Summer 2015

Inflammation and central pain sensitization in Interstitial Cystitis/Bladder Pain Syndrome

Andrew David Schrepf
University of Iowa

INFLAMMATION AND CENTRAL PAIN SENSITIZATION IN INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

by

Andrew David Schrepf

A thesis submitted in partial fulfillment
of the requirements for the Doctor of Philosophy
degree in Psychology in the
Graduate College of
The University of Iowa

August 2015

Thesis Supervisor: Professor Susan Lutgendorf

Graduate College
The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D. thesis of

Andrew David Schrepf

has been approved by the Examining Committee for
the thesis requirement for the Doctor of Philosophy degree
in Psychology at the August 2015 graduation.

Thesis Committee:

Susan K. Lutgendorf, Thesis Supervisor

Alan Kim Johnson

Karl J. Kreder

Kristian E. Markon

Mark W. Vander Weg

To my mother, Kris Vervaecke

I could hear the human noise we sat there making, not one of us moving, not even when the room went dark.

Raymond Carver
What We Talk About When We Talk About Love

ACKNOWLEDGEMENTS

I wish to gratefully acknowledge my dissertation committee for their dedicated service to the advancement of this thesis, The Multidisciplinary Approach to the Study of Chronic Pelvic Pain research network, the National Institute of Diabetes and Digestive and Kidney Diseases, and the Institute for Clinical and Translational Science at the University of Iowa, without which the collection of these data would not have been possible, and the following individuals whose support helped sustain this work: Mary Eno, Lauren Clevenger, and Katherine Jonas.

ABSTRACT

Central sensitization refers to abnormal pain modulation present which is characterized by non-aversive or mildly aversive stimuli promoting feelings of pain. Many conditions referred to as Functional Somatic Syndromes (FSS)s are characterized by abnormal pain modulation, including pain in areas of the body not thought to be related to the specific FSS with which the patient has been diagnosed.

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a diagnosis of exclusion characterized by pelvic pain and urologic symptoms that shares many environmental and psychosocial correlates with FSSs. Treatment is generally non-satisfactory for patients despite substantial healthcare expenditures. Preliminary evidence suggests abnormal pain modulation in IC/BPS. Inflammatory dysregulation is an underexplored mechanism in the pain experience in IC/BPS and FSSs. The purpose of the current project is to explore the role of dysregulated inflammatory processes in IC/BPS with an emphasis on painful symptoms in three distinct papers.

Paper one examines the role of inflammation in IC/BPS patients with particular emphasis on the association of Toll-Like Receptor (TLR)-4 mediated inflammation with symptoms of pelvic pain. Paper two expands on the findings of paper one by exploring the association of TLR-4 mediated inflammation with the presence of comorbid FSSs and widespread pain. Paper three evaluates the predictive ability of these previously explored baseline inflammatory measures by testing the association between TLR-mediated inflammation and diurnal cortisol rhythms with symptom trajectories and symptom flares over a year of observation. Finally, the significance of these novel findings is explored.

PUBLIC ABSTRACT

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a condition that affects many women in the United States. The primary symptoms are frequent and urgent urination accompanied by pain. It is not currently known what causes IC/BPS and this makes treating the condition difficult. IC/BPS is known to be associated with greater sensitivity to pain. The purpose of these research projects is to determine if painful symptoms in IC/BPS are associated with different aspects of the immune system that control a broad process known as inflammation.

The first project of this research project examined differences in inflammatory processes between women with IC/BPS and a healthy comparison group. Women with IC/BPS displayed distinct differences: higher levels of inflammation, different patterns of inflammation-controlling hormones, and greater inflammation responses by immune cells challenged with immune-stimulating substances. These responses were associated with the level of pain reported by women with IC/BPS. The second project examined IC/BPS patients with and without other pain conditions; inflammation responses were associated with having other pain conditions, more widespread pain, and greater sensitivity to painful pressure. The third project examined IC/BPS symptoms over 48 weeks; inflammation responses at baseline were associated with less symptom improvement.

These findings suggest that IC/BPS is marked by altered inflammation, and these alterations are associated with painful symptoms. It is possible that inflammation plays a role in the development of IC/BPS and greater sensitivity to pain. These findings may help to identify patients that could benefit from systemic anti-inflammatory treatments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BMI ..... Body Mass Index
BPI ..... Brief Pain Inventory
FSFI ..... Female Sexual Functioning Inventory
GUPI ..... Genitourinary Pain Index
IL ..... Interleukin
IC/BPS ..... Interstitial Cystitis/Bladder Pain Syndrome
ICSI ..... Interstitial Cystitis Symptom Index
LPS ..... Lipopolysaccharide
QST ..... Quantitative Sensory Testing
SAC ..... Staphylococcus aureus Cowan
SYMQ ..... Symptom and Health Care Utilization Questionnaire
TLR ..... Toll Like Receptor
TNF-α. ..... Tumor Necrosis Factor-Alpha

BACKGROUND AND REVIEW

Pain and Central Sensitization

At a basic level pain is an unpleasant feeling or sensation associated with tissue damage or injury. Much of the pain experience is due to nociceptive neurons capable of discerning noxious stimuli. These neurons are specialized to respond to high threshold input such as intense heat or sharp pressure. When stimuli reach this high threshold first order nociceptive neurons relay the signal to second order nociceptive neurons in the dorsal horn of the spinal cord. These subsequently relay the signal to various regions of the thalamus and neocortex (Woolf, 2011).

Until the 1960's the prevailing view of pain sensory networks was that they operated in a relatively passive state, relaying – but not modulating – these signals (Woolf, 2011). In 1965 Melzack and Wall first demonstrated that this relay system could, in fact, be inhibited in the spinal cord (Melzack & Wall, 1965) and further discoveries in the ensuing decades demonstrated that pain signaling is modulated by complex and interactive processes in both the peripheral and central nervous systems (Willis, 1985). The experience of pain can be both inhibited and promoted. The state of heightened responsiveness to painful stimuli is referred to as pain "sensitization".

Sensitization following injury is both normal and adaptive; when tissue damage occurs heightened pain sensitivity prevents an organism from exacerbating the injury and promotes self-care. In this case, high threshold responding sensory neurons (nociceptors) transmit the pain signal but at a lower threshold of input, a phenomena termed "hyperalgesia." The parallel sensory process by which low-threshold sensory neurons transmit non-aversive signals is unaffected. However, it is possible for these parallel processes to converge, resulting in low-threshold input leading to pain signals, a phenomenon termed "allodynia." This convergence is referred to as *central sensitization,* as it is believed to take place in the spinal cord and in pain networks in the brain (Woolf, 2011). Mechanistic and biological correlates of central

sensitization are the subject of intense investigation as chronic pain affects approximately 100 million people in the United States and the annual cost of treating pain exceeds expenditures for heart disease, cancer and diabetes (Gaskin & Richard, 2012).

Functional Somatic Syndromes

At the most basic level a Functional Somatic Syndrome (FSS) is a collection of symptoms experienced persistently by an individual for which no medical explanation can be found. Various names have been applied to symptoms of FSSs, including Medically Unexplained Symptoms (MUS)s. Some published studies use the terms somatization and somatoform disorders interchangeably with FSSs or MUSs, though these terms explicitly implicate psychological processes in symptom expression. Common FSSs include Irritable Bowel Syndrome (IBS), Chronic Fatigue Syndrome (CFS), fibromyalgia, Temperomandibular Disorder (TMD), and Chronic Pelvic Pain (CPP), as each affects millions of men and women in the United States (Nimnuan, Rabe-Hesketh, Wessely, & Hotopf, 2001).

The view that FSSs are essentially expressions of somatization finds significant support in the medical community (Barsky & Borus, 1999). This perception is fueled by in part by high rates of psychiatric comorbidities in FSSs (discussed below) and in part because most patients with FSSs do not have tissue damage or inflammation in the areas where they report symptoms. For instance, there are no definitive structural abnormalities or biomarkers for IBS, CFS or fibromyalgia (Daniel J Clauw, 2014; Morris & Maes, 2013; Soares, 2014). However, this view does not take into account the possibility that many patients with FSSs may, in fact, be physiologically sensitized to the experience of pain in a manner that is not manifested in end organ abnormalities but in peripheral and central nervous system structures that are not easily monitored. Perhaps in recognition of ongoing debate about the etiology of FSSs, the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) introduces the term Somatic Symptom Disorder (SSD) to replace or augment prior diagnoses such as somatoform disorders and no longer requires that symptoms have no medical basis for the diagnosis to be applied.

Prevalence and Burden

While the prevalence of FSSs is difficult to estimate given changing and alternative criteria for diagnoses, recent meta-analyses and large surveys place the prevalence of IBS at 11% (Canavan, West, & Card, 2014), CPP at 20% (Burkman, 2004) , CFS at 9% (Skapinakis, Lewis, & Meltzer, 2003) and fibromyalgia at 3% (Queiroz, 2013) in community samples. Attendant costs in lost economic productivity and health care spending are substantial. For instance, in the United States direct costs of treating an individual with IBS are estimated to be $1,562 to $7,547 annually, with an additional $791 to $7,737 estimated in indirect costs (Nellesen, Yee, Chawla, Lewis, & Carson, 2013). Similarly, estimated annual direct costs of treating CPP are approximately $7000 and nearly 20% of patients report lost wages due to the condition (Clemens, Markossian, & Calhoun, 2009).

FSSs are strongly associated with reduced quality of life (QOL). Compared to healthy controls (HC), women with CPP report worse physical functioning, more bodily pain, loss of vitality, worse general health and social functioning, and greater role limitations due to emotional and physical difficulties than healthy controls (Nan E. Rothrock, Lutgendorf, Hoffman, & Kreder, 2002). Patients with IBS evince largely the same pattern of QOL impairments (Whitehead, Burnett, Cook, & Taub, 1996) as do patients with fibromyalgia (Gormsen, Rosenberg, Bach, & Jensen, 2010) and CFS (Schweitzer, Kelly, Foran, Terry, & Whiting, 1995). These findings suggest that FSSs represent a substantial burden to sufferers, their families, and the health care systems they use.

Are FSSs discrete?

Three primary observations suggest that FSSs are not entirely discrete syndromes. The first observation concerns the high degree of comorbidity between FSSs. For instance, cross-sectional analyses reveal that IBS is present in 39% of CPP patients, and fibromyalgia in 19% (Nickel et al., 2010). In patients with fibromyalgia, nearly 66% also report CPP (Waylonis & Heck, 1992) and in CFS patients 63% meet criteria for IBS (Gomborone, Gorard, Dewsnap, Libby, & Farthing, 1996). Additionally, the presence of FSSs are associated with future diagnoses of other FSSs; a recent study found that having any FSS was

associated with 2.4 greater odds (95% CI, 1.3-4.7) of developing a new FSS in the following year (Warren, Langenberg, & Clauw, 2013). Principal Components Analyses (PCA) of MUSs have been employed to determine if the independence of putatively discrete syndromes can be confirmed. Subjects referred to outpatient clinics (n=550) were evaluated on 37 distinct cardinal symptoms of 13 FSSs and these symptoms were used in a PCA to determine the optimal factor structure. A two factor solution best fit the data, and subsequent analyses with FSS categorization revealed that 8 of the 13 FSSs including IBS, CFS, fibromyalgia, TMD and CPP were best explained by a single factor, suggesting that independence of these syndromes cannot be confirmed by the symptoms used to diagnose them (Nimnuan, Rabe-Hesketh, Wessely, & Hotopf, 2001).

The second primary observation is that FSSs share many psychosocial and demographic correlates. For instance, FSSs have been convincingly linked to a history of maltreatment and adversity, both in childhood and adulthood. A meta-analysis of 23 studies comprising 4640 subjects found that lifetime history of sexual abuse was associated with significantly greater odds of developing functional gastrointestinal disorders (OR 2.43, 95% CI, 1.36-4.31) and CPP (OR 2.73, 95% CI 1.73-4.30) (Paras et al., 2009). Similarly, a recent meta-analysis of 71 studies examined multiple forms of psychological trauma including emotional, physical, and sexual abuse, and found that exposure to any trauma was associated with 2.7 greater odds (95% CI, 2.27-3.10) of meeting criteria for a FSS (Afari et al., 2014). Meta-analyses have also confirmed that mood disorders, including depression and anxiety disorders, are more common in patients with FSSs compared to patients with organic disease and healthy comparison groups (Henningsen, Zimmermann, & Sattel, 2003). Large scale twin studies suggest a common genetic component for risk of developing FSSs (Kato, Sullivan, Evengård, & Pedersen, 2009; Vehof, Zavos, Lachance, Hammond, & Williams, 2014). Additionally, almost all MUSs and FSSs are more common in women than in men (Kroenke & Price, 1993). Given high rates of psychiatric comorbidity, it may be tempting to suggest that symptoms are largely due to catastrophizing, somatization and negative affect. However, mediation modeling approaches demonstrate that psychological and environmental factors (e.g.

neuroticism, abuse history, life events, anxiety, somatization and catastrophizing) account for just 36% of the variance in IBS severity and 42% of the variance in pain experienced by fibromyalgia patients (Malt, Olafsson, Lund, & Ursin, 2002; van Tilburg, Palsson, & Whitehead, 2013). This suggests that considerable variation in the symptoms of FSSs is not attributable to psychological factors.

The third primary observation supporting the relatedness of FSSs is the presence of medically unexplained pain. IBS is characterized by pain in the abdomen and pain associated with bowel movements (Drossman & Dumitrascu, 2006), fibromyalgia by widespread pain in the back, shoulders and extremities (Wolfe et al., 2010), CFS by headache, muscle pain, and joint pain without redness or swelling (Fukuda et al., 1994), and TMD by pain in or around the ear, face, jaw and temple (Dworkin et al., 2002). Additionally, pain is the most common complaint driving patients with FSSs to seek consultation (Hungin, Chang, Locke, Dennis, & Barghout, 2005; Kersh et al., 2001).

Taken together, these observations strongly support the conceptualization of FSSs as closely related constructs. This position argues for investigation of mechanisms promoting the development of FSSs that are not confined to the end organs with which each FSS is most closely associated. This is a particularly salient directive when considering the painful facets of FSSs, as the pain experience is complex and involves the interplay of both the peripheral and central nervous system.

Pain Modulation in FSSs: Central Pain Sensitization?

A substantial body of research suggests that many FSSs are characterized by abnormal pain modulation. In addition to visceral pain hypersensitivity (i.e. pain during rectal distension) both adults and adolescents with IBS demonstrate reduced thresholds for heat and pressure pain in areas not associated with IBS symptoms (i.e. the shoulder and forearm) compared to HCs (Piché, Arsenault, Poitras, Rainville, & Bouin, 2010; Stabell et al., 2014). Similarly, TMD patients demonstrate reduced pressure pain thresholds in the forearm and calf compared to HCs (Fernández-de-las-Peñas et al., 2009). A case control study of pain sensitivity at 7 non-specific sites in CFS revealed that CFS sufferers experience pain at less than half the pressure level of the HC group (3.30 kg/cm2 for CFS vs. 8.09 kg/cm2 for HC). In each of these

studies, psychological distress did not mediate the relationship between the FSS and pain sensitivity. These findings suggest that abnormal pain modulation in FSSs is, to some degree, a systemic phenomenon. Therefore, central sensitization is a useful framework in which to consider the etiology of FSSs. However, mechanisms of central sensitization and relevant biological correlates are only beginning to be investigated in human clinical populations with most work thus far focusing on brain imaging of pain processing networks in individuals with chronic pain (Seifert & Maihöfner, 2009).

Interstitial Cystitis/Bladder Pain Syndrome

Definition and Diagnosis

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a chronic condition characterized by pain in the bladder and or/pelvic region and urinary symptoms such as urgency, frequency, and nocturia (waking to urinate) (Hanno et al., 2011a). IC/BPS falls under the larger category of urologic chronic pelvic pain syndromes (UCPPS)s and is often considered in relation to other conditions characterized by pain and urinary symptoms such as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). A recent systematic investigation of IC/BPS prevalence in women, which contacted over 140,000 households and used structured follow-up interviews, found that between 3-7% of all women in the U.S. meet criteria (Berry et al., 2011a). This translates to 3.3 to 7.9 million women in the U.S. alone. Annual direct healthcare expenditures for IC/BPS exceed $7,900 per patient (Anger et al., 2011).

IC/BPS is a diagnosis of exclusion. The American Urologic Association (AUA) recommends a three part diagnostic procedure which includes: A) a review of the patient's medical history, a physical examination (i.e. pelvic exam), and laboratory tests, including urinalysis and urine culture. This allows the physician to exclude confounding conditions including bladder cancer and active infection. B) Measurement of baseline urinary and painful symptoms for monitoring treatment efficacy. C) Cystoscopy and urodynamics when diagnoses are uncertain. However, there are no agreed upon findings that indicate IC/BPS, excepting the presentation of Hunner's lesions, inflammatory lesions or ulcerations found in the wall of the bladder (Hanno et al., 2011a). Interestingly, Hunner's lesions are present in only

approximately 10% of IC/BPS patients (Simon, Landis, Erickson, & Nyberg, 1997). Other differences in bladder physiology have been posited and associated with IC/BPS to vary degrees. These include injury or deficiencies in the defensive mucosal layer or the urothelium, mast cell or vascular abnormalities, undetected infection, neurogenic inflammation, and upregulation of sensory nerves in the bladder (Offiah, McMahon, & O'Reilly, 2013). However, these putative etiologic factors have been inconsistently linked to IC/BPS and have failed to produce successful treatment strategies.

Treatment

Front line treatments of IC/BPS primarily consist of behavioral modifications (e.g. altering fluid intake, dietary restrictions, stress management) and education on availability and efficacy of second and third line treatments. Second line treatments include manual physical therapy techniques, multi-modal pain management (possibly including pharmacologic intervention), and a variety of classes of oral medications with few adverse side effects but questionable effectiveness (e.g. tri-cyclic antidepressants). Third line treatments include low-pressure hydrodistension of the bladder and/or electrocautery/fulguration of Hunner's lesions discovered during cystoscopy. Fourth, fifth and sixth line treatments are also available and may include implantation of neuro-stimulatory devices, oral administration of cyclosporine A, and major surgery (Hanno et al., 2011a). More than 20% of patients are treated with hydrodistension or intravesical infusions (Anger et al., 2011).

Despite the diversity of treatment options and large expenditures, IC/BPS patients are generally dissatisfied with outcomes. One large-scale longitudinal investigation of actively treated IC/BPS patients (n= 637, median follow-up 31 months) found no detectable improvement in symptom severity over the period of observation (Propert et al., 2000). In another survey of 750 IC/BPS patients, 27-50% felt that there had been no improvement in their condition across all treatments, and another 26-31% felt their condition had deteriorated over the preceding 6 months (Hill, Isom-Batz, Panagopoulos, Zakariasen, & Kavaler, 2008). This is unsurprising given the results of clinical investigations of individual available treatments. For instance, a recent randomized, double-blind, placebo-controlled study of the effectiveness

of pentosan polysulfate sodium, the only FDA approved oral medication for IC/BPS, found no differences in symptom reduction between active and placebo groups (Nickel et al., 2014). Similarly, in what appears to be the only placebo-controlled study of intravesical infusion of botulinum toxin, one AUA third-line treatment used commonly in IC, there was no observed benefit of the treatment (Gottsch, Miller, Yang, & Berger, 2011).

IC/BPS: a FSS?

