF, CXCL9 and UPK3A in bladder biopsy specimens from controls, and patients with nonclassic and classic IC to explore IC pathophysiology.

NGF mRNA levels were increased in each type of IC, suggesting that increased NGF expression may be a common IC change. Although the 1.5-fold increase in ulcerative lesions was not statistically significant in our study, it is comparable to a previous observation.[14] NGF, which is produced by bladder smooth muscle and the urothelium,[15] was implicated in altered bladder sensory function and the development of referred hyperalgesia in response to bladder inflammation[5] as well as in pathological conditions, such as idiopathic detrusor overactivity, neurogenic bladder and inflammatory bladder disease.[16]

In addition to NGF, TRPV2 mRNA levels were slightly but significantly increased in each type of IC. A few reports have shown a possible role for TPRV2 in bladder function. TRPV2 mRNA was found in cultured urothelial cells, urothelial tissue, dissociated smooth muscle cells and deepithelialized bladder tissue.[17] Since it is a heat and stretch activated channel, the function of TRPV2 could be to detect bladder filling or nociceptive sensation.

Table 1. *Patient background*

	Bladder Ca Control	Nonclassic IC	Classic IC
No. male/female	8/3	6/11	2/20
Mean ± SD age at diagnosis (range)	69.7 ± 12.3 (48-82)	56.1 ± 18.5 (20–73)	70.6 ± 10.6 (36–83)
Median yrs symptom history (range)		3.5 (1.0–28.0)	4.0 (1.5–20.0)
Mean ± SD score:			
OSSI	2.73 ± 1.0	13.1 ± 3.15*	14.1 ± 4.06*
OSPI	2.73 ± 1.27	11.5 ± 3.37*	12.4 ± 3.24*
VAS	0.55 ± 0.69	5.25 ± 2.77*	7.55 ± 1.85*

*p <0.05 vs control.