While peripheral inflammation and tissue pathology continue to be investigated in IC/BPS and related conditions in hope of developing effective treatments, there is considerable evidence that the focus on peripheral pathology is inadequate. For instance, a review of laparoscopic examinations for IC/BPS symptoms revealed that approximately 35% of patients have no visible pelvic pathology (Hammoud, Gago, & Diamond, 2004), and patients with negative laparoscopic findings continue to have persistent pelvic pain (Cox, Ayers, Nala, & Penny, 2007). Furthermore, the presence of peripheral pathology appears to be weakly related or unrelated to symptoms. Case-control studies have revealed that women with IC/BPS symptoms are not more likely than asymptomatic women to have endometriosis or pelvic adhesions (Thornton et al., 1997; Walker et al., 1995) and correlational studies show inconsistent or weak relationships between severity of lesions and the presence of pain (Sinaii et al., 2008; Vercellini et al., 2007). A recent investigation of ulcerative vs. non-ulcerative IC/BPS found no differences in factors that exacerbated symptoms, extent of pain, or symptom scores (Killinger, Boura, & Peters, 2013).

Factors that are associated with IC/BPS are similar to those associated with FSSs. For instance IC/BPS sufferers are more likely to have depression (Novi et al., 2005), anxiety (Chung, Liu, Lin, & Chung, 2014), childhood sexual trauma (Nickel et al., 2011), and antecedent MUSs than healthy comparison groups (Warren et al., 2009). FSSs such as IBS, CFS and fibromyalgia are common comorbidities in IC/BPS. A comparison of IC/BPS patients with matched controls found that IBS was present in 39% of IC/BPS patients vs. 5% of controls, that fibromyalgia was present in 17% of IC/BPS patients vs. 3% of controls, and CFS present in 10% of IC/BPS patients vs. 2% of controls (Nickel et al., 2010). A recent

review of the IC/BPS literature with an emphasis on shared characteristics of FSSs found that 16 of 18 features of FSSs were present in IC/BPS (Warren, 2014). Additionally, pain modulation appears to be altered in IC/BPS. IC/BPS patients tested with Quantitative Sensory Testing (QST) techniques show reduced thermal pain tolerance, measured at the lower right ankle (Ness, Lloyd, & Fillingim, 2014a), and mechanical hyperalgesia in the suprapubic region (Lai, Gardner, Ness, & Gereau, 2014), compared to controls.

Taken together, these findings strongly suggest that IC/BPS shares much in common with FSSs, and that the evidence for peripheral etiology in IC/BPS is lacking. These observations, coupled with altered pain perception in IC/BPS, argue for a more systemic evaluation of factors that might influence the experience of pain in IC/BPS – the role of central sensitization.

Putative Mechanisms of Central Sensitization – The Role of Inflammation

A Brief Overview of Inflammatory Processes

The four cardinal signs of inflammation, heat, redness, swelling and pain, reflect the migration of leukocytes from blood vessels into the adjacent tissue (Medzhitov, 2010) . These neutrophils, macrophages, and mast cells then act to clear invading pathogens. Small cellular messengers, called cytokines (e.g. Interleukin-6 [IL-6], Tumor Necrosis Factor- alpha [TNF-α]), are primary actors driving this migration. The release of the cytokines often occurs when elements of bacteria, viruses and damaged or dying cells are recognized by Toll-Like Receptors (TLR)s, highly conserved receptors on sentinel immune cells (Takeda, Kaisho, & Akira, 2003). When the concentration of these pro-inflammatory cytokines is sufficient, they begin to elicit systemic effects, including release of acute phase proteins from the liver and prostaglandins from the brain, which can then induce behavioral changes (Medzhitov, 2010). Inflammation is a beneficial process up to a point, but sustained inflammatory signaling can lead to damage of healthy tissue, organ failure, and even death. It is increasingly recognized that inflammatory process are subtly altered in many individuals not presenting the cardinal signs of acute inflammation. Elevated low-grade inflammation is associated with a number of chronic diseases including

cardiovascular disease, diabetes and cancer (Coussens & Werb, 2002; Ridker, 2003; Wellen & Hotamisligil, 2005).

The hypothalamic-pituitary-adrenal (HPA) axis is an endocrine system that acts as one of the primary regulators of inflammation. One of the chief products of the HPA axis is cortisol, a glucocorticoid with potent anti-inflammatory and metabolic properties. The release of cortisol typically follows a diurnal pattern with peak levels secreted shortly after waking and nadirs reached in the second half of the night (Chrousos, 1995). Chronic inflammation and chronic psychosocial stress are both associated with alterations in this pattern and reduced effectiveness of inflammatory regulation (Silverman & Sternberg, 2012).

Inflammation and Pain

Both observational and experimental data support the role of dysregulated inflammatory processes in pain modulation. Animal models conclusively demonstrate that acute inflammatory immune challenges, such as injection of lipopolysaccharide (LPS; a component of gram-negative bacteria) and injuries that generate damage-associated molecular patterns (DAMP)s, produce hyperalgesia (Grace, Hutchinson, Maier, & Watkins, 2014; M R Hutchinson et al., 2009). Additionally, longer duration inflammatory insults, such as chronic constriction injuries, produce chronic pain that is reversible by pharmacologic inhibition of inflammation-promoting TLRs (Lewis et al., 2012). Animal models also demonstrate that prior exposure to glucocorticoids sensitizes animals to inflammation-induced chronic pain (Hains et al., 2011; Loram et al., 2011). Human studies of experimental endotoxemia, in which a small dose of LPS is injected into a participant, also demonstrate the role of inflammation in pain sensitization. In one study, intravenous LPS injected in healthy participants induced reduced pain thresholds after 3 hours and these reductions were associated with peak increases in pro-inflammatory cytokine levels in blood (Wegner et al., 2014). Similarly, another study of healthy participants found that intravenous LPS reduced visceral pain thresholds (measured by rectal distension) two hours post injection and that these reductions were correlated with IL-6 increases in blood (Benson et al., 2012).

Inflammation in FSSs

Abnormalities in inflammatory processes have been identified in many FSSs. For instance, meta-analyses and systematic reviews find a general pattern of elevated pro-inflammatory cytokines in the blood of patients with IBS (Ortiz-Lucas, Saz-Peiró, & Sebastián-Domingo, 2010), CFS (Patarca, 2001) and fibromyalgia (Uçeyler, Häuser, & Sommer, 2011). Similarly, abnormalities of the Hypothalamic-Pituitary-Adrenal (HPA) axis have been noted in a number of FSSs. IBS and fibromyalgia patients show blunted cortisol responses to socially stressful tasks (Suárez-Hitz et al., 2012; Wingenfeld et al., 2008) , and patients with CFS show a blunted adrenocorticotropic hormone (ACTH) response to social stress (Gaab et al., 2005). Abnormal responses to TLR stimulation in the peripheral blood of FSS patients have also been noted. In IBS patients, stimulation of whole blood with TLR agonists resulted in a stronger response of pro-inflammatory cytokines compared to HCs (McKernan, Gaszner, Quigley, Cryan, & Dinan, 2011) and CFS patients display lower production of the anti-inflammatory cytokine transforming growth factor-beta (TGF-β) following TLR stimulation of peripheral blood mononuclear cells (PBMC)s (Tomoda et al., 2005). Taken together these findings suggest that inflammatory dysregulation may play a role in the etiology of FSSs, though it is unknown if such dysregulation is associated with painful symptoms. Furthermore, different facets of the inflammatory response have rarely been explored simultaneously in patients with FSSs.

Summary of Literature Review and Purpose of the Current Project

Several important conclusions follow from the preceding review.

a) FSSs are highly prevalent and burdensome to the patient and health care system. This conclusion stems from the high estimated prevalence of FSSs in community samples, the substantial reductions in QOL reported by patients with FSSs, and large direct and indirect annual costs of treating patients with FSSs.

b) *IC/BPS is a condition characterized urologic symptoms and pelvic pain for which current treatments are inadequate.* This conclusion stems from clinical trials and observational studies of patients with

IC/BPS that find little or no improvement in symptoms over time and weak efficacy of available treatments despite substantial expenditures.

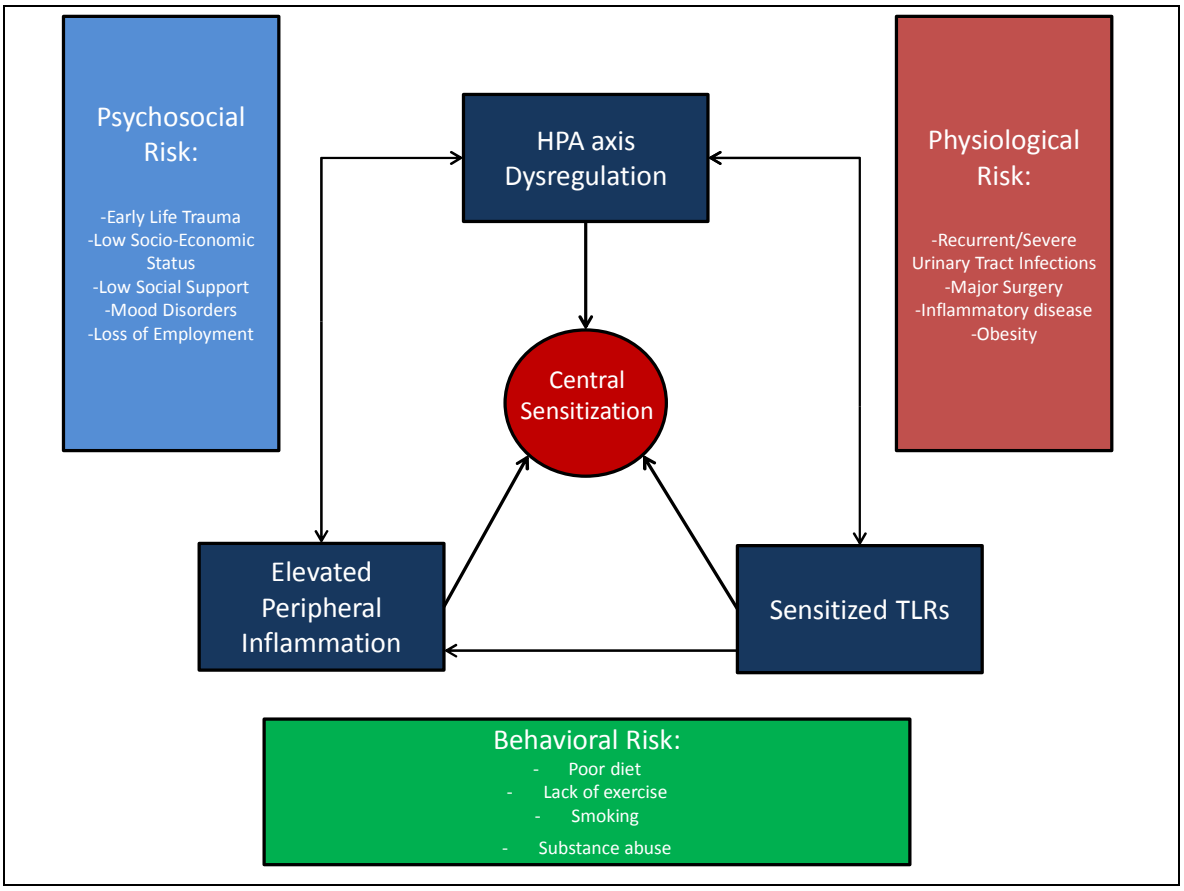
c) *IC/BPS shares many features of FSSs.* This conclusion is supported by the limited evidence of peripheral pathology in IC/BPS, the high degree of comorbid FSSs observed in IC/BPS, the prevalence of psychosocial correlates of FSSs such as mood disorders and early life adversity, and evidence suggesting altered pain modulation in IC/BPS.

d) Patients with FSSs and IC/BPS display abnormal pain modulation that may be governed by a common mechanism, central pain sensitization. This conclusion follows the results of previous studies using QST in patients with FSSs and IC/BPS indicating that pain sensitivity is higher in patients with FSSs than in healthy comparison samples, and by the significant overlap in presentation of symptoms, including pain, in FSSs.

e) *Inflammatory dysregulation may play a role in central pain sensitization in FSSs and IC/BPS.* This conclusion is derived from the observation that many patients with FSSs have high levels of markers of inflammation in peripheral blood, abnormal functioning of the HPA axis, and heightened inflammatory responses to immune challenges mediated by TLRs.

These conclusions support a new conceptual model of painful symptoms in IC/BPS that emphasizes the contribution of dysregulated inflammatory processes to altered pain modulation. Exploring inflammatory mechanisms of pain in IC/BPS has the potential to identify sensitized pathways that are relevant to other FSSs. Furthermore, identifying these mechanisms may lead to novel treatment approaches that emphasize central and systemic facets of pain sensitization. See Figure 1 for the conceptual model of the role of inflammation in central sensitization in IC/BPS.

Figure 1. A conceptual model of inflammation and pain sensitization in IC/BPS.



The goal of this dissertation is to explore the role of inflammation and HPA-mediated inflammatory regulation in IC/BPS with an emphasis on pain. The conceptual model described in Figure 1 is investigated in the context of inflammatory dysregulation, IC/BPS symptoms and symptom trajectories, and comorbid pain.

Paper 1: Brief Summary and Purpose

The first paper explores three primary facets of inflammatory processes as correlates of the IC/BPS syndrome. These are basal *systemic inflammation*, measured by IL-6 in blood plasma, *immune cell responsiveness to challenge*, measured by a composite score of the IL-6 and IL-1β response to TLR-2 and 4 stimulation, and *diurnal rhythm of an inflammation suppressing hormone,* measured by salivary cortisol collected 9 times over 3 days. These measures are compared between female IC/BPS sufferers and healthy controls recruited from the community. Additionally, these inflammatory measures are analyzed

for their association with the magnitude of painful and urinary symptoms in IC/BPS sufferers. Findings provide insight into the utility of these measures as biomarkers of IC/BPS, and as correlates of IC/BPS symptomology in particular. Mechanisms suggested by these findings are interpreted and discussed.

Paper 2: Brief Summary and Purpose

The second paper expands on the findings of paper one by explicitly considering the above inflammatory processes as correlates of widespread pain in IC/BPS. This paper explores TLR-2 and 4 inflammation, diurnal cortisol, and IL-6 in plasma as markers of comorbid pain conditions (IBS, CFS, fibromyalgia, TMD and vulvodynia). Additionally, analyses are undertaken to explore these markers as correlates widespread pain measured on a body map in which IC/BPS sufferers are asked to endorse painful regions outside the bladder/pelvic region. Finally, QST is undertaken in a subsample of patients in which pressure pain thresholds are determined in a non-symptomatic region of the body (the thumb) and these results compared with inflammatory measures. Findings provide insight about whether the above markers are associated with comorbid (non-bladder/pelvic) pain, with implications for the presence, and possibly development, of additional syndromes. QST provides more objective evidence of central sensitization by using a standardized and validated measure of dolorimetry in IC/BPS. Positive findings would suggest a role for inflammatory dysregulation in the central sensitization phenomenon and support the conceptualization of IC/BPS as a FSS.

Paper 3: Brief Summary and Purpose

The third paper extends the cross-sectional findings of the first two papers by exploring TLR-2 and 4 inflammation as well as diurnal cortisol rhythms as predictors of longitudinal symptom trajectories (pain and urinary) and symptom flares over a 48 week period of observation. Symptom change is modeled with random and fixed effects with primary outcomes including time X inflammatory variable interactions (indicating different symptom trajectories) and inflammatory variables as predictors of increased likelihood of symptom flares. Findings provide insight about whether inflammatory measures have predictive utility and the potential to identify patients with recalcitrant symptoms. Longitudinal

findings provide additional evidence for altered pain modulation processing in some patients with IC/BPS.

CHAPTER 1. INFLAMMATION AND INFLAMMATORY CONTROL IN INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME: ASSOCIATIONS WITH PAINFUL SYMPTOMS

Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a debilitating chronic condition associated with urinary symptoms including urgency, frequency, nocturia and pelvic pain. Berry and co-authors report the prevalence of IC/BPS at between 3 and 8 million women in the United States (Berry et al., 2011b). Few patients achieve long-term remission of symptoms despite a range of treatments (Anger et al., 2011; Propert et al., 2000). A substantial body of research has explored bladder pathology and other peripheral tissue abnormalities, however, a large number of IC/BPS patients lack any discernible end organ inflammation, and do not respond to treatment of peripheral tissues (e.g. hydrodistension of bladder) (Potts & Payne, 2012). This suggests that investigation of more systemic factors (e.g., inflammation, inflammatory control) may yield important insights. IC/BPS patients have a high prevalence of comorbid Functional Somatic Syndromes (FSSs), such as irritable bowel syndrome (IBS) and fibromyalgia (FM), which are also characterized by chronic pain in the absence of clearly identifiable peripheral pathology(D J Clauw et al.; Novi et al., 2005). This suggests that altered central pain processing (e.g. hyperalgeisa, allodynia), termed central sensitization (Woolf, 2011) could be a contributory factor to the chronic pain seen in IC. Consistent with this notion, a recent study found that IC/BPS patients have reduced pain thresholds and pain tolerance compared to healthy controls (Ness, Lloyd, & Fillingim, 2014b).

Toll-Like Receptors (TLR), particularly TLR-4, have been identified as critical factors in central pain sensitization. Evidence in animal models of chronic pain suggests that inflammatory signaling secondary to TLR-4 stimulation plays a critical part in the development of hyperalgesia and allodynia (Ellis et al., 2014; Mark R. Hutchinson et al., 2008; Milligan & Watkins, 2009). Preliminary evidence suggests that heightened inflammatory responses to TLR stimulation may be a feature of human pain syndromes as well (Kwok, Hutchinson, Gentgall, & Rolan, 2012), but these have not been evaluated in IC/BPS.

Further, no studies have determined if inflammatory responses to TLR stimulation are associated with the magnitude of painful symptoms. Endogenous control of inflammation, one of the central functions of the Hypothalamic-Pituitary-Adrenal (HPA) axis may also be a critical factor in IC/BPS. We previously found that higher levels of morning salivary cortisol, an endogenous HPA axis glucocorticoid with anti-inflammatory properties, are associated with less severe symptoms in IC/BPS patients (Lutgendorf et al., 2002). TLR-mediated inflammation and HPA axis activity may be interactive, as glucocorticoids have been shown to potentiate inflammatory responses in animal models of pain (Loram et al., 2011). The goals of the current study were to identify differences in inflammatory processes, including those mediated by TLRs, and HPA axis function as assessed by diurnal cortisol between IC/BPS patients and healthy controls, and to determine whether these factors were associated with IC/BPS symptoms.

Methods

MAPP Study and Recruitment

The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) study is a multi-site research effort sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases to better characterize syndrome phenotypes, syndrome etiology, and the natural history of chronic urologic pelvic pain patients (Landis et al., 2014). The University of Iowa is a participating institution collecting site-specific data on biomarkers of chronic pelvic pain. Inclusion criteria for IC/BPS included being at least 18 years of age and reporting pain, pressure or discomfort associated with the bladder or pelvic region present the majority of the time during the most recent 3 months. Additionally, IC/BPS participants were negative on urine culture for any uropathogens. Exclusion criteria included a history of urethral stricture, neurological disorder affecting the bladder or bowel, cystitis caused by tuberculosis, radiation therapy or Cytoxan/cyclophosphamide therapy, augmentation cystoplasty or cystectomy, active autoimmune or infectious disorder, history of cancer, major psychiatric illness, or cardiac, pulmonary, renal, or hepatic disease, or pregnancy. Of the 98 female Iowa IC/BPS and HC participants enrolled in the broader trans-MAPP study at the University of Iowa, 86 (88%) agreed to an additional blood draw for

isolation of PBMCs, and 72 (73%) agreed and were able to collect salivary cortisol. Study participants were 58 (48 for cortisol analyses) IC/BPS patients and 28 (24 for cortisol analyses) HCs who met study criteria. In addition to meeting exclusion criteria, healthy controls had no urinary, pelvic, or bladder symptoms, and met no criteria for common FSSs IBS, FM, or Chronic Fatigue Syndrome (Drossman & Dumitrascu, 2006; Fukuda et al., 1994; Wolfe et al., 2010). All participants provided informed consent and all procedures were cleared by the Institutional Review Board of the participating MAPP institutions.