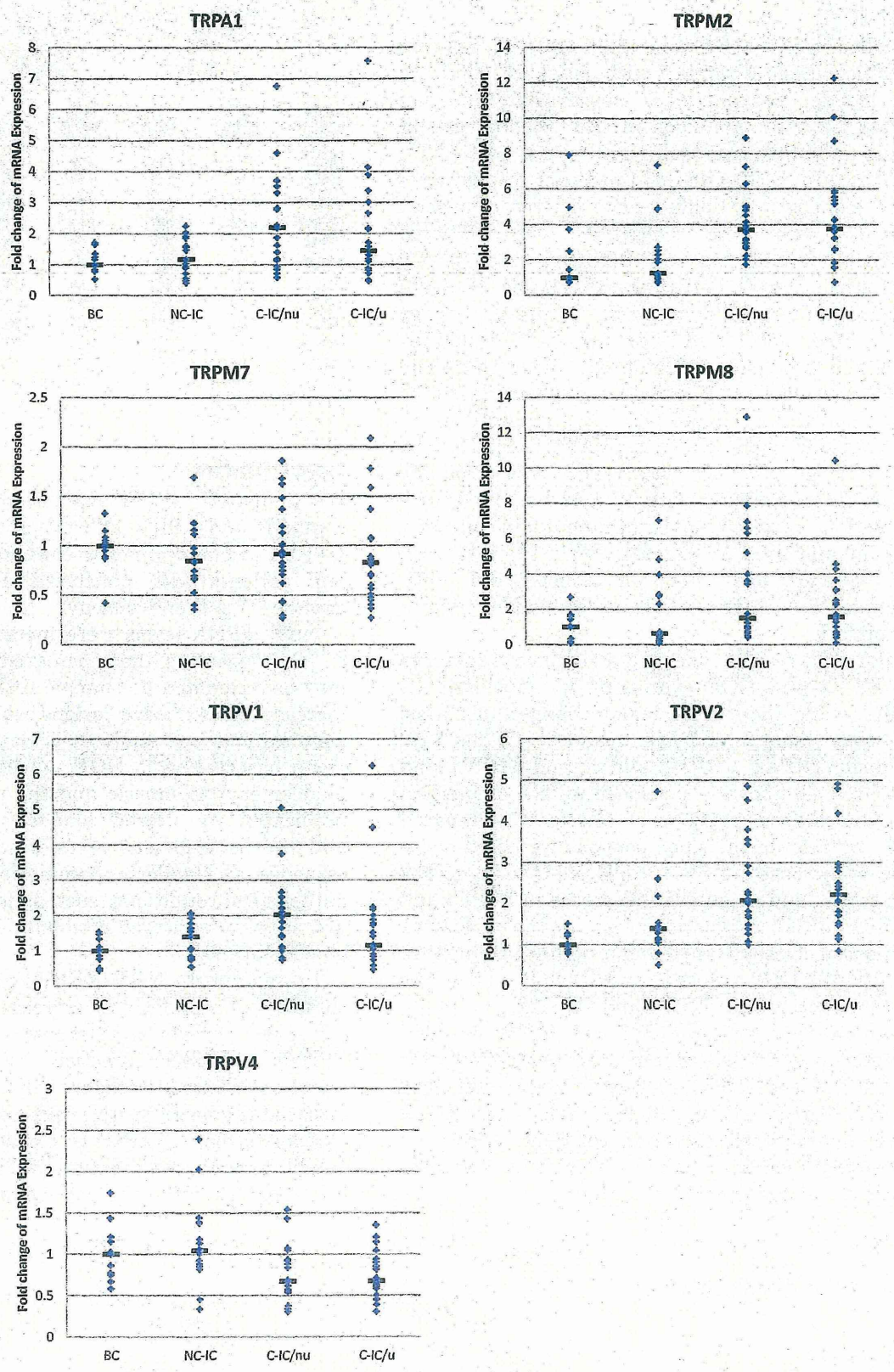

Figure 1. TRP channel mRNA expression in bladder tissue of each patient (dots), normalized to GAPDH and expressed as fold relative to median (horizontal lines) of controls. *BC*, bladder cancer. *NC-IC*, nonclassic IC. *C-IC/nu*, classic IC nonulcerative lesions. *C-IC/u*, classic IC ulcerative lesions.