Demographic and Symptom Information

Participants provided demographic information at the time of eligibility screening. Upon study entry, participants had a blood draw, urine collection, physical examination and completed a battery of trans-MAPP questionnaires relating to pelvic and bladder symptoms which have been previously used to assess pain and symptom severity in urologic conditions. These included the 9-item Genitourinary Pain Index (GUPI)(Clemens, Calhoun, et al., 2009), the 19-item Female Sexual Functioning Inventory (FSFI) (Rosen et al., 2000), and the 4-item Interstitial Cystitis Symptom Index (ICSI) (O'Leary, Sant, Fowler, Whitmore, & Spolarich-Kroll, 1997) . The GUPI includes pain and urinary symptom subscales. The pain subscale contains questions indicating the number of painful areas in the genitourinary region (e.g. the bladder, urethra), the number of activities (i.e. filling, voiding of bladder) that are painful, and two questions relating to the frequency and intensity of pain experience regardless of area or activity. The FSFI contains 3 questions relating specifically to the frequency and intensity of pain during and after intercourse. The ICSI contains questions specific to bladder pain, and to the urgency and frequency of urination and nocturia.

Cortisol

Salivary cortisol was collected in salivettes by participants at 3 time points (upon waking: 4-9am, afternoon: 4-6:30pm, and bedtime: 8pm-12am) for three consecutive days. Samples collected outside this time frame were excluded to maintain homogeneity. Participants were instructed not to eat, exercise or consume caffeine for thirty minutes prior to collecting a sample. Self-report of collection time has been

demonstrated to be reliable and salivary cortisol is stable at room temperature (Kraemer et al., 2006). Salivettes were analyzed by chemiluminescence immunoassay (IBL, Hamburg, Germany) at the Technical University of Dresden. The lower detection limit is 0.41 nmol/L and inter-assay and intra-assay coefficients of variance are less than 10%.

Inflammatory Measures

Blood samples were collected between approximately 11:30am and 12:30pm. PBMCs were separated by Ficoll-paque gradient centrifugation within 30 minutes of blood collection and cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 ug/ml streptomycin for 3 days at 37°C in a humidified incubator with 5% CO2 and TLR agonists. TLR-2 and 4 agonists were selected on the basis of the role of these receptors in chronic pain in animal models (Bastos et al., 2013; Mark R. Hutchinson et al., 2008). For stimulation of TLR-4, 50 ng/ml of Lipopolysaccharide (LPS) was used; For TLR-2 stimulation, 0.04 ng/ml of Staphylococcus aureus Cowan I (SAC) was used. Conditioned media was then harvested and frozen at -80°C prior to batch ELISA analysis. Each well contained 1X10^6 cells in 24 well plates, with one well per subject for TLR-4 and TLR-2 stimulation. Cytokines were assayed in duplicate by DuoSet ELISAs (R&D Systems) according to instructions included with the kit. Plasma cytokines were assayed with high sensitivity Quantikine ELISAs. Similar approaches in populations with complex presentations of pain have been reported and are able to distinguish patients from healthy controls (Chao et al., 1991; Kovarik et al., 2011; Kowalski et al., 2008; Kwok et al., 2012).

Statistical analyses

Statistical analyses were performed using SPSS v. 21. Inflammatory variables (plasma interleukin-6 (IL-6), pro-inflammatory cytokine response to TLR stimulation in PBMCs) were log-10 transformed to normalize their distribution. Composite inflammation scores for stimulated cytokine responses were calculated by summing the z-scores ([individual score- group mean]/ group standard deviation) for the IL-6 and interleukin-1 beta (IL-1β) response in PBMCs following either LPS or SAC stimulation. Both cytokines have been identified as mediators of pain amplification by spinal glia and are released

following TLR-2 and 4 stimulation, in part, by transcription of nuclear factor-kappaB (NF□□) (Milligan & Watkins, 2009). Cortisol values were normalized using natural log transformations. Distributions of transformed variables were examined for confirmation of normality. Salivary cortisol values at each of the collection points were regressed on the time of collection over the three-day period to calculate cortisol slope, a measure of the average hourly decrease in cortisol over the course of the day as described previously (Kraemer et al., 2006). To determine if diurnal patterns of cortisol secretion differed between groups, a repeated measures ANOVA was used with time of cortisol sample (morning, afternoon, nocturnal) and group membership as factors. A significant time by group interaction indicates different patterns of diurnal secretion between groups. Post-hoc comparisons with Sidak adjustment were used to determine which, if any, time-points differed in salivary cortisol concentrations between groups.

BMI was used as a covariate in all analyses of biomarkers due to well-established relationships between adiposity and inflammation (Festa et al., 2001; Wirtz, Ehlert, Emini, & Suter, 2008). While all participants were free of major psychiatric diagnoses, levels of negative affect (i.e. anxious and depressive symptoms) have previously been linked to self-reported pain measures in chronic pain populations so the participant score on the Positive and Negative Affect Schedule (PANAS) negative affect scale was included as a covariate in analyses of symptoms (Smith & Zautra, 2008; Tang et al., 2008). Group differences between IC/BPS and HC in demographic and symptom data were tested by one-way analysis of variance (ANOVA) and chi-squared tests. Within IC/BPS participants, One-way ANOVAS were used to test mean differences in inflammatory variables for the use of tri-cyclic antidepressants, pentosan polysulfate, opioids, SSRI/SNRIs, and NSAIDs and the presence of a comorbid FSS. Differences in inflammatory variables between IC/BPS and HC were tested with General Linear Models controlling for BMI. Relationships between inflammatory variables and symptom scores were assessed using multiple regression controlling for BMI and negative affect.

Because use of tricyclic anti-depressants and the presence of a comorbid FSS were each associated with marginal differences in TLR responses, these variables were also controlled for in additional analyses to

determine if the magnitude of the association between inflammatory variables and self-reported painful symptoms remained statistically significant and comparable to reduced models. Similarly, because duration of symptom in years was associated with TLR-4 inflammatory response, models were also tested which controlled for duration of symptoms. To determine which inflammatory measures had the strongest association with painful symptoms, all inflammatory variables (IL-6, cortisol slope, TLR-2 inflammatory score, and TLR-4 inflammatory score) were used simultaneously as predictors of painful symptom scores in models with BMI and negative affect.

To determine if dysregulated HPA activity and heightened TLR-4 inflammatory responses combine to exacerbate symptoms of pain, we divided patients on median splits of cortisol slope (steep, i.e. healthier; flat, i.e. less healthy) and TLR-4 inflammatory response, and compared measures of pain in the resulting 4 groups by One-Way ANOVA. Post-hoc comparisons were conducted with Sidak adjustment.

Results

Participant Characteristics

The mean age of participants was approximately 42 years (range 20-68). IC/BPS patients and healthy controls did not differ on potential confounding variables such as income, education, race, ethnicity, employment status or age (all $p>.15$). BMI of HCs was significantly elevated compared to that of IC/BPS patients ($p=.021$). As expected, IC/BPS participants reported more genitourinary symptoms including pain and urinary dysfunction, elevated IC symptoms, and more compromised sexual functioning (all $p<.004$) when compared to HCs. In IC/BPS patients the average duration of symptoms was 7.7 (SD=7.3) years. See Table 1.

Table 1. Participant Characteristics, Chapter 1.

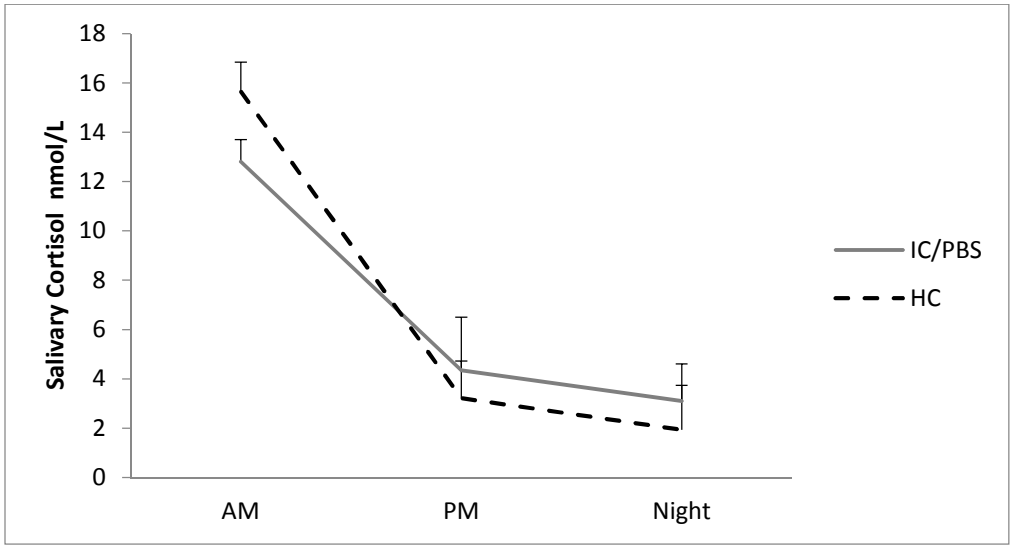
	IC/BPS *n*=58	Healthy Controls *n*=28	*p*
Age Mean(SD)	41.1 (14.8)	42.9 (13.1)	.59
BMI Mean(SD)	27.2 (5.8)	30.5 (6.9)	.021
Race % (*n*) *White* *Asian* *Multi Race*	 97 (56) 2 (1) 2 (1)	 100 (28) 0 0	.61
Ethnicity % (*n*) *Non-Hispanic*	 100 (58)	 100 (28)	N/A
Education % (*n*) *High School or GED* *Some College* *Graduated College* *Graduate Degree*	 14 (8) 24 (14) 33 (19) 29 (17)	 11 (3) 25 (7) 39 (11) 25 (7)	.93
Employment % (*n*) *Employed* *Unemployed* *Disabled* *Retired* *Full Time Homemaker*	 60 (35) 12 (7) 9 (5) 7 (4) 10 (6)	 90 (25) 4 (1) 0 (0) 4 (1) 4 (1)	.15
Annual Income/$ % (*n*) *<10,000* *<25,000* *<50,000* *<100,000* *>100,000* *Prefer not to answer*	 14 (8) 7 (4) 21 (12) 36 (21) 17 (10) 5 (3)	 7 (2) 7 (2) 18 (5) 46 (13) 18 (5) 4 (1)	.92
Comorbid Conditions % (*n*) None Irritable Bowel Syndrome Fibromyalgia Chronic Fatigue Syndrome	 33 (47) 23 (40) 2 (3) 8 (14)	 0 (100)	N/A

IC/BPS participants on tricyclic antidepressants (n=29, 50%) showed marginally lower IL-6 responses following TLR-4 stimulation (p=.056) and lower IL-6 following TLR-2 stimulation (p=.044) but no differences in any other inflammatory measure (p values>.42). IC/BPS participants on opioids (n=11, 19%), pentosan polysulfate (n=29, 50%), SSRI/SNRIs (n=9, 16%), or NSAIDs (n=7, 12%) did not differ on any inflammatory variable from patients not taking those medications (all p>.12). The presence of a comorbid FSS was associated with marginally higher IL-1β following TLR-4 stimulation (p=.07) but no differences in any other inflammatory variable (p values>.27).

Inflammatory Markers and Cortisol in IC/BPS patients and Healthy Controls

Cortisol slope was significantly flatter (p=.010) in IC/BPS participants compared to HCs, indicating a smaller hourly decrease in salivary cortisol for IC/BPS patients. Additionally, the results of the repeated measures ANOVA revealed a significant time by group interaction (p=.016). Post-hoc comparisons revealed no significant differences in morning cortisol (p=.14) but marginally higher afternoon cortisol (p=.07) and significantly higher nocturnal cortisol (p=.019) in the IC/BPS group, indicating a blunting of the diurnal cortisol rhythm. See Figure 2.

Figure 2. Means and standard deviations of diurnal salivary cortisol concentrations in IC/BPS participants and HCs.



As seen in Table 2, IC/BPS participants had significantly elevated plasma IL-6 compared to HC's ($p=0.040$), suggesting an elevated level of basal inflammation. When stimulated with TLR-2 receptor agonist SAC, PBMCs isolated from IC/BPS participants demonstrated greater IL-1β responses ($p=.040$), but no difference in IL-6 responses ($p=.10$), compared to PBMCs of HC participants. There were no statistically significant differences between IC/BPS and HC participants in the pro-inflammatory cytokine response in LPS stimulated PBMCs (see Table 2.)

Table 2. Symptoms and Biomarkers in IC/BPS patients and Healthy Controls.

Variable Mean (95% CI)	IC/BPS *n*=58	Healthy Controls *n*=28	*p*
GUPI score total	24.7 (22.7,26.8)	1.4(.5,2.3)	<.001
GUPI score pain	12.0(10.8,13.1)	.3(-.1,.6)	<.001
GUPI score urinary subscale	5.6(4.8,6.4)	.7(.3,1.1)	<.001
IC Symptom Index	10.5(9.3,11.6)	2.5(1.6,3.5)	<.001
FSFI total	16.5(13.78,19.1)	25.3(21.2,29.5)	<.001
FSFI Pain	6.00(4.5,7.4)	11.9(9.7,14.1)	<.001
Cortisol Slope (ln transformed)	-.10(-.12, -.08)	-.14(-.17, -.12)	.010
IL-6 plasma (log_{10} transformed)	.45(.39,.52)	.34(.25,.43)	.040
IL-1β + LPS	3.34(3.16,3.50)	3.53(3.27,3.77)	.24
IL-1β + SAC	1.22(.93,1.52)	.67(.24,1.10)	.039
IL-6 + LPS	4.20(4.09,4.31)	4.13(3.96,4.29)	.48
IL-6 + SAC	1.71(1.35,2.07)	1.15(.63,1.67)	.09

Associations between Inflammation and IC/BPS Symptoms

The TLR-4 inflammation score was associated with multiple measures of non-specific pain intensity and frequency. The composite TLR-4 inflammation score (calculated from the response of IL-6 and IL-1β to LPS) was significantly associated with higher total GUPI scores (p=.005), GUPI pain subscale scores (p=.010), and marginally with GUPI urinary symptom subscale scores (p=.062). On the GUPI pain

subscale, the relationship with TLR-4 inflammation score was strongest for two items; pain frequency (p=.001) and intensity (p=.008). The TLR-4 inflammation score was also associated with reduced FSFI sexual functioning (p=.001); the strongest relationships were seen with pain variables in this scale: e.g., pain frequency during intercourse (p=.002), pain frequency after intercourse (p=.002), and sexual pain severity (p=0.003). The TLR-4 inflammation score was marginally associated with higher IC symptom index scores (p=.068). However, there were no significant relationships between the TLR-4 inflammation score and the ICSI urinary urgency, frequency, or nocturia items (p-values > 0.17) or with the pain/burning specific to the bladder item (p=.13). Interestingly, TLR-4 inflammatory response was associated with longer duration of symptoms (years; p=.047) and with flatter cortisol slopes (p=.043). In contrast to these findings, TLR-2 inflammatory response for SAC-stimulated PBMCs, plasma IL-6, and cortisol slope were not significantly associated with any symptom scale (all p>.10). See Table 3 for associations of TLR-4 inflammatory response with scales. See Table 4 for associations of TLR-4 inflammatory response with specific items.

Table 3. Relationship of TLR-4 inflammatory response with symptom scores.

TLR-4 (LPS) inflammation score		
Measure	β	*p*
GUPI score total	.333	.005
GUPI score pain	.310	.010
GUPI score urine	.252	.062
FSFI total	-.407	.001
FSFI pain	-.393	.002
IC symptom Index	.246	.068
Cortisol slope	.299	.040
Duration of symptoms (years)[a]	.256	.059
TLR-2 (SAC) inflammation score	.236	.080
Plasma IL-6 pg/mL	.037	.78

[a] adjusted for age

Table 4. Relationships between TLR-4 inflammatory response and scale items.

TLR-4 (LPS) inflammation score		
Item	β	*p*
GUPI pain frequency	.401	.001
GUPI pain intensity	.339	.008
FSFI pain frequency during intercourse	-.405	.002
FSFI pain frequency after intercourse	-.389	.002
FSFI pain severity	-.368	.003
Urinary Urgency (ICSI Q1)	.174	.18
Urinary Frequency (ICSI Q2)	.117	.40
Nocturia (ICSI Q3)	.182	.18
Burning/Pain in Bladder (ICSI Q4)	.201	.13

The relationships between TLR-4 inflammation scores and symptom scales were not attenuated or rendered insignificant when analyses adjusted for use of tri-cyclic anti-depressants and presence of comorbid FSSs, nor were either of these associated with differences in painful symptoms (p values >.19). Adjusting for the duration of symptoms in years similarly did not affect the relationship between TLR-4 inflammation and painful symptoms and duration of symptoms was not itself associated with pain (p values > 0.14). The relationship between TLR-4 inflammation score and painful symptoms was similarly unaffected when all other inflammatory variables were included simultaneously as predictors of painful symptoms, and no other inflammatory variable was associated with pain in these models (p values >.16).

Secondary Analyses

Consistent with the hypothesis that HPA dysregulation and TLR-4 inflammation may act synergistically on measures of pain, we found that, amongst the four groups, the most severe pain scores on the GUPI pain subscale, GUPI pain frequency and severity items, and FSFI pain subscale were all reported in the group with flatter cortisol slopes and high TLR-4 inflammatory responses (Table 5).

Table 5. Means and standard deviations of selected scales and items by patient groups.

Group	GUPI pain scale	GUPI pain frequency**	GUPI pain severity	FSFI pain scale ** +
steep slope/ low TLR-4 inflammation (n=13)	11.055 (5.29)	2.31 (1.03)*	4.38 (2.63)	10.54 (4.86)*
flat slope/ low TLR-4 inflammation (n=10)	10.50 (4.35)	2.1 (1.2)*	4.00 (1.89)	4.4 (5.03)
steep slope/ high TLR-4 inflammation (n=11)	11.42 (4.45)	2.64 (1.29)	4.82 (1.89)	4.36 (4.18)
flat slope/high TLR-4 inflammation (n=14)	13.81 (3.32)	3.71 (1.33)	5.86 (1.46)	3.00 (4.22)

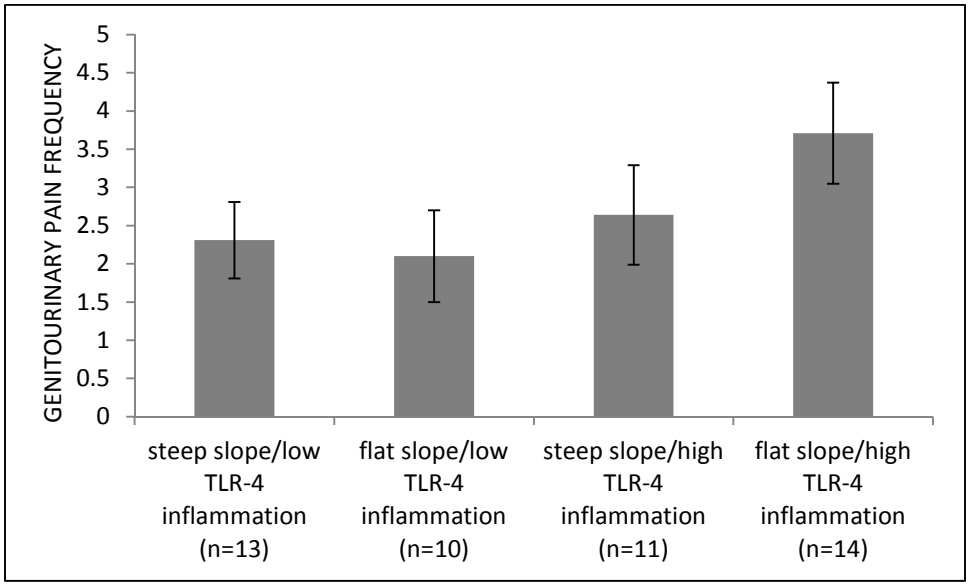
** omnimbus test, $p<.05$

* post-hoc comparison vs. flat slope/high TLR-4 inflammation group, $p<.05$

+ lower scores indicate greater pain

Despite relatively small groups, post-hoc comparisons demonstrated that differences between the flat cortisol slope/high TLR-4 inflammation group and steep cortisol slope/low TLR-4 inflammation group were statistically significant for the GUPI pain frequency and FSFI pain scores (both $p<.027$). See Figure 3.

Figure 3. Means and standard deviations of GUPI pain frequency item for IC/BPS participants grouped by median split of cortisol slope and TLR-4 inflammation response.



Discussion

One of the major findings of this study is that TLR-4 stimulated inflammatory cytokine responses are robustly associated with measures of painful symptoms in women with IC/BPS. These findings were not altered when controlling for multiple other measures of inflammation and potential confounding variables. The symptoms most strongly associated with TLR-4 stimulated inflammatory responses were non-specific pain severity and pain frequency. Further, IC/BPS is marked by inflammatory dysregulation, indicated by flattened diurnal cortisol rhythmicity, elevated plasma IL-6 and an exaggerated IL-1□ response to TLR-2 stimulation in PBMCs. TLR-2 stimulated IL1-□□responses in PBMCs were recently shown to be capable of differentiating a population of chronic pain patients from HCs (Kwok et al., 2012). This study extends that finding to a chronic pelvic pain population. More generally, these

findings are in agreement with the results of other studies demonstrating that TLR stimulated PBMCs are able to differentiate patients with Inflammatory Bowel Disease, recent onset rheumatoid arthritis, and persistent fatigue from healthy controls (Chao et al., 1991; Kovarik et al., 2011; Kowalski et al., 2008). Importantly, none of the aforementioned studies determined if the magnitude of the response to TLR stimulation was associated with the magnitude of reported painful symptoms, or examined HPA activity in conjunction with TLR responses. Ours is the first study we know of to report these associations between pain and TLR inflammatory response in a clinical population; importantly, this sample was screened extensively for medical comorbidities and conditions which could simultaneously affect both inflammation and pain (e.g. recurrent bacterial infection) and many potential confounding variables were controlled. For instance, while the use of tri-cyclic anti-depressants was associated with lower TLR inflammatory responses, its inclusion as a covariate had no effect on the relationship between TLR-4 inflammation and pain. In models controlling for all other measures of inflammation, the relationships between TLR-4 inflammatory responses and pain scales were not attenuated; this provides strong evidence that TLR-4 mediated inflammation specifically plays a critical role in IC/BPS pain symptoms. These findings may be related to recent work demonstrating that experimental induced endotoxemia via LPS administration reduces pain thresholds in healthy subjects (de Goeij et al., 2013).

The present findings indicate that HPA dysregulation is a feature of IC/BPS. Compromised GC diurnal variation has been associated with a reduced ability to respond to acute stressors with rapid GC release (Scheff, Calvano, Lowry, & Androulakis, 2012). This may be of particular importance in IC/BPS, where symptom flares are often preceded by acute psychosocial stress, and these findings suggest one potential process by which this may occur (N E Rothrock, Lutgendorf, Kreder, Ratliff, & Zimmerman, 2001). Thus, HPA dysregulation may limit the ability of IC/BPS sufferers to control pain-promoting inflammation; the association of flattened cortisol slope with greater TLR-4 inflammatory cytokine responses suggests that this is true. Early life traumatic events, such as sexual trauma, are associated with

HPA axis dysregulation, and there is evidence that chronic pain patients, including pelvic pain patients, report more early life trauma (Paras et al., 2009).

It is now well established that pro-inflammatory TLR-4 activation of spinal cord glial cells is a key factor in the development and maintenance of chronic pain (Ellis et al., 2014; Mark R. Hutchinson et al., 2008; Milligan & Watkins, 2009). It has been hypothesized that PBMC cytokine responsiveness to TLRs may mirror the cytokine responsiveness to TLRs in the glial cells of the spinal cord, and a recent study in a sciatic constriction animal model of pain demonstrated concordance between TLR-2 and TLR-4 stimulated PBMCs and inflammation assayed in the supernatant of lumbar spinal cultures (Kwok et al., 2013, 2012). Therefore, it is possible that the difference in TLR-2 mediated inflammation between IC/BPS and HCs reflects underlying processes in the spinal cord. That TLR-mediated inflammation in PBMCs would distinguish pain populations as different in presentation as IC/BPS patients and the pain group used in Kwok, et al. (primarily chronic back, shoulder and leg pain, and osteoarthritis) is noteworthy (Kwok et al., 2012). Inflammation in PBMCs may therefore serve as a useful biomarker of persistent pain in clinical settings and may help phenotype pain patients. In animal models, TLR-4 stimulation specifically in spinal glial cells has been shown to lead to amplified ascending pain signaling via, in part, the release of pro-inflammatory cytokines This TLR-4-stimulated glial activation is thought to underlie the initiation of chronic pain and its extension beyond the original site (Milligan & Watkins, 2009). However, recent experiments in preclinical models of pain call into question the role of TLR-4 in female pain. These found that TLR-4 stimulation in the spinal cord only produced a heightened pain response in male mice, an effect possibly mediated by testosterone (Sorge et al., 2011), and that TLR-4 knockout did not reliably attenuate the pain response in female mice (Stokes, Cheung, Eddinger, Corr, & Yaksh, 2013). As administration of TLR-4 agonists to the brain and hindpaw still produced heightened pain in both female and male mice, this sex difference may be limited to direct stimulation of the spinal cord (Sorge et al., 2011). Clearly, further mechanistic investigation with an animal model of IC/BPS would be required to determine relevant sex differences in TLR activity for IC/BPS.

That TLR-4 mediated inflammation was a robust predictor of painful symptoms in female IC/BPS patients is interesting, as interactions between TLR-4 mediated inflammation and sex hormones have been posited as a one potential mechanism in the greater prevalence of pain conditions in women (Nicotra, Loram, Watkins, & Hutchinson, 2012). Glial cells are known to express estrogen receptors (Nicotra et al., 2012), and chronic estrogen stimulation in vivo enhances pro-inflammatory gene expression in microglia following LPS stimulation (Calippe et al., 2010). Further, IC/BPS symptoms are known to differ according to phases of the menstrual cycle (Powell-Boone et al., 2005). Therefore, exploring the interaction between TLR mediated inflammation and sex hormones/phases of the menstrual cycles may reveal insight into the painful symptoms experienced by female IC/BPS patients.

Importantly, TLR-4 responsiveness was associated most strongly with pain frequency and intensity, rather than specific urologic symptoms (i.e. pain on filling of the bladder; urinary frequency) or anatomical regions (i.e. the urethra). This suggests that peripheral tissue inflammation or damage is not solely responsible for the initiation of painful signaling, and is consistent with the hypothesis that some pain in IC/BPS may be mediated by central pain amplification. The marginal difference in IL-1β response to TLR-4 stimulation between IC patients with pelvic pain only and comorbid FSSs is intriguing and requires further investigation. That TLR-4 stimulated inflammatory cytokine responses are associated with longer symptom duration and flattened cortisol slopes raises the possibility that that TLR-4-mediated pain may be progressive. Other studies have demonstrated that longer symptom duration is associated with a greater number of problematic symptom domains in IC/BPS (Nickel, Shoskes, & Irvine-Bird, 2009). These findings suggest possible parallel physiologic processes. Glucocorticoid signaling is known to mediate sensitization of microglial pro-inflammatory responses, a pathway that may additionally provoke stress-induced symptom flares in IC/BPS (Loram et al., 2011). Analyses demonstrating that the most severe pain was experienced by those with flat cortisol slopes and high TLR-4 inflammatory responses provides some evidence that inflammatory responses and poor endogenous inflammatory control may contribute to pain exacerbation synergistically, though longitudinal analyses

are required to clarify this relationship. Compromised inflammatory control may permit sensitization of TLRs in immune cells and the spinal cord, and repeated inflammatory insults from reactive immune cells may disrupt HPA activity; more likely, the relationship is bi-directional.

These novel findings offer compelling evidence that the inflammatory response to TLR-4 stimulation is associated with pain in IC/BPS patients, and suggest that PBMC responsiveness to TLR-2 stimulation holds promise as a biomarker for IC/BPS pain. Importantly, these findings also suggest that TLR-4 may be a therapeutic target in IC/BPS. Animal research has demonstrated that TLR4 antagonism can reverse neuropathic pain in a sciatic nerve constriction model (Mark R. Hutchinson et al., 2008). Further, recent work has demonstrated that TLR-4 antagonism may increase the effectiveness of opioids while reducing tolerance and dependence (Mark R. Hutchinson et al., 2007).

Limitations

These analyses were cross-sectional and thus do not provide information about causality or about the longitudinal course of IC/BPS symptoms, nor can temporal precedence for any measure of inflammatory dysregulation be established. We used self-report measures of pain; future work should incorporate quantitative sensory pain tests. We used a single concentration of LPS; thus it is possible that examination of dose-response kinetics with higher concentrations may have revealed that TLR4 cytokine response can differentiate between IC/BPS and HCs as reported by Kwok et al. in other chronic pain populations (Kwok et al., 2012).

Future Directions

Understanding the longitudinal course of TLR-4-mediated pain is a critical endeavor. In particular, it would be important to determine if TLR-4-mediated pain is an early or late feature of IC/BPS pain, and if it is only an important mechanism in a subset of patients. Animal models need to be developed to allow for greater mechanistic investigation of TLR-mediated pain in IC/BPS, and to confirm if PBMC cytokine responsiveness is reflective of glial cytokine responsiveness in the spinal cord. Additionally, the role of TLR-2 inflammation, which differentiates IC/BPS patients from HCs, but does not predict painful

symptoms, needs to be elucidated. Characterizing TLR-2 and TLR-4 receptor expression in IC/BPS patients may provide important insights, as might investigation of cytokine and TLR polymorphisms. Finally TLR-4 antagonists may provide an important avenue of future treatment for those suffering from IC/BPS.

CHAPTER 2. TOLL-LIKE RECEPTOR 4 AND COMORBID PAIN IN INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a highly prevalent debilitating chronic condition characterized by pelvic/bladder pain and urinary symptoms such as frequency, urgency, and nocturia (Hanno et al., 2011b). Additionally, IC/BPS patients have a high prevalence of psychiatric comorbidities including depression and anxiety disorders (Clemens, Brown, & Calhoun, 2008). While some patients present with Hunner's ulcers, inflammatory lesions found on the wall of the bladder, approximately 90% do not (Simon, Landis, Erickson, & Nyberg, 1997). IC/BPS is therefore a diagnosis of exclusion, and is sometimes considered a cluster of medically unexplained symptoms.

It has been proposed that there may be distinct subtypes of IC/BPS, as some patients appear to experience pain and discomfort in the pelvic/bladder region only (i.e. local pain), while others report extra-pelvic pain consistent with somatic syndromes like fibromyalgia, Irritable Bowel Syndrome (IBS), Chronic Fatigue Syndrome (CFS), or Temporomandibular joint disorder (TMD), suggesting a condition mediated by the central nervous system. A recent investigation found that that comorbid IBS and CFS were present in 39% and 19% of IC/BPS patients, respectively (Nickel et al., 2010). These findings are consistent with the results of many studies finding high degrees of comorbidity between somatic syndromes (Wessely, Nimnuan, & Sharpe, 1999). This suggests that there may be common physiological factors that support global changes in central pain pathways and increased pain perception (Phillips & Clauw, 2011). Furthermore, IC/BPS patients with comorbid somatic syndromes (e.g. CFS) appear to be at risk of developing additional somatic syndromes in the future, suggesting a progressive element of altered pain perception in some patients (Warren, Langenberg, & Clauw, 2013).

Identifying markers of pain sensitization in IC/BPS may improve early phenotyping of vulnerable patients and lead to novel therapeutic targets with the potential to prevent disease progression. Furthermore, identifying markers of central sensitization in chronic pain patients may further prevent psychiatric

comorbidity as chronic pain has recently been shown to induce dysfunction in the locus coeruleus and subsequent depression and anxiety like behaviors in an animal model (Alba-Delgado et al., 2013). Much research has been devoted to identifying altered mechanisms of pain perception (i.e. sensitized pathways), and whether reliable markers of these alterations can be identified. Candidate markers include changes in pain processing networks identified through functional magnetic resonance imaging (fMRI), and hyperalgesia/allodyina identified by quantitative sensory testing (QST), both of which have identified abnormal responses to stimuli in chronic pain patients, including patients with IC/BPS (Kilpatrick et al., 2014; Ness et al., 2014b). Another promising biomarker is the inflammatory response to Toll-Like Receptor (TLR) stimulation in peripheral immune cells, as we have recently found these responses to be associated with heightened pelvic pain in IC/BPS (Schrepf et al., 2014).

TLRs are highly conserved receptors on sentinel immune cells that respond to both Microbe Associated Molecular Patterns (MAMPs) and Damage Associated Molecular Patterns (DAMPs; Hutchinson et al., 2009). We have recently reported that TLR-2 inflammatory responses distinguish IC/BPS patients from healthy controls, and that the magnitude of TLR-4 inflammatory responses in stimulated PBMCs are associated with the extent of painful urinary and pelvic symptoms reported by IC/BPS patients. PBMCs have been hypothesized to mark pain sensitization in humans since it was demonstrated that proliferation of PBMCs incubated with morphine is strongly associated with tolerance for noxious cold stimuli (Hutchinson, La Vincente, & Somogyi, 2004). Additionally, we found that IC/BPS patients had higher serum levels of Interleukin (IL)-6, a marker of systemic inflammation, and altered diurnal cortisol patterns (Schrepf et al., 2014). These finding echo a recent investigation that found that TLR-2 and TLR-4 inflammatory responses in PBMCs differentiate chronic pain patients from healthy controls (Kwok et al., 2012) and other work identifying altered TLR inflammatory responses as features of other conditions characterized by persistent pain such as Inflammatory Bowel Disease and Rheumatoid arthritis (Kovarik et al., 2011; Kowalski et al., 2008). However, it is unknown if inflammatory responses in PBMCs can

differentiate subtypes of painful syndromes such as IC/BPS, particularly those characterized by pain not typically considered part of the IC/BPS syndrome (i.e. widespread, extra-pelvic pain.) .

The purpose of the current study was to determine if inflammatory processes, especially TLR-2 and TLR-4 inflammatory responses in PBMCs, are differentially associated with pelvic vs. extra-pelvic pain in IC/BPS. Additionally, we examined relationships between TLR-mediated inflammation, pain intensity/ interference with daily life, and pressure pain sensitivity determined by QST. We also examined the relationship between TLR-mediated inflammation and the presence of comorbid pain conditions in IC/BPS patients, as these conditions are characterized by pain outside the pelvic region, and contribute substantially to the difficulty of treating the IC/BPS syndrome (Nickel et al., 2010). In line with the results of our earlier work and results from animal models of chronic pain, we hypothesized that greater TLR inflammatory responses in PBMCs would be associated with pain outside the pelvic region, increased pain sensitivity by QST, and comorbid somatic syndromes.

Methods

MAPP Study and Recruitment

The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) is a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) sponsored research initiative comprising several sites with the objective of characterizing the epidemiology, symptom trajectories, phenotypes and biological correlates of chronic pelvic pain (Clemens et al., 2014). The University of Iowa is a participating institution emphasizing biomarker research. Participants were eligible if they were at least 18 years of age, female, not pregnant, and reported chronic pain/pressure/discomfort associated with the bladder or pelvic region in the preceding three months. Participants had negative urine cultures for uropathogens. Exclusion criteria included conditions which might result in tissue damage to areas relevant to IC/BPS symptomology (e.g. history of urethral stricture, neurological disorder affecting the bladder or bowel). Additional information about the MAPP project, including patient characterization,

study aims, and full exclusion criteria is available (Clemens et al., 2014; Landis et al., 2014; Schrepf et al., 2014).

Demographic and Symptom Information

The sample was composed of an expanded group of participants from a previously reported study (Schrepf et al., 2014) who provided additional information on pain and comorbid syndromes. In addition, a subsample completed QST. Sixty-six women provided demographic information at the time of eligibility screening, including information about income, education, employment, race and ethnicity. Upon study entry, participants had a blood draw, urine collection, physical examination and completed a battery of questionnaires relating to pain and urological symptoms. These included the Brief Pain Inventory (BPI), a measure of pain intensity, interference with daily life, and a body map for selection of painful areas (Cleeland & Ryan, 1994) which has previously been validated in chronic pain populations (Tan, Jensen, Thornby, & Shanti, 2004). The body map was modified so that patients could select regions where pain was experienced from a standardized form of 45 distinct areas. Participants were also administered self-report screens to assess the presence of comorbid somatic syndromes. These included the Rome III criteria for IBS (Drossman & Dumitrascu, 2006), the American College of Rheumatology diagnostic criteria for Fibromyalgia (Wolfe et al., 2010), International Chronic Fatigue Syndrome Study Group criteria for CFS (Fukuda et al., 1994), an 8 question MAPP specific diagnostic tool for symptoms of vulvodynia (e.g. "experience constant burning or raw feeling at the opening of the vagina,") and the Research Diagnostic Criteria for TMD (Dworkin et al., 2002). These diagnostic criteria show adequate reliability and validity (Dworkin et al., 2002; Ford et al., 2013; Komaroff et al., 1996; Wolfe et al., 2010) excepting the criteria for vulvodynia, which is necessarily exploratory. Additionally, participants completed the reliable and validated Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988). Use of non-steroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, opioids, selective serotonin/norepinephrine reuptake inhibitors (SSRI/SNRI), and pentosan polysulfate, and duration of symptoms in years, were collected by patient self-report.

Cortisol

Salivary cortisol was collected in salivettes by participants at 3 time points (upon waking: 4-9am, afternoon: 5 pm, and bedtime: 8pm-12am) for three consecutive days prior to the baseline visit. Samples collected outside this time frame were excluded to maintain homogeneity. Individual waking and bed times have been demonstrated to better approximate diurnal cortisol rhythms than scheduled collection times (Kraemer et al., 2006). Participants were instructed not to eat, exercise or consume caffeine for thirty minutes prior to collecting a sample. Participants wrote the time of each collection on the salivette tubes. Self-report of collection time has been demonstrated to be reliable and salivary cortisol is stable at room temperature (Kraemer et al., 2006). Salivettes were analyzed by chemiluminescence immunoassay (IBL, Hamburg, Germany) at the Technical University of Dresden. The lower detection limit is 0.41 nmol/L and inter-assay and intra-assay coefficients of variance are less than 10%.