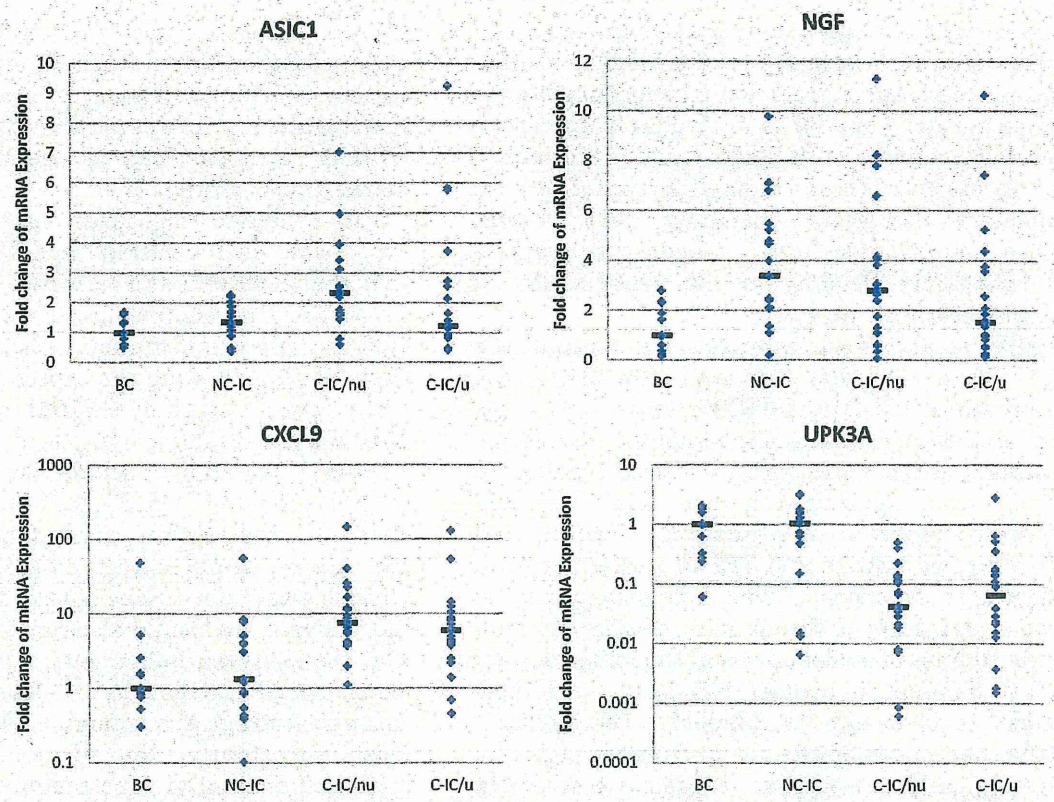

Figure 2. NGF, ASIC1, UPK3A and CXCL9 mRNA expression in bladder tissue, normalized to GAPDH and expressed as fold relative to median (horizontal lines) of controls. *BC*, bladder cancer. *NC-IC*, nonclassic IC. *C-IC/nu*, classic IC nonulcerative lesions. *C-IC/u*, classic IC ulcerative lesions.

We found the same expression pattern for TRPA1 and TRPM8. That is, each was increased only in nonulcerative lesions of classic IC. TRPA1 is found in sensory nerve fibers that innervate the bladder trigone[18] and pharmacological blockade of TRPA1 attenuated bladder overactivity in rats with spinal cord injury.[19] TRPM8 immunoreactive staining was observed in the urothelium and afferent nerve fibers in the human bladder and the number of positive nerve fibers increased in cases of idiopathic detrusor overactivity and painful bladder syndrome.[20] A selective TRPM8 channel inhibited the cold evoked pain response in mice.[21] These observations and our results indicate that increased expression of TRPA1 and TRPM8 mRNA may be involved in the nociceptive sensitization of classic IC.

Table 3. *Symptom severity correlated with RNA expression*

Gene Symbol	OSSI		OSPI		VAS	
	r	p Value	r	p Value	r	p Value
TRPA1	0.035	0.776	0.178	0.143	0.228	0.060
TRPM:						
2	0.331	0.005*	0.363	0.002*	0.316	0.008*
7	−0.032	0.791	0.068	0.582	0.003	0.983
8	0.017	0.888	0.156	0.202	0.130	0.288
TRPV:						
1	0.162	0.183	0.289	0.016†	0.185	0.128
2	0.266	0.027†	0.339	0.004*	0.516	0.000*
4	−0.291	0.015†	−0.273	0.023†	−0.233	0.055
ASIC1	0.194	0.109	0.308	0.010†	0.213	0.079
NGF	0.161	0.181	0.277	0.021†	0.172	0.145
CXCL9	0.282	0.019†	0.236	0.051†	0.002	0.988
UPK3A	0.006	0.966	−0.103	0.443	−0.061	0.607

*p <0.01.
†p <0.05.

TRPM2 mRNA was increased in each portion from patients with classic IC with the most pronounced increase of all of the TRP channels. To our knowledge this is the first study showing a relationship between increased TRPM2 expression and a bladder disorder. TRPM2 is a Ca^{2+} permeable, nonselective cation channel that is expressed highly in the brain and broadly in other tissues, and acts as a sensor for reactive oxygen species.[22] TRPM2 up-regulation in macrophages and microglia aggravates peripheral and spinal pronociceptive inflammatory responses, facilitating inflammatory and neuropathic pain.[23] These findings support the view that TRPM2 may be a primary factor in the proinflammatory neuropathic pain associated with classic IC.

Urinary pH strongly influences IC pain symptoms.[24] ASICs have emerged as key sensors of extracellular pH. Recent studies suggest that these channels have pivotal roles in the pathophysiology

of the initiation of inflammation and chronic pain.[25] ASICs were over expressed in the bladder urothelium and detrusor of rats with cyclophosphamide induced cystitis[13] as well as in bladder biopsy specimens from patients with bladder pain syndrome.[26] The expression of these channels is induced by inflammatory mediators, including NGF, brain-derived neurotrophic factor, 5-hydroxytryptamine and bradykinin, leading to the sensitization of bladder sensory pathways.

TRPV1 receptors are expressed on urothelial cells and C fibers,[9] and they respond to low pH. In the current study ASIC1 and TRPV1 expression levels were significantly increased in nonulcerative lesions of classic but not nonclassic IC. These results are partially inconsistent with those of a previous study indicating significant up-regulation of ASIC2a and ASIC3 but not ASIC1a and TRPV1 mRNA levels in bladder pain syndrome.[26] The discrepancy may be partly explained by differences in selection criteria.

Several lines of evidence reveal the importance of TRPV4 channels in bladder physiology and their possible involvement in bladder overactivity.[27] TRPV4 channels facilitate the micturition reflex by activating mechanosensitive, capsaicin insensitive C fibers in rats.[10] In our study we detected decreased TRPV4 mRNA expression levels in classic IC samples. The reason for the decrease is unclear but it may be clinically related to the abnormal bladder filling sensation in patients with IC.

The increase in CXCL9 levels was consistent with a previous report that CXCL9 together with other CXCR binding chemokines, such as CXCL10 and 11, is up-regulated in the bladder tissue of patients with ulcerative IC.[4] Serum levels of these substances are also increased.[28] A series of CXCR binding proteins attracts and stimulates monocytes/macrophages, and T, natural killer, mast and dendritic cells, leading to mucosal inflammation and tissue destruction.[28] Our finding that CXCL9 mRNA was increased in classic but not nonclassic IC suggests that this chemokine and the resultant inflammation are events specific to classic IC.

UPKs are barrier proteins expressed by urothelial cells and their expression is altered in IC.[29] We found remarkable down-regulation of UPK3A in classic IC, essentially the same as in a previous report.[6] Heavy suppression of UPK3A expression may be pathognomonic to IC, in that the increased permeability of the urothelium enhances the penetration of urinary substances into the bladder wall and results in inflammatory changes. Decreased UPK3A expression can be useful as a diagnostic marker for ulcerative IC.

All symptom measures correlated with the expression level of TRPM2 and TRPV2, and partly with that of TRPV1 and 4, ASIC1, NGF and CXCL9, supporting the significance of expressed genes in symptomatic manifestation. It is tempting to anticipate that modulating the expression or function of these genes, especially TRPM2 and TRPV2, may alleviate the disabling symptoms of IC.

Study limitations include the lack of healthy controls, the unmatched ages of patients with nonclassic IC, the possible effect of treatments used at the time of sample taking and the undefined pathophysiological interactions of increased expression of the mRNAs. We should also consider the possibility that the observed inflammatory reactions reflected physiological reactions as a defense mechanism, as well as pathological reactions caused by the disease. Most importantly, the direct evidence for an increased amount of gene products and their function, and the precise localization of the substances in bladder tissue should be explored. Further studies using patients with overactive bladder as a comparative control, or examining possible changes along with clinical progression or therapeutic responses are also warranted in the future.

CONCLUSIONS

This study demonstrated increased expression of the genes involved in pronociceptive inflammatory reactions, including TRPM2 for the first time to our knowledge, and TRPV1, TRPV2, TRPV4, ASIC1, NGF and CXCL9, in classic IC cases. Different expression patterns suggest distinct classic and nonclassic IC pathophysiologies. The genes or their products are potential candidates for use as biomarkers or novel therapy targets.

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REFERENCES

1. van de Merwe JP, Nordling J, Bouchelouche P et al: Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. Eur Urol 2008; **53:** 60.
2. Homma Y, Ueda T, Tomoe H et al: Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome. Int J Urol 2009; **16:** 597.
3. Gillenwater JY and Wein AJ: Summary of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases Workshop on Interstitial Cystitis, National Institutes of Health,

Bethesda, Maryland, August 28-29, 1987. J Urol 1988; **140:** 203.