Inflammatory Measures

Blood samples were collected between approximately 11:30am and 12:30pm. PBMCs were separated by Ficoll-paque gradient centrifugation within 30 minutes of blood collection and cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 ug/ml streptomycin for 3 days with TLR agonists at 37°C in a humidified incubator with 5% CO2. TLR-2 and TLR-4 agonists were selected on the basis of the role of these receptors in chronic pain in animal models and our previous findings linking them to symptoms in IC/BPS (Bastos et al., 2013; Mark R. Hutchinson et al., 2008; Schrepf et al., 2014). For stimulation of TLR-4, 50 ng/ml of Lipopolysaccharide (LPS) was used; for TLR-2 stimulation, 0.04 ng/ml of Staphylococcus aureus Cowan I (SAC) was used. Conditioned media was then harvested and frozen at -80°C prior to batch ELISA analysis. Each well contained 1X106 cells in 24 well plates, with one well per subject for TLR-4 and TLR-2 stimulation. Cytokines IL-6 and IL-1β were assayed in duplicate by DuoSet ELISAs (R&D Systems) according to instructions included with the kit. Plasma IL-6 was assayed with a high sensitivity Quantikine ELISA (R & D Systems).

Quantitative Sensory Testing

A subsample of participants completed the MAPP network QST protocol. Pressure pain sensitivity was evaluated at the thumbnail (As-Sanie et al., 2013; Giesecke et al., 2004; Petzke et al., 2001) using the Multimodal Automated Sensory Testing (MAST) system (Harte et al., 2013). The MAST system consists of a control computer that executes testing algorithms and stores testing data, and a touch-screen interface for participant feedback. Computer-controlled pressure stimuli are applied to the thumbnail bed via a 1 cm2 rubber probe housed within a wireless, pistol-grip style handset. Probe movement is driven by a miniature servo-motor. A closed-looped control system measures applied pressures and dynamically self-adjusts motor output to the resistance of the thumb and any movement to ensure accurate and repeatable force delivery.

Participants received scripted instructions, and MAST system familiarization and practice testing prior to data collection. During testing, an ascending series of 5-s duration pressures were delivered at a rate of 4 kg/cm2/s to the dominant thumbnail beginning at 0.50 kg/cm2 and increasing in 0.50 kg/cm2 steps, with a minimum inter-stimulus interval of 20 s. Pain intensity was rated after each stimulus on a 0-100 numerical rating scale (NRS) displayed on the interface screen (0 = no pain; 100 = worst pain imaginable). Testing terminated when the first of three possible stop conditions were met: 1) participant reached her personal pain tolerance (i.e., requested to stop the test), 2) patient reported a pain intensity rating of > 80/100, or 3) the maximum pressure of 10 kg/cm2 was delivered. A modified three-parameter logistic model was used to fit the stimulus-response data obtained from this procedure. The midpoint between the minimum and maximum stimulus intensity was estimated within-person using the SAS NLIN procedure to derive an overall measure of supra-threshold pressure pain sensitivity, referred to as Pain50. Additional outcome variables included pressure pain threshold, defined as the first pressure in a string of at least two consecutive pressures that elicited a NRS pain rating > 0, and pressure pain tolerance, defined as the last pressure recorded in the stimulus response profile.

Data Analysis

Statistical analyses were performed using SPSS v. 21 and R v. 3.1.1. Cytokine values were log-10 transformed, and cortisol values natural log transformed, to normalize their distribution. The Inflammatory response scores for stimulated cytokines were calculated by summing the z-scores ([individual score- group mean]/ group standard deviation) for the IL-6 and interleukin-1 beta (IL-1β) response in PBMCs following stimulation with LPS (TLR-4) or SAC (TLR-2. This inflammatory response score was then standardized for ease of interpretation. Both IL-6 and IL-1β have been implicated in enhanced pain processing when released by spinal glia, and both are released following TLR-2 and 4 stimulation, in part, by transcription of nuclear factor-kappaB (Milligan & Watkins, 2009). The composite score, therefore, is likely more reflective of the inflammatory response to TLR stimulation than either cytokine alone. Distributions of transformed variables were examined for confirmation of normality. Salivary cortisol values at each of the collection points were regressed on the time of collection over the three-day period to calculate cortisol slope, a measure of the average hourly decrease in cortisol over the course of the day as described previously (Kraemer et al., 2006).

To determine if inflammatory variables were associated with a greater likelihood of endorsing extra-pelvic sites as painful, mixed-effects logistic regression models were used. Higher probabilities of endorsing pain outside the pelvic region reflect pain not typically considered part of the IC/BPS syndrome. Thus, endorsement of pain at any of the 44 sites outside the pelvic region was considered indicative of extra-pelvic pain. In these models the dependent variable of interest was the probability of a patient selecting any extra-pelvic site (44 sites) as painful, with inflammatory variables as predictors. Subject and site specific intercepts were tested as random effects, with the maximum random effects structure retained by likelihood testing. Modeling subject specific variance (e.g. if some patients are more likely to endorse any site as painful) and site-specific variance (e.g. if lower back pain is more likely to be endorsed than pain in the hands across subjects) can allow more accurate estimation of fixed effects (Baayen, Davidson, & Bates, 2008). Random intercept terms were retained for subject and pain site.

BMI, age, use of medications, presence of a comorbid condition, and inflammatory variables were used in univariate analyses to determine which, if any variables, were associated with a greater likelihood of endorsing extra-pelvic pain. Significant variables ($p < .05$) were retained in multivariate analyses. Relationships between pain severity/interference (from the BPI) and inflammation were tested in multivariate General Linear Models with the same set of covariates. To explore the relationships between inflammatory variables (e.g. TLR-4 inflammatory responses and cortisol slope) and duration of symptoms in years Pearson correlations were used.

Group differences between IC/BPS only and IC/BPS comorbid patients (IBS, CFS, fibromyalgia, TMD, vulvodynia) with respect to inflammatory variables were tested with one-way ANOVAs, and between IC/BPS only and IC/BPS comorbid patients with individual conditions (IBS, fibromyalgia, CFS, TMD and vulvodynia). The association between number of comorbid conditions and inflammatory variables was tested by Spearman's Rank correlations. Relationships between inflammatory variables and pain intensity and interference were assessed using General Linear Models controlling for negative affect, comorbid condition status, and use of SSRI/SSNIs, following the results of the univariate analyses. Due to the non-normal distribution of QST Spearman's rank correlation tests were used to test the association with inflammatory variables.

Results

Demographic Characteristics and Covariates

Participants were on average approximately 42 years old (range 20-74), and the vast majority were non-Hispanic and white. See Table 6.

Table 6. Participant Characteristics, Chapter 2.

Participant Characteristics	N=66
Age Mean(SD)	42.03 (15.12)
BMI Mean(SD)	27.18 (5.65)
PANAS negative affect	22.27 (8.56)
Race % (*n*)	
White	97 (64)
Asian	1 (1)
Multi Race	1 (1)
Ethnicity % (*n*)	
Non-Hispanic	98 (65)
Hispanic	1 (1)
Education % (*n*)	
High School or GED	13 (9)
Some College	27 (18)
Graduated College	32 (21)
Graduate Degree	27 (18)
Employment % (*n*)	
Employed	62 (41)
Unemployed	11 (7)
Disabled	8 (5)
Retired	9 (6)
Full Time Homemaker	9 (6)
Not Answered	1 (1)

Table 6. Continued

Annual Income/$ % (*n*)	
<10,000	14 (9)
<25,000	6 (4)
<50,000	23 (15)
<100,000	33 (22)
>100,000	20 (13)
Prefer not to answer	5 (3)
Comorbid Conditions	
% (*n*)	
None	39 (26)
Irritable Bowel Syndrome	42 (28)
Fibromyalgia	3 (2)
Chronic Fatigue Syndrome	12 (8)
TMD	35 (23)
Vulvodynia	21 (14)
Number of Comorbid Conditions	
% (*n*)	
	39 (26)
0	26 (17)
1	23 (15)
2	6 (4)
3	6 (4)
4	
Tricyclic anti-depressants	
No	56 (37)
Yes	44 (29)

Table 6. Continued

Opioids	
No	83 (55)
Yes	17 (11)
Pentosan Polysulfate	
No	56 (37)
Yes	44 (29)
NSAIDs	
No	89 (59)
Yes	11 (7)
SSRI/SNRIs	
No	86 (57)
Yes	14 (9)

Higher TLR-4 inflammatory responses were associated with a greater likelihood of endorsing extra-pelvic pain (p= .006). SSRI/SNRI use was associated with a greater likelihood of endorsing extra-pelvic pain (p < .001). Tricyclic antidepressant use was marginally associated with a lower likelihood of endorsing extra-pelvic pain (p = .081) whereas older age was marginally associated with a greater likelihood of endorsing extra-pelvic pain (p =.071). Greater negative affect and presence of a comorbid condition were both associated with a greater likelihood of endorsing extra-pelvic pain (both p <.001). BMI, opioid use, NSAID use, Pentosan Polysulfate use, , cortisol slope, and duration of symptoms in years were not associated with the likelihood of endorsing extra-pelvic pain (all p > .15; univariate analyses for covariate selection not shown). Additionally, the inflammatory response to TLR-2 stimulation was not associated with a greater likelihood of endorsing pain outside the pelvic region (Odds Ratio = 1.03, 95% CI = .72, 1.49, p = .86). Therefore, multivariate analyses included comorbid status, SSRI/SNRI use, and negative affect in addition to the TLR-4 inflammatory response.

TLR-4 Inflammatory Response and Pain

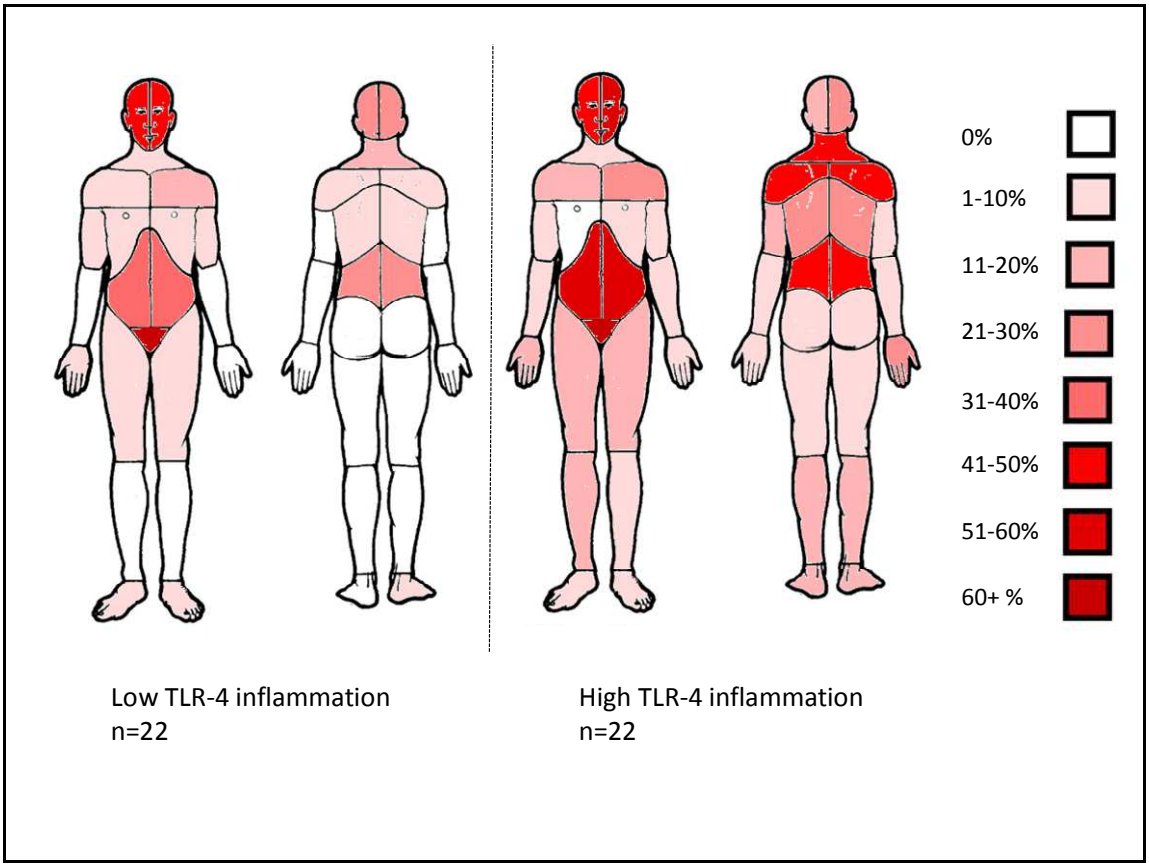
In multivariate analyses, a one standard deviation increase in the TLR-4 inflammatory response was associated with 1.59 greater odds (95% CI = 1.08, 2.33) of endorsing pain outside the pelvic area on the body map (p = .019), controlling for negative affect, comorbid status, and SSRI/SNRI use. This one standard deviation increase in TLR-4 inflammatory response corresponds to a 63% increase in the likelihood of a participant endorsing pain outside the pelvic region at any site on the body map. See Table 7.

Table 7. Relationship of TLR-4 inflammatory response with extra-pelvic pain.

Outcome Measure	Predictor	Est.	S.E.	Z value	Odds Ratio 95% CI	*p*
Extra-Pelvic Pain						
	TLR-4 inflammation response (one SD above mean)	.46	.20	2.34	1.59 1.08, 2.33	.019
	Use of SSRI/SNRI	.41	.57	.73	1.51 .50, 4.59	.47
	Comorbid Condition	.39	.43	.92	1.48 .64, 4.59	.36
	PANAS negative affect (one SD above mean)	.66	.21	3.12	1.94, 1.28, 2.95	.002

Figure 4 illustrates the higher likelihood of endorsing extra-pelvic pain among those with higher TLR-4 inflammatory response.

Figure 4. Prevalence of pain reported in the first tertile (low) of the TLR-4 composite inflammatory score versus the third tertile (high) for each site on the body map.



The TLR-4 inflammatory score was associated with greater self-report of pain severity ($p = .013$) and pain interference ($p = .046$) on the BPI, controlling for negative affect, comorbid status, and SSRI/SNRI use. See Table 8.

Table 8. General Linear Models testing relationship of TLR-4 inflammatory response with BPI symptom scores.

Outcome Measure	Predictor	B.	S.E.	$F_{1,65}$	95% CI	*p*
Pain Severity (BPI)						
	TLR-4 inflammatory response	.503	.197	6.53	.109,.896	.013
	Comorbid condition	.204	.429	.23	-1.063, .655	.64
	Use of SSRI/SNRI	-.156	.615	.06	-1.075, 1.386	.97
	PANAS negative affect	.096	.025	14.83	.046,.145	<.01
Pain Interference (BPI)						
	TLR-4 inflammatory response	.567	.278	4.17	.012, 1.123	.046
	comorbid condition	.200	.607	.50	-1.414, 1.014	.74
	Use of SSRI/SNRI	-.028	.832	.01	-1.635, 1.691	.97
	PANAS negative affect	.171	.035	23.67	.101,.241	<.01

Additionally, the TLR-4 inflammatory response was associated with higher TLR-2 inflammatory responses (r = .304, p = .013) and longer duration of symptoms in years (r = .295, p=.017) but not with cortisol slope (r = .165, p = .23 (n=56) or plasma IL-6 (r = .064, p = .61).

Quantitative Sensory Testing

In the subsample of 32 patients (13 IC/BPS only, 19 IC/BPS comorbid) who underwent QST, higherTLR-4 inflammation scores were marginally associated with lower pressure pain thresholds (Spearman's Rho= -.334, p = .062) and increased levels of IL-6 in plasma were significantly associated with lower pressure pain thresholds (Spearman's Rho = -.381, p = .031). In contrast, the TLR-2 inflammation score was not associated with pressure pain thresholds (p =.30), nor was cortisol slope (p = .84). Pain50 and pressure pain tolerance were not associated with inflammatory variables (all p > .19).

Inflammatory Measures and Comorbid Conditions

The TLR-4 inflammatory response was significantly greater in IC/BPS comorbid patients (p = .016) compared to the IC/BPS patients without comorbid conditions. Additionally, a higher TLR-4 inflammatory response distinguished IC/BPS + IBS patients (n=28, p = .018), IC/BPS + TMD (n=23, p = .007), and IC + vulvodynia (n=14, p = .049), from IC/BPS only participants (n=26). The TLR-4 inflammatory response was higher in IC/BPS + CFS participants (n=8) and IC/BPS + fibromyalgia participants (n=2) but not significantly so (both p > .18), possibly due to small sample sizes. IL-1β measured in supernatant of LPS (TLR-4) stimulated PBMCs was significantly higher in IC/BPS patients with comorbid conditions (p = .008) whereas IL-6 was not significantly elevated (p = .21). IL-1β and IL-6 measured in the supernatant of SAC (TLR-2)-stimulated PBMCs did not differ significantly between groups (both p > .20) nor did the TLR-2 composite inflammatory score (p = .50). IL-6 measured in plasma was marginally higher in IC/BPS comorbid participants (p = .097, while cortisol slopes did not differ between groups (p = .96). See Table 9.

Table 9. Means and standard deviations of biomarkers in participants with IC/BPS only and those with additional comorbid conditions.

Variable Mean (S.D.) 95% CI Inflammatory Variables	IC Only *n*=26	IC Comorbid *n*=40	*p*
TLR-4 inflammatory response	-.36 (1.13) -.82, .09	.24 (.85) -.03, .51	.016
IL-1β + LPS	3.11 (.93) 2.73,3.48	3.58 (.44) 3.44, 3.72	.008
IL-6 + LPS	4.15 (.40) 3.99, 4.31	4.28 (.42) 4.14, 4.41	.205
TLR-2 inflammatory response	-.10 (1.06) -.53, .32	.07 (.97) -.24, .38	.501
IL-1β + SAC	1.17 (1.19) .69, 1.65	1.44 (1.14) 1.08, 1.81	.365
IL-6 + SAC	1.68 (1.37) 1.13, 2.24	1.79 (1.22) 1.40, 2.19	.719
Cortisol Slope (ln transformed)	*n*=22 -.11 (.06) -.14, -.08	*n*=34 -.11 (.07) -.13, -.08	.955
IL-6 plasma (log_{10} transformed)	.33 (.35) -.75, 0.84	.46 (.26) .00, .97	.097
Duration of Symptoms (years)	6.91 (7.48) 3.89, 9.93	8.3 (8.00) 5.71, 10.90	.48

Greater numbers of comorbid syndromes were associated with a greater TLR-4 inflammatory response (Spearman's Rho = .311, p = .011). No other inflammatory variable was associated with number of comorbid conditions or presence of individual comorbid conditions (all p >.10).

Discussion

The key finding of this study is that TLR-4-mediated inflammatory responses in PBMCs are associated with extra-pelvic pain in a chronic pelvic pain population. This is demonstrated in the association between LPS evoked inflammation and an increasing likelihood of endorsing pain outside the pelvic region, and by the ability of this inflammatory response to distinguish between patients meeting criteria for IC/BPS only and patients meeting criteria for IC/BPS who also had comorbid syndromes. Use of medications was not associated with measures of pain, while greater negative affect was strongly associated with pain measures independent of TLR-4 inflammation. While exploratory, QST data suggest that TLR-4 inflammation may also be associated with lower pressure pain thresholds measured at a non-symptomatic site remote from the pelvic region (i.e., the thumb), further suggesting a central mechanism of pain hypersensitivity in this population. These findings build on our previous finding that TLR-4 inflammation is associated with pelvic pain in IC/BPS (Schrepf et al., 2014) by demonstrating that TLR-4 inflammation is associated with comorbid pain not typically considered part of the IC/BPS syndrome.