4. Ogawa T, Homma T, Igawa Y et al: CXCR3 binding chemokine and TNFSF14 over expression in bladder urothelium of patients with ulcerative interstitial cystitis. J Urol 2010; **183:** 1206.
5. Ochodnický P, Cruz CD, Yoshimura N et al: Nerve growth factor in bladder dysfunction: contributing factor, biomarker, and therapeutic target. Neurourol Urodyn 2011; **30:** 1227.
6. Zeng Y, Wu XX, Homma Y et al: Uroplakin III-delta4 messenger RNA as a promising marker to identify nonulcerative interstitial cystitis. J Urol 2007; **178:** 1322.
7. Birder LA: TRPs in bladder diseases. Biochim Biophys Acta 2007; **1772:** 879.
8. Yu W, Hill WG, Apodaca G et al: Expression and distribution of transient receptor potential (TRP) channels in bladder epithelium. Am J Physiol Renal Physiol 2011; **300:** F49.
9. Birder LA, Nakamura Y, Kiss S et al: Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci 2002; **5:** 856.
10. Aizawa N, Wyndaele JJ, Homma Y et al: Effects of TRPV4 cation channel activation on the primary bladder afferent activities of the rat. Neurourol Urodyn 2012; **31:** 148.
11. Kobayashi H, Yoshiyama M, Zakoji H et al: Sex differences in the expression profile of acid-sensing ion channels in the mouse urinary bladder: a possible involvement in irritative bladder symptoms. BJU Int 2009; **104:** 1746.
12. Kullmann FA, Shah MA, Birder LA et al: Functional TRP and ASIC-like channels in cultured urothelial cells from the rat. Am J Physiol Renal Physiol 2009; **296:** F892.
13. Corrow K, Girard BM and Vizzard MA: Expression and response of acid-sensing ion channels in urinary bladder to cyclophosphamide-induced cystitis. Am J Physiol Renal Physiol 2010; **298:** F1130.
14. Liu HT and Kuo HC: Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology 2007; **70:** 463.
15. Steers WD and Tuttle JB: Mechanisms of disease: the role of nerve growth factor in the pathophysiology of bladder disorders. Nat Clin Pract Urol 2006; **3:** 101.
16. Lowe EM, Anand P, Terenghi G et al: Increased nerve growth factor levels in the urinary bladder of women with idiopathic sensory urgency and interstitial cystitis. Br J Urol 1997; **79:** 572.
17. Birder LA: Involvement of the urinary bladder urothelium in signaling in the lower urinary tract. Proc West Pharmacol Soc 2001; **44:** 85.
18. Nagata K, Duggan A, Kumar G et al: Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. J Neurosci 2005; **25:** 4052.
19. Andrade EL, Forner S, Bento AF et al: TRPA1 receptor modulation attenuates bladder overactivity induced by spinal cord injury. Am J Physiol Renal Physiol 2011; **300:** F1223.
20. Mukerji G, Yiangou Y, Corcoran SL et al: Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. BMC Urol 2006; **6:** 6.
21. Knowlton WM, Daniels RL, Palkar R et al: Pharmacological blockade of TRPM8 ion channels alters cold and cold pain responses in mice. PLoS One 2011; **6:** e25894.
22. Kaneko S, Kawakami S, Hara Y et al: A critical role of TRPM2 in neuronal cell death by hydrogen peroxide. J Pharmacol Sci 2006; **101:** 66.
23. Haraguchi K, Kawamoto A, Isami K et al: TRPM2 contributes to inflammatory and neuropathic pain through the aggravation of pronociceptive inflammatory responses in mice. J Neurosci 2012; **32:** 3931.
24. Koziol JA, Clark DC, Gittes RF et al: The natural history of interstitial cystitis: a survey of 374 patients. J Urol 1993; **149:** 465.
25. Araki I, Du S, Kobayashi H et al: Roles of mechanosensitive ion channels in bladder sensory transduction and overactive bladder. Int J Urol 2008; **15:** 681.
26. Sánchez-Freire V, Blanchard MG, Burkhard FC et al: Acid-sensing channels in human bladder: expression, function and alterations during bladder pain syndrome. J Urol 2011; **186:** 1509.
27. Janssen DA, Hoenderop JG, Jansen KC et al: The mechanoreceptor TRPV4 is localized in adherence junctions of the human bladder urothelium: a morphological study. J Urol 2011; **186:** 1121.
28. Sakthivel SK, Singh UP, Singh S et al: CXCL10 blockade protects mice from cyclophosphamide-induced cystitis. J Immune Based Ther Vaccines 2008; **6:** 6.
29. Graham E and Chai TC: Dysfunction of bladder urothelium and bladder urothelial cells in interstitial cystitis. Curr Urol Rep 2006; **7:** 440.