This is the first study to our knowledge which has shown TLR-mediated inflammation to be associated with comorbid pain in a chronic pain population. Further, these differences in TLR-4 mediated inflammation were not associated with a particular comorbid condition, as each condition that was well-represented in our sample (IBS, TMD, and vulvodynia) was characterized by higher TLR-4 mediated inflammation compared to patients with IC/BPS only. This suggests that TLR-4 inflammation may reflect a broad mechanism by which pain signaling is enhanced in IC/BPS. This extends our earlier finding that TLR-4 inflammation predicts non-specific pain severity and frequency of pelvic pain symptoms in IC/BPS but was not associated with pain in particular anatomical regions or during particular activities. A recent investigation in an animal model of visceral pain found that TLR-4

regulates stress-induced pain (Tramullas et al., 2014). This is notable given the high prevalence of IBS in IC/BPS patients, and because stressful events frequently precipitate symptom flares (N E Rothrock et al., 2001). QST data indicating lower pressure pain thresholds (increased pain sensitivity) in patients with higher TLR-4 inflammatory responses and higher IL-6 in blood suggest that these inflammatory processes are related to global pain sensitivity, not pain associated with IC/BPS only, and are consistent with other findings that higher pro-inflammatory cytokines in blood are associated with altered pain sensitivity on QST in osteoarthritis patients (Lee et al., 2011). While the presence of a comorbid syndrome was associated with an increased likelihood of endorsing extra-pelvic pain in a univariate model, this was no longer true in the multivariate model including TLR-4 inflammation and negative affect. This provides further evidence that TLR-4 inflammation may play a critical role in the painful symptoms associated with comorbid conditions in IC/BPS, though it is not possible to determine from these results what role, if any, TLR-4 inflammation may play in centrally mediated pain sensitization.

The fact that TLR-2 stimulation distinguished patients from comparison participants in our previous study, but did not distinguish between patients with and without comorbid syndromes, is of interest. One possibility is that hypersensitivity to TLR-2 is an early or universal feature of IC/BPS while TLR-4 sensitivity is not. Previous research has demonstrated that higher TLR-2 density on PBMCs, but not TLR-4 density, distinguishes IBS patients from controls (Ohman et al., 2012). Another recent study found that TLR-2 and TLR-4 mRNA were up-regulated in the colonic mucosal tissue of IBS patients with heterogeneous symptom presentation compared to those with symptoms of diarrhea or constipation only (Belmonte et al., 2012). It is unknown if TLR-4 density on PBMCs or other tissue may differ between IC/BPS patients with and without comorbid conditions, or if TLR-4 responses are heightened in patients with comorbid syndromes due to a "priming" effect of prior MAMP or DAMP exposure. Another possibility is that intracellular signaling and subsequent cytokine production may differ significantly following TLR-4 vs. TLR-2 stimulation; a recent investigation of stimulated human whole blood found that both TLR-2 and TLR-4 stimulation resulted in NF-κB family activity, but that a distinct IFN

upregulation occurred following TLR-4 stimulation only (Blankley et al., 2014). Another recent investigation used principal component analysis to analyze various cytokine and chemokine responses to both TLR-2 and TLR-4 receptor stimulation in human whole blood; the results indicated that different TLR agonists (including TLR-2 and TLR-4), evoked distinct protein signatures, suggesting divergent intracellular signaling pathways (Duffy et al., 2014). Thus it appears that though TLR2 and 4 ligands may signal through the same receptor, each cytokine is capable of eliciting unique signaling patterns. Another possibility is that unexplored vulnerabilities (e.g. genetic factors) could mask the relationship between TLR-2 mediated inflammation and pain, if such a relationship exists. Clearly, more research is needed to identify relevant cellular and intracellular differences in TLR-4 vs. 2 responses in the context of IC/BPS.

Microglia and astrocytes express TLRs including TLR-2 and TLR-4, and stimulation of TLR-4 on microglia induces release of pro-inflammatory cytokines IL-6, IL-1β- and TNF-α in the spinal cord (Milligan & Watkins, 2009). TLR-4-mediated inflammation released by glia cells in the dorsal horn of the spinal cord is thought to be one contributing mechanism for central pain amplification in rodent models of chronic pain (Ellis et al., 2014; Grace, Hutchinson, Maier, & Watkins, 2014; Hutchinson et al., 2008), though this effect may be sex specific, as recent work suggests that LPS promotes hyperalgesia when delivered to the brain or periphery, but not the spinal cord, in female mice (Sorge et al., 2011). Regardless, TLR-4 is essential in LPS induced hyperalgesia as LPS injection fails to promote hyperalgesia in TLR-4 deficient mice (Mattioli et al., 2014). Importantly for this study, the amplification of pain signaling sometimes involves extension of pain from the original site of injury, termed "extra-territorial" pain (Wieseler-Frank, Maier, & Watkins, 2005). Whether circulating PBMCs reflect neuro-inflammatory processes remains an open question; recent work suggests that TLR-mediated inflammation in PBMCs corresponds to the same TLR-mediated inflammation in the spinal cord, in a rodent model of chronic pain (Kwok et al., 2013). However, relevant animal models of IC/BPS will need to be developed

before concordance between inflammatory responses in PBMCs and spinal microglia can be formally tested.

While rodent models that investigate the role of TLRs in pain have typically used neuropathic injury models (e.g. sciatic constriction), studies of human pain populations with little evidence of peripheral tissue damage have also identified differential responses to TLR stimulation in PBMCs (Kowalski et al., 2008; Schrepf et al., 2014). If sensitization of TLR-induced inflammation is a mechanism for pain amplification in IC/BPS, this raises the question of what the initiating events may be in this population, given the generally low proportion of patients with evidence of peripheral tissue damage. One large twin study implicated both genetic factors (approximately one third) and non-shared environmental factors (approximately two thirds) in the risk of IC/BPS (Altman et al., 2011). At least two large studies have identified an increased number of antecedent urogenital infections as a risk factor for IC/BPS in women raising the possibility that recurrent or severe infections might serve as initiating events in IC/BPS (Díaz Mohedo, Wärnberg, Barón López, Mera Velasco, & Cabello Burgos, 2014; Li et al., 2010). TLRs including TLR-4 are pattern recognition receptors that respond to PAMPs and DAMPs; they play a critical role in expelling bacteria from the urinary tract and are expressed on both bladder epithelial cells and phagocytic cells that migrate into the bladder during infections (Song & Abraham, 2008). Purified LPS infused directly into the bladder induces pain in the pelvic region in a rodent model of urinary tract infection (Rudick et al., 2010). One possibility, therefore, is that sustained local inflammatory events precipitate TLR sensitization in migrating immune cells that then begin to modulate central pain processing after evidence of local infection is gone. This concept is supported by experiments demonstrating that a single peripheral inflammatory challenge (e.g. formalin) or peripheral trauma (e.g. laparotomy) can prime spinal microglia activation and subsequent LPS induced allodynia for as long as two weeks (Hains et al., 2011). More direct implications for IC/BPS symptoms have been demonstrated in an animal study that found a demyelination injury to the sciatic nerve increased the sensitivity of

bladder-associated sensory neurons to chemokines and increased the frequency of micturition (Foster et al., 2011).

Limitations

This sample of IC/BPS patients was disproportionately non-Hispanic and white compared to the general population. These findings require replication in a more diverse sample. Isolated PBMCs were stimulated at a single dose-level of LPS and SAC. It is possible that characterizing TLR-2 inflammatory responses at a wide range of doses might reveal differences between IC/BPS only patients and those suffering comorbid conditions (Kwok et al., 2012). These analyses are cross-sectional and cannot determine causal directions between pain measures and TLR-mediated inflammation. A large number of statistical tests were performed in exploring these novel hypotheses; these associations require replication in other samples of IC/BPS patients.

Conclusions and Future Directions

TLR-4 mediated inflammation is a promising biomarker of comorbid pain in IC/BPS patients and may be associated with pain in other somatic syndromes. Putative TLR-4 antagonists that have been shown effective at suppressing pain in animal models may have application in IC/BPS populations. In addition to characterizing TLR-mediated inflammatory responses at a wider range of concentrations, more research is required to delineate the relationship between local inflammatory events and central pain sensitivity. As IC/BPS is often characterized by symptom fluctuation, termed "flares," future studies should consider TLR-mediated inflammation in relation to these events.

CHAPTER 3. INFLAMMATION AND SYMPTOM TRAJECTORIES IN INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a debilitating chronic condition characterized by urinary symptoms such as frequency, urgency and nocturia, as well as genitourinary pain. Though the condition is prevalent, affecting between 3-8 million women in the United States by some estimates (Berry et al., 2011b), the etiology and course of the disease is not well understood. For instance, one large scale longitudinal study of IC/BPS sufferers found that most did not experience improvement in symptoms over a long period of observation (up to four years) despite a variety of different treatment modalities (Propert et al., 2000). Another recent longitudinal investigation found that 62% of IC/BPS sufferers experienced symptom persistence during at least three of four time points when assessed every three months over a year (Suskind, Berry, Suttorp, Elliott, & Clemens, 2014). However, to date, no biomarkers have been identified that are associated with the course of the disease.

The clinical presentation of IC/BPS often does not include signs of local inflammation and tissue damage (e.g. in the bladder; Potts & Payne, 2012), therefore recent investigations have focused on more systemic physiological risk factors for IC/BPS and associated symptoms. We have recently identified Toll-Like Receptor (TLR) inflammatory responses in peripheral blood mononuclear cells (PBMCs), diurnal cortisol dysregulation as robust correlates of the IC/BPS syndrome (Schrepf et al., 2014, 2015). Specifically, we found that TLR-2 inflammatory response and diurnal cortisol dysregulation served as biomarkers of IC/BPS while TLR-4 inflammatory responses were associated with greater IC/BPS pain severity (Schrepf et al., 2014), and the extent of comorbid pain (Schrepf et al., 2015).

The purpose of the current study is to determine if these inflammatory biomarkers measured at study entry are related to longitudinal symptom outcomes in in IC/BPS over a 48 week period. We

hypothesized that elevated TLR inflammatory responses and dysregulated diurnal cortisol patterns would be associated with less improvement in symptoms over time.

Materials and Methods

MAPP study and recruitment

The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP), sponsored by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), is a multi-site research effort intended to characterize various aspects of chronic pelvic pain including the identification of biomarkers associated with the disease and symptom trajectories (Clemens et al., 2014). Eligible participants were at least 18 years of age, female, not pregnant, and reported chronic pain, pressure, or discomfort in the bladder or pelvic region for the preceding three months or during the majority of the last six months. Participants' urine was screened for uropathogens. Exclusion criteria were designed to identify conditions which could result in tissue damage relevant to IC/BPS symptomology (e.g. history of urethral stricture, neurological disorder affecting the bladder or bowel). Further information about the full scope of the MAPP project, participants and inclusion/exclusion criteria is available (Clemens et al., 2014; Landis et al., 2014; Schrepf et al., 2014).

Demographic and symptom information

Participants were 24 women, representing a subsample of a larger cohort study previously reported5,6 who participated in a longitudinal study of symptom trajectories. Demographic and symptom information was collected during a baseline visit, after which symptom data was collected bi-weekly for 48 weeks via an online module (25 time-points, including baseline). At the baseline visit participants had a blood draw, urine collection, and physical examination, and completed a battery of questionnaires. Baseline and longitudinal symptom measures included the Symptom and Health Care Utilization Questionnaire (SYMQ; Landis et al., 2014), and the 9-item Genitourinary Pain Index (GUPI; Clemens et al., 2009) The SYMQ contains a question about symptom flare status (i.e., "Are you currently experiencing a flare of your urologic or pelvic pain symptoms? By this we mean, are

you currently experiencing symptoms that are much worse than usual?"), and the GUPI contains both pain and urinary subscales. The Rome III criteria for irritable bowel syndrome(Drossman & Dumitrascu, 2006), the American College of Rheumatology diagnostic criteria for Fibromyalgia (Wolfe et al., 2010), the International Chronic Fatigue Syndrome (CFS) Study Group criteria for CFS (Fukuda et al., 1994), an 8 question MAPP specific diagnostic tool for symptoms of vulvodynia (e.g. "experience constant burning or raw feeling at the opening of the vagina,") and the Research Diagnostic Criteria for temporomandibular disorder (TMD; Dworkin et al., 2002) were administered to determine if participants suffered comorbid pain conditions. Participants also completed the Positive and Negative Affect Scale (Watson et al., 1988). Medications including non-steroidal anti-inflammatory drugs (NSAIDs), tricyclic antidepressants, opioids, selective serotonin/norepinephrine reuptake inhibitors (SSRI/SNRI), and pentosan polysulfate were collected by patient self-report, as was duration of symptoms in years.

Cortisol

Participants collected saliva in provided salivettes at 9 time points: upon waking(4–9 am), afternoon (5 pm), and bedtime, (8 pm–12 am) for three consecutive days prior to the first visit. Samples collected outside this time frame were excluded to maintain homogeneity. Individual waking and bed times are preferable for approximating diurnal cortisol rhythms than scheduled collection times (Kraemer et al., 2006). For thirty minutes prior to collecting a sample participants were instructed not to eat, exercise, or consume caffeine. Time of each collection was indicated on the salivette tubes by the participants, a procedure which has been demonstrated to be reliable (Kraemer et al., 2006). Salivary cortisol is stable at room temperature.15 Salivettes were analyzed by chemiluminescence immunoassay (IBL, Hamburg, Germany) at the Technical University of Dresden. The lower detection limit is 0.41 nmol/L and inter-assay and intra-assay coefficients of variance are less than 10%.

Toll-Like Receptor Inflammatory Responses

Blood draw occurred between approximately 11:30 am and 12:30 pm. PBMCs were separated by Ficoll-paque gradient centrifugation within 30 min of blood collection and cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin for 3 days with TLR agonists at 37 °C in a humidified incubator with 5% CO2. TLR-2 and TLR-4 agonists were selected based on our previous findings linking them to symptoms in IC/BPS (Schrepf et al., 2014, 2015). For stimulation of TLR-4, 50 ng/ml of the classic TLR-4 agonist Lipopolysaccharide (LPS) was used; for TLR-2 stimulation, 0.04 ng/ml of staphylococcus aureus cowan I (SAC) was used. Conditioned media was harvested and frozen at −80 °C prior to batch ELISA analysis. Each well contained 1×10^6 cells in 24 well plates, with one well per subject for TLR-4 and TLR-2 stimulation. Cytokines IL-6 and IL-1β were assayed in duplicate by DuoSet ELISAs (R&D Systems) according to instructions included with the kit.

Statistical Analyses

Statistical analyses were performed using SPSS v. 21 and R v. 3.1.1. Cytokine and cortisol values were log transformed to normalize their distributions. The inflammation response to TLR-2 and TLR-4 stimulation in PBMCs was calculated as the sum of each cytokine z-score for each individual: ([individual score − group mean]/group standard deviation) for the IL-6 and interleukin-1 beta (IL-1β). This inflammatory response was then standardized for ease of interpretation. Using both cytokine responses is thought to better reflect the inflammatory response to TLR stimulation as both have been implicated in enhanced central pain sensitization and both are controlled, in part, by NFκB transcription (Milligan & Watkins, 2009). Salivary cortisol values at each of the collection points were regressed on the time of collection over the three-day period to calculate cortisol slope, a measure of the average hourly decrease in cortisol over the course of the day as described previously (Kraemer et al., 2006).

Primary outcomes included symptom trajectories of GUPI urinary and pain subscale scores, probability of symptom flares during the full 48 week period of observation, during the initial 12 weeks following the baseline visit, and time to the first reported symptom flare. Missing data points were estimated using the Restricted Estimation of Maximum Likelihood (REML) method.

To determine if symptom trajectories differed by inflammatory markers, mixed effects longitudinal models were used. The effect of time was evaluated with both linear and quadratic terms and the results compared by likelihood testing. The linear effect of time was retained following those results. See supplemental Figure 1 for linear symptom trajectories of each participant. Optimal random effects structures were determined by likelihood testing of nested models with and without each random term, beginning with maximum complexity. Modeling subject specific variance in intercepts and slopes can allow more accurate estimation of fixed effects (Baayen et al., 2008). For models of symptom trajectories (both GUPI subscales) random intercepts for subjects and random slopes for the effect of time for each subject were retained. Analyses were first conducted with age, BMI, duration of symptoms, PANAS negative affect score, use of medications (SSRI/SNRIs, opioids, NSAIDs, and pentosan polysulfate), the composite as predictors of GUPI subscales with time and a time by variable interaction. Significant main effects indicated an effect of the variable on the outcome measure across the period of observation, while significant time by variable interaction terms indicate an effect on the slope of the symptom trajectory. Significant main and interaction effects were retained in multivariate models that included TLR-4 inflammation scores, TLR-2 inflammation score, and cortisol slope.

For models of flare probability, mixed effects logistic regression models were used with random intercepts for subject retained following model evaluation. Bivariate analyses were conducted on the likelihood of a symptom flare during 1) the full 48 week period, 2) the 12 week period following the baseline visit with age, duration of symptoms, PANAS negative affect score, use of medications (SSRI/SNRIs, opioids, NSAIDs, and pentosan polysulfate). Significant main effects were retained in

multivariate models that included TLR-4 inflammation scores, TLR-2 inflammation score, and cortisol slope.

Regression analyses and one-way ANOVAs were conducted to determine if inflammatory variables were associated with time to first flare. Significant covariates were retained in multivariate regressions with inflammatory variables as main predictors of interest.

Results

Participants

Participants were largely White and non-Hispanic (96%), with college or graduate degrees (67%). A majority of participants were using Tricyclic antidepressants (62%) or pentosan polysulfate (67%). Demographic and medical information is presented in Table 10.

Table 10. Demographic and medical characteristics of participants., Chapter 3.

Participant Characteristics	N=24
	Mean(SD)
Age	42.09 (13.23)
BMI	27.43 (5.66)
PANAS negative affect	21.67 (9.74)
Duration of symptoms in years	7.21 (7.97)
	% (n)
Race *White* *Asian*	 96 (23) 4 (1)
Ethnicity % (*n*) *Non-Hispanic*	 100 (24)
Education % (*n*) *High School or GED* *Some College* *Graduated College* *Graduate Degree*	 4 (1) 30 (7) 33 (8) 33 (8)
Employment *Employed* *Disabled* *Retired* *Full Time Homemaker*	 83 (20) 8 (2) 4 (1) 4 (1)

Table 10. Continued

Annual Income/$ % (*n*)	
<10,000	21 (5)
<25,000	4 (1)
<50,000	17 (4)
<100,000	46 (11)
>100,000	12 (3)
Comorbid Conditions % (*n*)	
None	33 (8)
One or More (IBS, Fibromyalgia, TMD, vulvodynia, CFS)	67 (16)
Tricyclic anti-depressants	
No	38 (9)
Yes	62 (15)
Opioids	
No	75 (18)
Yes	25 (6)
Pentosan Polysulfate	
No	33 (8)
Yes	67 (16)
NSAIDs	
No	96 (23)
Yes	4 (1)

Table 10. Continued.

SSRI/SNRIs	
No	88 (21)
Yes	12 (3)

On average, participants completed 89% of longitudinal symptom questionnaire time-points (range 56%-100%).

Symptom Trajectories

Covariate Selection

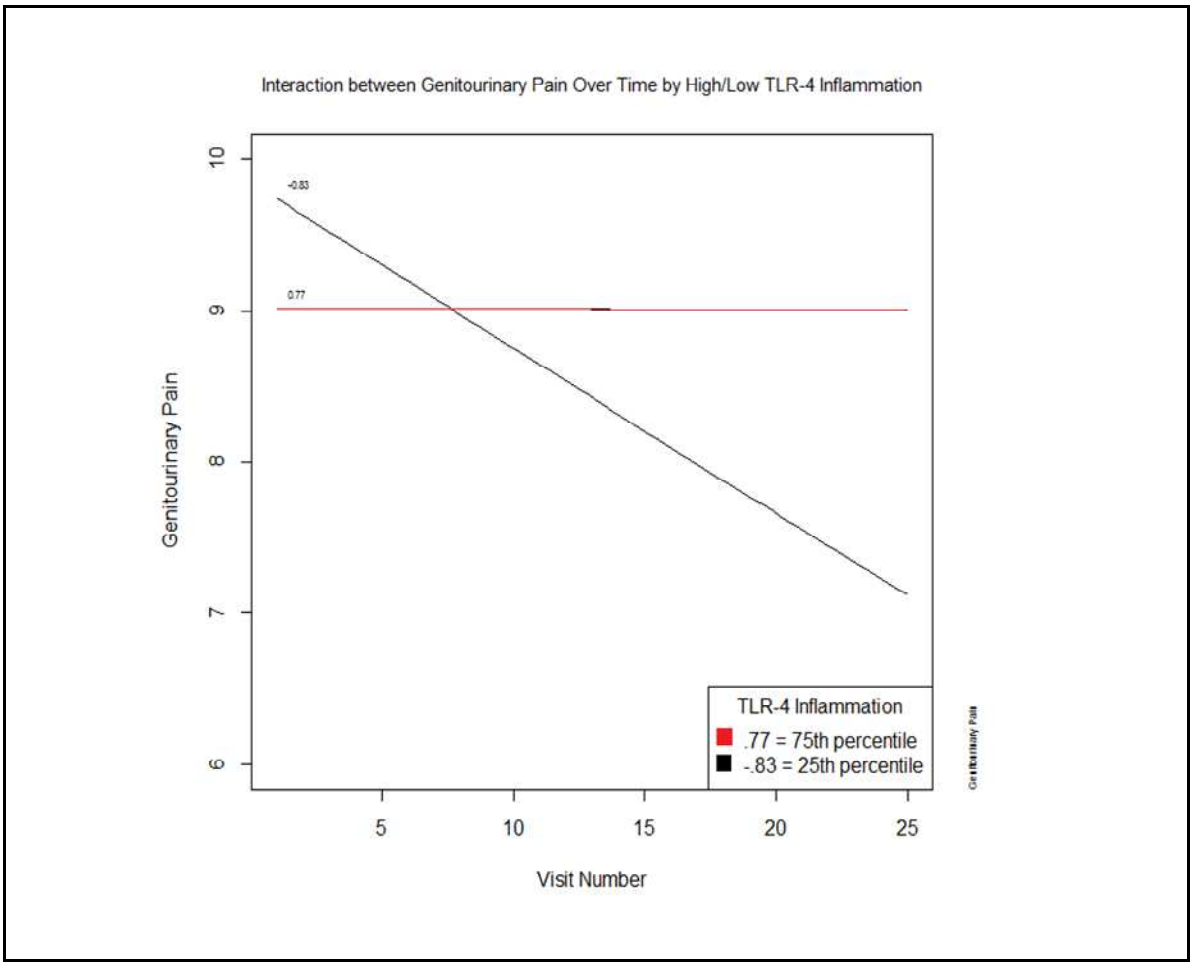
Patient age, BMI, duration of symptoms in years, and use of any medication were not associated with symptom levels or trajectories (all $p > .08$). Levels of negative affect were associated with higher levels of GUPI pain subscale and urinary symptom scores (both $p < .01$) but were not associated with symptom trajectories for either subscale (both $p = .74$). Comorbid status was associated with higher levels of GUPI urinary subscale scores ($p = .045$) but not symptom trajectory ($p = .13$). Therefore, negative affect was retained as a main effect in multivariate models predicting the GUPI pain subscale trajectories, and negative affect and comorbid condition status were retained as main effects in multivariate models predicting GUPI urinary subscale trajectories with inflammatory variables as main predictors.

Multivariate Models

Elevated TLR-4 inflammation score ($p = 0.031$), elevated TLR-2 inflammation score ($p = .045$), and flattened diurnal cortisol slope ($p = .012$) were each associated with less symptom improvement on the GUPI pain subscale over the 48 week period. See Table 2 for parameters of multivariate models. These findings predict that a patient one standard deviation above the mean on TLR-4 inflammation would experience a slight worsening of symptoms over the period (.36 points) while a patient one

standard deviation below the mean would experience moderate improvement (-2.89 points) on the 20 point scale. Results for TLR-2 inflammation and diurnal cortisol slope were similar. See Figure for fitted symptom trajectories on the GUPI pain subscale of patients at the 25th and 75th percentile of TLR-4 inflammation scores.

Figure 5. Predicted painful symptom trajectory for a participant at the 25th and 75th percentile of TLR-4 inflammation over the 25 visit (48 week) period.



Elevated TLR-4 inflammation (p = .018) was associated with less symptom improvement on the GUPI urinary subscale, while TLR-2 inflammation (p = .16) and diurnal cortisol slope were not (p = .17). See Table 11.

Table 11. Relationship between inflammatory variables and and genitourinary pain and urinary symptoms over the 48 week observation period.

GUPI Pain	Estimate	S.E.	DF	Z value	p
Time	-0.053	0.029	22.294	-1.791	0.087
TLR-4 composite score	0.519	0.871	21.586	-0.596	0.56
Negative Affect	0.445	0.087	21.131	5.096	<.001
Time*TLR-4 composite score	0.068	0.030	23.347	2.289	0.031
GUPI Pain	Estimate	S.E.	DF	Z value	*p*
Time	-0.054	0.030	22.089	-1.825	0.082
TLR-2 composite score	-0.784	0.838	21.245	-0.936	0.36
Negative Affect	.450	0.085	21.204	5.291	<.001
Time*TLR-2 composite score	0.063	0.030	22.078	2.123	0.045
GUPI Pain	Estimate	S.E.	DF	Z value	*p*
Time	-0.046	0.030	20.174	-1.555	0.14
Diurnal Cortisol Slope	-0.692	- 0.802	19.332	0.864	0.40
Negative Affect	0.445	0.079	19.229	5.622	<.001
Time*Slope	0.083	0.030	20.702	2.763	0.01
GUPI Urinary Symptoms	Estimate	S.E.	DF	Z value	*p*
Time	-0.017	0.011	22.153	-1.561	0.13

Table 11. Continued

Comorbid Condition	2.015	1.212	19.999	1.663	0.11
TLR-4 composite score	-0.446	0.559	20.128	-0.797	0.44
Negative Affect	0.122	0.059	20.074	2.073	0.051
Time*TLR-4 composite score	0.027	0.011	23.677	2.521	0.019

This finding predicts that a patient one standard deviation above the mean on TLR-4 inflammation would experience slight worsening of symptoms over the 48 week period (.25 point increase) while a patient one standard deviation below the mean would experience mild improvement (-1 point) on the 10 point scale.

Probability of Symptom Flares

Covariate Selection

Age, BMI, duration of symptoms, and use of any medication were not associated with an increased probability of reporting symptom flares over the full 48 week period, or the 12 weeks following the baseline visit (all $p > .12$). Higher levels of negative affect ($p < .001$) and the presence of a comorbid condition ($p = .013$) were each associated with a higher probability of reporting a symptom flare over the 48 week period. Age, duration of symptoms, presence of a comorbid condition, and use of any medication were not associated with a greater probability of reporting a symptom flare in the 12 weeks following the baseline visit (all $p > .08$). Higher levels of negative affect were associated with a greater probability of reporting a symptom flare in the 12 weeks following the baseline visit ($p < .001$). Thus, negative affect and comorbid status were retained as covariates for multivariate models

of flare probability over the full 48 weeks with inflammatory variables as main predictors, and negative affect for the multivariate models of flare probability in the 12 week period following the baseline visit with inflammatory variables as main predictors.

Multivariate Models

Neither TLR-4 inflammation, TLR-2 inflammation, nor diurnal cortisol slope was significantly associated with greater flare probability over either the full 48 week period or the 12 week period following the baseline visit (all $p > .29$).

Time to First Flare

Covariate Selection

Neither, age, BMI, duration of symptoms, comorbid status, nor use of any medications was associated with time to first flare in weeks (all $p > .24$). Higher levels of negative affect were associated with shorter time to first flare ($p = .016$), and thus negative affect was retained as a covariate in multivariate models of time to first flare with inflammatory variables as predictors.

Multivariate Models

Neither TLR-4 inflammation, TLR-2 inflammation, or diurnal cortisol slope were associated with time to first flare (all $p > .25$).

Conclusions

The primary findings of this study are that elevated levels of TLR-mediated inflammation, and dysregulated diurnal cortisol slope, are associated with less improvement in genitourinary pain in a sample of women suffering from IC/BPS over a 48 week period. Additionally, elevated TLR-4 inflammation was also associated with less improvement in urinary symptoms. In contrast, these inflammatory markers were not associated with the probability of self-reported symptom flares over the period of observation (48 weeks) or in the 12 weeks following the baseline visit. Similarly, these biomarkers were not associated with the time to first symptom flare. These findings echo our

previous investigations indicating that TLR inflammation and cortisol slope serve as biomarkers of IC/BPS and associated symptoms (Schrepf et al., 2014, 2015), and extend them by demonstrating that these markers are associated with less improvement in IC/BPS symptoms over time. Projected symptom trajectories using a one standard deviation difference in TLR-4 inflammation predicted a decline of more than 3 points on the GUPI pain subscale when comparing low vs. high TLR-4 inflammation – this amounts to a greater than 30% decrease in self-reported pain from study entry (mean = 10.4 over weeks 0-4). This suggests that TLR-mediated inflammation is associated with clinically relevant changes in symptoms in this sample.

To our knowledge, this is the first study to report on a biomarker of symptom trajectories in IC/BPS. Previous research has primarily focused on whether individuals met case definitions at subsequent visits following initial diagnosis with IC/BPS and demographic correlates of this likelihood (Suskind et al., 2014). The main effect of time was not statistically significant in any of the final multivariate models – this is consistent with the results of a large prospective study of IC/BPS patients assessed at approximately three month intervals for up to four years that found no significant change in symptoms over the period of observation (Propert et al., 2000). Examining predicted trajectories of patients at high and low levels of TLR-4 and TLR-2 inflammation, as well as cortisol slope, indicates that dysregulated inflammatory factors were primarily associated with a lack of symptom improvement over time, while low levels of TLR inflammation and more normal cortisol slopes were associated with modest symptom improvement. This suggests that these inflammatory factors could have utility in identifying patients who struggle to improve with conventional treatment. However, a prospective study with treatment-matched participants, or an investigation of treatment modalities on inflammatory markers, would be necessary to address this question.

Flatter diurnal cortisol slopes have previously been linked to glucocorticoid insensitivity following the dexamethasone suppression test (Jarcho, Slavich, Tylova-Stein, Wolkowitz, & Burke, 2013), therefore it is possible that IC/BPS patients with flattened cortisol slopes have a compromised ability

to control endogenous levels of inflammation. Loss of pulsatility in glucocorticoid rhythms has similarly been linked to a difficulty responding to acute stressors with sufficient glucocorticoid release (Scheff et al., 2012). The release of inflammatory cytokines following stimulation of TLR-4 in spinal glia has been identified as mechanistic component of centrally mediated pain sensitization (Grace et al., 2014; Mark R. Hutchinson et al., 2008; Milligan & Watkins, 2009). A recent investigation found some concordance between levels of inflammatory cytokines following TLR stimulation in both spinal glia and PBMCs (Kwok et al., 2013). However, whether PBMCs can serve as accessible proxies for central inflammatory processes remains an open question worthy of additional investigation. It is possible, for instance, that centrally acting treatment strategies, particularly those that might target TLR-mediated inflammation, could be effective in a subset of IC/BPS patients. Interestingly, while we did not find TLR-2 inflammation to be associated with either genitourinary or comorbid pain in our previous investigations (Schrepf et al., 2014, 2015), elevated TLR-2 inflammation was associated with less improvement in genitourinary pain in this study. Given that we have previously reported associations between cortisol slope, TLR-4, and TLR-2 inflammation, it is possible that one inflammatory marker is more intrinsically related to the longitudinal course of symptoms than the others. However, we did not have a sufficient sample size to detect the independent influence of each marker.

Strengths and Limitations

Strengths of this study include the frequency of longitudinal time points (bi-weekly for 48 weeks), inclusion of data on medication use, and the use of relatively sophisticated modeling techniques. Limitations include the relatively small sample size, the heterogeneous nature of study entry (i.e. participants had experienced symptoms for varying lengths of time) different treatment strategies employed over the period of observation (e.g. different uses of medications), and the ethnic/racial homogeneity of the sample. While the results of this study are promising, larger samples with greater frequency of inflammatory data collection are required to determine if these processes are

mechanistically related to symptom trajectories and/or are affected by treatment. While the differences in symptom trajectories might be considered clinically meaningful, the lack of associations with symptom flares is noteworthy, as these flares are known to be strongly related to reduced quality of life (Sutcliffe et al., 2014).

Future Directions

These results should be replicated in a larger and more diverse sample of IC/BPS patient. Prospective studies should be conducted that monitor the change in inflammatory processes following treatment changes. Structural and functional neuroimaging conducted in conjunction with collection of inflammatory data might aid in determining if these processes are associated with central pain sensitization.

DISCUSSION AND CONCLUSIONS

Viewed together, the findings of the above research program support several novel conclusions about the IC/BPS syndrome. IC/BPS patients are characterized by a pro-inflammatory phenotype; multiple facets of inflammatory processes appear to be altered. Each of these measures of inflammatory processes were derived from systemic indices – peripheral blood cytokines, peripheral blood mononuclear cells, and salivary cortisol (which has been shown to strongly reflect serum cortisol levels; Kirschbaum & Hellhammer, 1994). This body of work supports the conceptual framework of these research projects as outlined in Figure 1; specifically, that IC/BPS should be considered, at least in part, a systemic condition with important parallels to more widely researched FSSs. It is possible that with further replication and extension of these findings, clinicians may be able to use these measures of inflammation to algorithmically indicate the IC/BPS syndrome, phenotype subtypes of IC/BPS, and/or draw inferences about the disease trajectory. In addition, some preliminary conclusions about the pathophysiology of IC/BPS may be drawn.

In the discussion that follows, the chief findings of these research projects are summarized by each facet of the inflammatory response studied, followed by implications for IC/BPS symptomology. This is followed by discussion of the pathophysiology of IC/BPS and its relationship to FSSs in the context of these results, limitations of the current projects, and future directions for research.

Summary of Findings: Inflammatory Mechanisms in IC/BPS

Plasma IL-6

Elevated IL-6 in blood plasma differentiates IC/BPS from healthy controls. This elevation primarily indicates heightened basal systemic inflammation. IL-6 is part of the acute phase response following infection or injury; it is also induces release of C-reactive protein from the liver and adipose tissue (Pepys & Hirschfield, 2003). While these are primarily pro-inflammatory functions, IL-6 also serves to negatively regulate inflammation by inhibiting TNF-α and IL-1β at sufficient concentrations as well as inducing the release of IL-1 receptor antagonist and the anti-inflammatory cytokine IL-10 (Tilg, Trehu,

Atkins, Dinarello, & Mier, 1994; Xing et al., 1998). Elevated plasma IL-6 in IC/BPS suggests that the condition is characterized in part by heightened basal levels of inflammation. The observed concentration of IL-6 in blood plasma of IC/BPS patients (>2.5 pg/mL) is above a level that has previously been identified as conferring heightened risk for future adverse health events (Ferrucci et al., 1999). This may have particular importance in IC/BPS, as the condition has also been linked, for instance, to future occurrences of coronary artery disease (Chen et al., 2014).

Diurnal Cortisol

Dysregulated patterns of diurnal cortisol secretion, including flattened cortisol slopes and elevated nocturnal cortisol differentiate IC/BPS from healthy controls. Such alterations have previously been linked to functional measures of the HPA axis. For instance, flattened diurnal cortisol slopes have been associated with a loss of responsiveness to the dexamethasone suppression test in both healthy and depressed women (Jarcho et al., 2013). This strongly implies a loss of receptor responsiveness at one or more levels of the HPA axis (Silverman & Sternberg, 2012). This dysregulation was also found to be associated with the magnitude of the TLR-4 inflammatory response. This suggests that a broader loss of inflammatory control is a feature of IC/BPS, at least in some sufferers, though additional experiments are required to determine if glucocorticoid insensitivity in PBMCs or other circulating immune cells is a factor in IC/BPS and its symptomology. That this dysregulation was primarily driven by elevated afternoon and night levels of cortisol rather than morning levels, compared to healthy controls, is of interest as these are not believed to be heritable (Kupper et al., 2005). This provides inferential support for the idea that diurnal cortisol dysregulation in IC/BPS may be precipitated by environmental factors. One possibility, given high levels of nocturia, is that HPA dysregulation in IC/BPS reflects sleep disruption. Previous research has also demonstrated that cortisol rhythmicity is influenced by health behaviors, socioeconomic status, the extent of social networks, lifetime exposure to trauma, and chronic stress (Deverts et al., 2010; Ganzel et al., 2007; Miller, Chen, & Zhou, 2007). HPA dysregulation has

also been found in other conditions characterized by medically unexplained pain (Suárez-Hitz et al.; Wingenfeld et al., 2008)

Toll-Like Receptor Inflammatory Responses

Heightened inflammatory response to TLR-2 stimulation in PBMCs differentiates IC/BPS from healthy controls. TLR-2 primarily responds to gram-positive bacteria (e.g. *staphylococcus*; Underhill et al., 1999). Contrary to popular belief, the urogenital tract and bladder are not sterile environments and are populated with both gram-positive and gram-negative bacteria (Whiteside, Razvi, Dave, Reid, & Burton, 2015). One possibility, then, is that TLR-2 sensitized PBMCs migrating to the bladder and urogenital tract trigger immune-responses to non-infectious resident bacteria, promoting urinary symptoms, pain and symptom flares. However, this possibility is not consistent with the general absence of peripheral signs of inflammation in IC/BPS patients (Potts & Payne, 2012). TLR-4 mediated inflammation serves to differentiate IC/BPS patients with comorbid pain conditions from patients with IC/BPS alone. This is demonstrated most potently by elevated TLR-4 inflammation in every comorbid condition studied (IBS, CFS, fibromyalgia, TMD, vulvodynia) when compared to participants with IC/BPS alone. TLR-4 inflammation, therefore, seems to be related to an IC/BPS phenotype characterized by the presence of FSSs.

How PBMCs become sensitized to particular pathogen-classes is not yet well understood; however, TLR expression and upregulation in PBMCs have been identified in a variety of conditions including cancer, autoimmune disease, and acute infections (Ten Oever et al., 2014; Zhang et al., 2015). Perhaps most relevant to these findings, TLR-2 receptor density has been shown to be increased in IBS patients (Ohman et al., 2012). The current findings suggest the importance of examining TLR-2 and -4 receptor affinity and density in IC/BPS on PBMCs as an important next step in identifying the mechanism by which TLR stimulation is increased.

Summary of Findings: Inflammatory Correlates of IC/BPS and Comorbid Symptoms

IC/BPS symptoms

One of the primary findings of these research projects is that TLR-4 mediated inflammation, elicited by the classic TLR-4 agonist LPS in PBMCs, is a robust correlate of painful IC/BPS symptoms. While this composite score was associated with total scores on the Genitorurinary Pain Index (GUPI), which contains pain, urinary, and quality of life subscales, the most robust relationships were found with measures of pain. This pattern was born out when TLR-4 inflammation was associated with item-level data. For instance, the relationship with pain frequency (β=.40) was much stronger than with urinary frequency (β=.11). Additionally, TLR-4 inflammation was most strongly associated with pain frequency and intensity measures, rather than with pain in particular anatomical regions such as the urethra or bladder. This suggests that TLR-4 inflammation is linked to pain perception in a manner that is unlikely to be explained by particular local inflammatory dysfunction (i.e. inflammation in the urethra). This also provides preliminary evidence that TLR-4- mediated inflammation may be related to central pain sensitization in IC/BPS, a possibility discussed in greater detail below.

Clinically, the magnitude of the associations observed suggests that TLR-4 inflammation can provide non-negligible information about symptom severity. When combined with measures of diurnal cortisol using simple median splits, those participants in the high TLR-4 inflammation/flat diurnal cortisol group vs. low TLR-4/steep cortisol slope had symptom levels 61% higher for pain frequency measures and 34% higher for pain intensity measures and each difference was consistent with "medium" to "large" effect sizes.

When longitudinal outcomes were considered, TLR-2/4 inflammation and diurnal cortisol slope were each associated with less symptom improvement over a long period of observation (48 weeks). The average level of pain reported on the GUPI pain subscale in the first four weeks of the study was 10.4 on a scale ranging 0-20, versus 9.0 over the last four weeks, a decrease of only 1.4 points. Projected symptom trajectories using a one standard deviation difference in TLR-4 inflammation predicted a

decline of more than 3 points on the same scale over a 48 week period for patients low in TLR-4 inflammation, or a greater than 30% decrease in self-reported pain from study entry. Results were similar for TLR-2 inflammation and cortisol slope. This suggests that TLR-mediated inflammation is associated with clinically relevant changes in symptoms. Elevated TLR-4 inflammation was also associated with less improvement in urinary symptoms – this is likely of interest to clinicians, as urinary symptoms are closely linked to reduced quality of life in IC/BPS.

Comorbid Pain

TLR-4 inflammation was a robust predictor of the likelihood of endorsing pain outside the pelvic region using a body map paradigm. A one standard deviation increase in TLR-4 inflammation was associated with an almost 60% greater probability of endorsing pain outside the pelvic region, controlling for negative affect and use of medications. Finally, TLR-4 inflammation was marginally associated with lower pain thresholds on QST measured at a non-symptomatic site (thumb) in a subsample of participants.

Implications and Conclusions

It is possible to reach several conclusions about the IC/BPS syndrome in accordance with the above novel results. First, IC/BPS is characterized by systemic dysregulation of inflammatory processes (e.g., elevated peripheral blood IL-6, flattened diurnal cortisol slope, heightened inflammatory responses to TLR-2 inflammation). Second, IC/BPS patients can be further categorized into local vs. widespread symptom phenotypes by elevated inflammatory responses to TLR-4 stimulation. Third, this inflammatory dysregulation -- particularly TLR-4 inflammatory sensitization -- may be mechanistically related to symptoms. Each facet of pain in IC/BPS under investigation – genitourinary pain, comorbid pain, and QST pressure pain thresholds – was associated with heightened TLR-4 inflammation. Fourth, inflammatory dysregulation measured at a single time-point may provide clinically useful information about symptom trajectories.

Implications for the Pathophysiology of IC/BPS and Related Symptoms

While cross-sectional, these results can provide tentative conclusions about the pathophysiology of IC/BPS. While most definitions of IC/BPS refer explicitly to urogenital/bladder/sexual pain and urinary symptoms, epidemiological investigations make clear that pain outside the pelvic region is both common and debilitating in IC/BPS.

Several aspects of the current work support a notion of central sensitization in IC/BPS that is consistent with pain presentation in other FSSs that have been more fully characterized. For instance, such studies revealed relationships between TLR-4 inflammation and pain that were strongest when measured in terms of 1) intensity, 2) frequency, 3) and extent. TLR-4 inflammation has been associated with comorbid presentations as diffuse as orofacial pain (TMD) and pain in the bowel (IBS). Examination of the body map paradigm in project 2 strongly suggests diffuse pain increases as TLR-4 inflammatory responses increase. Reduced pain thresholds in a non-symptomatic area (the thumb) is consistent with other FSSs in which patients show reduced pain tolerance in areas not associated with the diagnosed FSS (i.e., IBS, TMD; Fernández-de-las-Peñas et al., 2009; Piché, Arsenault, Poitras, Rainville, & Bouin, 2010; Stabell et al., 2014). The results of the current studies suggest that IC/BPS is characterized by central pain sensitization like other FSSs, and that TLR-4 mediated inflammation may be a critical component of this aspect of the syndrome. One of the most intriguing results of the current studies was that elevated TLR-4 inflammation was associated with a longer duration of symptoms in years; this suggests a potential progressive element of the disease.

Results from nearly fifteen years of research in animal models implicate TLR-4 mediated inflammation in spinal glial cells as one mechanism by which central pain sensitization occurs (Milligan & Watkins, 2009). In these paradigms, initiating events such as peripheral nerve constriction and acute inflammatory challenge to peripheral tissue serve to increase inflammatory activity by microglia and astrocytes in the dorsal horn of the spinal cord (Hains et al., 2011; Mark R. Hutchinson et al., 2008; Tanga, Nutile-McMenemy, & DeLeo, 2005). The specific role of TLR-4 is demonstrated by the loss or

attenuation of hyperalgesia in TLR-4 knock-out mice and mice treated intrathecally with TLR-4 antisense oligodeoxynucleotide following these challenges (ODN) (Tanga et al., 2005). Additionally, a recent study using a rodent model of pain suggests that TLR inflammatory responses in PBMCs may reflect the same responses in spinal glia cells (Kwok et al., 2013). Therefore, one particularly intriguing hypothesis is that TLR-4 inflammation in PBMCs from IC/BPS patients reflects spinal glial sensitization, promoting a state of hyperalgesia/allodynia. However, significant pre-clinical research efforts are necessary before this mechanism can be confirmed in IC/BPS.

While TLR-mediated inflammation is a clearly established mechanism of pain sensitization, the role of glucocorticoids has not been researched to the same degree. It is possible, for instance, that one precipitating event in IC/BPS is dysregulation of the HPA axis and loss of inflammatory control. CPP in general is associated with a greater frequency of childhood traumatic events, which in turn serve as a risk factor for HPA axis dysregulation (Paras et al., 2009). A small, previous study in women with CPP found a lower mean number of glucocorticoid receptors on circulating lymphocytes than in healthy controls; this could be related to the finding that flattened diurnal cortisol slope was associated with higher TLR-4 inflammatory responses in the current studies (Heim, Ehlert, Rexhausen, Hanker, & Hellhammer, 1997).

The potential influence of familial genetic and environmental transcriptional factors in IC/BPS should not be ignored. Small-scale studies have demonstrated up-regulation of pro-inflammatory and chemotactic genes such as IL-1β in tissue and PBMCs of IC/BPS patients (You, Yang, Anger, Freeman, & Kim, 2012), polymorphisms previously linked to auto-immune disorders and adrenergic receptor alteration (Sugaya et al., 2002), and familial clustering of the disorder (Weissman et al., 2004). It is very possible that IC/BPS results from the interaction of susceptible phenotypes with pro-inflammatory environmental factors such as early life stress or recurrent infections. One particular important avenue of research would involve collecting careful information on antecedent infections and early-life stress in IC/BPS patients and examining the association between these factors, inflammatory processes, and disease presentation.

Critically, animal models of IC/BPS need to be developed that mimic essential features of the disease. This is particularly important as the peripheral etiology of IC/BPS is in question, leading to considerable divergence between the presentation of IC/BPS and the models of pain used in most rodent research paradigms of spinal glial sensitization. For instance, most rodent models achieve pain sensitization via acute inflammatory challenges or acute injury (e.g. formalin exposure, sciatic constriction; Hains et al., 2010). Given that participants in the current research projects had no evidence of infection or peripheral injury and a mean duration of symptoms approaching seven years, animal models of chronic sensitization need to be developed. Of particular use would be animal models that allow for spinal glial sensitization and sensitivity in circulating immune markers to be assayed simultaneously. One previous animal study has identified prior glucocorticoid exposure as a risk factor for LPS induced spinal neuro-inflammation (Loram et al., 2011). Animal models capable of exploring this phenomenon in IC/BPS are pertinent. For instance, experimentally disrupting corticosterone rhythms in a rodent model of IC/BPS might help to determine the sequencing of inflammatory dysregulation observed in human clinical populations. Similarly, repeated acute peripheral challenges mimicking the impact of repeated urogenital/bladder infections (identified as antecedent risk factors in IC/BPS; Díaz Mohedo et al., 2014; Li et al., 2010) could help to determine if such events potentiate development of the IC/BPS syndrome.

Other research has identified other important factors as being related to IC/BPS. For instance, urinary nerve growth factor (NGF) has been meta-analytically associated with the condition (Hammoud, Gago, & Diamond, 2004). Additionally, the role of bladder and urogenital microbiota in the pathogenesis of IC/BPS has not been explored, but recent work suggests significant variation between individuals that may alter nociception or immune function (Whiteside et al., 2015). Altered function of the sympathetic nervous system (SNS) has also been linked IC/BPS, evidenced by higher levels of urinary and plasma catecholamines and abnormal responses to autonomic testing (e.g. TILT test; Charrua et al., 2013). It is not clear how systemic inflammatory processes may interact with these factors. Future research should

incorporate measures of systemic inflammation, NGF, SNS function, and characterization of the urogenital/bladder microbiome.

The current studies provide considerable information about central pain sensitization and inflammatory correlates in IC/BPS. A natural extension of the current research should be to attempt to replicate these findings in other FSSs, particularly syndromes that are characterized by an absence of peripheral inflammation. It is not difficult to imagine target populations: IBS patients who have been screened for inflammatory bowel disease, TMD patients who have undergone scanning of the face and jaw and tested negative for inflammatory substances in the synovial fluid around the jaw. In fact, the current findings are most pertinent to symptoms of IC/BPS that are not strictly related to pelvic/bladder dysfunction, as only marginal relationships were observed for urinary symptoms. It is an intriguing and potentially highly relevant possibility that TLR-4 mediated inflammation is a mechanism for pain sensitization in a variety of medically unexplained chronic pain conditions.

Clinical Implications

Some of the mechanisms implicated above either as correlates of IC/BPS etiology or symptomology may be amenable to clinical interventions. From a clinical perspective, the possibility that TLR-4 inflammation acts mechanistically in IC/BPS is intriguing. First, TLR-4 antagonists exist that have reasonable specificity and are generally well-tolerated. For instance, naltrexone HCL is a competitive opioid receptor antagonist that has also been demonstrated to act on TLR-4 (Hutchinson et al., 2008). At low doses (i.e. < 5mg), naltrexone HCL has been demonstrated effective in treating pain in fibromyalgia, Crohn's disease, multiple sclerosis, and complex regional pain syndrome (Younger, Parkitny, & McLain, 2014). Similarly, Naloxone, is blood-brain-barrier permeable TLR-4 antagonist that has been demonstrated to be effective in the reversal of neuropathic pain in multiple animal models (Mark R. Hutchinson et al., 2008; Lewis et al., 2012). In human pain populations, Naloxone has been used relatively sparsely by itself, though some promising effects have been noted in IBS (Hawkes et al., 2002). In addition to these compounds, other putative inhibitors of central glia exist, such as minocycline, which

has been demonstrated effective in reducing pain in rodent models of mechanical pain (Kannampalli et al., 2014; Pu et al., 2013) and in some human neuropathic pain studies (Sumitani et al., 2015; Vanelderen et al., 2015).

The fact that HPA axis dysregulation may contribute to the IC/BPS syndrome should also be of considerable interest to clinicians. Given the high prevalence of nocturia in IC/BPS, disruption of sleep cycles is an almost inevitable consequence of the syndrome. For instance, a recent investigation found that a majority of IC/BPS patients report poor sleep quality, sleep duration of less than six hours a night on average, or problems sleeping due to IC/BPS symptoms (Troxel et al., 2014). The severity of sleep disruption was strongly related to IC/BPS impairment and reductions in quality of life. If HPA dysregulation does contribute to loss of inflammatory control in IC/BPS, then sleep therapy should be a natural target of IC/BPS treatment. Every instance of waking is accompanied by a rise in cortisol that can itself reduce slow wave sleep after the individual returns to rest (Buckley & Schatzberg, 2005). These interactions need to be studied further, as experimental sleep disruption is also known to decrease nociceptive thresholds, possibly through disruption of the HPA axis (Schuh-Hofer et al., 2013). Therefore, one potentially important therapeutic avenue would be greater attention to nocturnal IC/BPS symptoms, with an emphasis on reducing nocturia, waking episodes and increasing sleep duration. There does not appear to be any extant literature on the effectiveness of sleep therapy, including pharmacologic interventions, in IC/BPS.

High levels of negative affect were observed in the IC/BPS participants in these studies, echoing the same finding from the broader literature (Chuang, Weng, Hsu, Huang, & Wu, 2015). One unexplored facet of negative affect and pain concerns the interactive effects of inflammation on both negative affective processes and pain perception. For instance, recent work has highlighted the ability of experimentally induced inflammation to lower pain thresholds via an increase in negative affectivity (Lacourt et al., 2015; Wegner et al., 2014). Additionally, recent work has demonstrated gray matter abnormalities in the brains of IC/BPS patients associated with both pain and increased negative affectivity. It remains to be

determined if inflammatory correlates of IC/BPS symptoms are also associated with brain circuitry related to pain perception and mood disturbances. Well-designed functional and structural neuroimaging studies are needed to address this question.

Limitations

The current research projects contain a number of limitations. First, the participants in these studies were recruited from a single research center (the University of Iowa) , thus limiting generalizability beyond the Midwestern region of the United States. Furthermore, as participants were largely non-Hispanic Caucasians, the demographic characteristics of the participants cannot inform questions about how IC/BPS may manifest differently in racial and ethnic minorities. The inflammatory measures used in these studies, while important measures of functional inflammatory activity, cannot speak to potentially important risk factors in IC/BPS, such as genetic polymorphisms or transcriptional alterations in circulating immune cells. Additionally, the use of single time-point for collection is a limitation, as few inferences can be drawn about whether the variation in inflammatory parameters represents a stable versus a transient phenomenon. Particularly with regards to longitudinal outcomes, a single time-point for inflammatory measures is not ideal. It is critically important moving forward that inflammatory measures be sampled throughout the disease trajectory (e.g., before and after treatment changes, during symptom flares and symptom remission). TLR stimulation occurred at a single dose level of each agonist; characterizing the kinetics of TLR inflammatory responses would likely provide more information and might reveal more subtle differences. The sample size of the longitudinal component of the third research project was relatively small. It may be that at larger sample sizes additional relationships with covariates of interest and/or additional interaction terms could be detected. In all three projects, recruitment was open to patients at different levels of symptom duration and intensity. Therefore, conclusions about inflammation and the time-course of the disease are necessarily exploratory. The lack of findings concerning symptom flares also limits the clinical usefulness of these findings. These studies were conducted entirely in female IC/BPS patients; related conditions such as chronic

prostatitis may involve different mechanisms and inflammatory phenotypes. More research is necessary before parallels can be established between these findings and other chronic pelvic pain conditions.

Major Findings and Recommendations

IC/BPS is characterized by systemic dysregulation of inflammatory systems; TLR-4 inflammation appears to be strong mechanistic correlate of painful IC/BPS and comorbid symptoms. Longitudinal outcomes in IC/BPS appear to be related to this inflammatory dysregulation. TLR-4 is found to be most closely related to non-specific pain symptoms and may reflect the extent of central pain sensitization in the disease. TLR-4 may also be an important correlate of painful symptoms in FSSs in general, as the degree of TLR-4 inflammation was related to non-symptomatic pain on QST and to the extent of pain outside the pelvic region.

Following replication and monitoring of inflammatory processes throughout the disease and treatment trajectory, and confirmation in animal models, these findings may have translational impact either through the use of selected pharmacological agents or behavioral interventions designed to normalize inflammatory processes. Animal models of IC/BPS need to be developed that can address the degree to which systemic measures of inflammation reflect inflammatory processes implicated in central pain sensitization. Initiating events in IC/BPS are a critical and largely poorly understood facet of the disease. Genome wide association studies, environmental and interpersonal events, and medical sequelae of the disease need to be better elucidated, followed by the implementation of experimental animal models capable of reproducing the same inflammatory dysregulation observed in IC/BPS. These inflammatory mechanisms need to be explored in other FSSs as correlates of pain sensitization.

REFERENCES

Afari, N., Ahumada, S. M., Wright, L. J., Mostoufi, S., Golnari, G., Reis, V., & Cuneo, J. G. (2014). Psychological trauma and functional somatic syndromes: a systematic review and meta-analysis. Psychosomatic Medicine, 76(1), 2–11.

Alba-Delgado, C., Llorca-Torralba, M., Horrillo, I., Ortega, J. E., Mico, J. A., Sánchez-Blázquez, P., … Berrocoso, E. (2013). Chronic pain leads to concomitant noradrenergic impairment and mood disorders. Biological Psychiatry, 73(1), 54–62. http://doi.org/10.1016/j.biopsych.2012.06.033

Altman, D., Lundholm, C., Milsom, I., Peeker, R., Fall, M., Iliadou, A. N., & Pedersen, N. L. (2011). The genetic and environmental contribution to the occurrence of bladder pain syndrome: an empirical approach in a nationwide population sample. European Urology, 59(2), 280–5. http://doi.org/10.1016/j.eururo.2010.10.028

Anger, J. T., Zabihi, N., Clemens, J. Q., Payne, C. K., Saigal, C. S., & Rodriguez, L. V. (2011). Treatment choice, duration, and cost in patients with interstitial cystitis and painful bladder syndrome. International Urogynecology Journal, 22(4), 395–400.

As-Sanie, S., Harris, R. E., Harte, S. E., Tu, F. F., Neshewat, G., & Clauw, D. J. (2013). Increased pressure pain sensitivity in women with chronic pelvic pain. Obstetrics and Gynecology, 122(5), 1047–55. http://doi.org/10.1097/AOG.0b013e3182a7e1f5

Baayen, R. H., Davidson, D. J., & Bates, D. M. (2008). Mixed-effects modeling with crossed random effects for subjects and items. Journal of Memory and Language, 59(4), 390–412.

Barsky, A. J., & Borus, J. F. (1999). Functional somatic syndromes. Annals of Internal Medicine, 130(11), 910–21.

Bastos, L. F. S., Godin, A. M., Zhang, Y., Jarussophon, S., Ferreira, B. C. S., Machado, R. R., … Moraes, M. F. D. (2013). A minocycline derivative reduces nerve injury-induced allodynia, LPS-induced prostaglandin E2 microglial production and signaling via toll-like receptors 2 and 4. Neuroscience Letters, 543, 157–162. http://doi.org/10.1016/j.neulet.2013.03.014

Belmonte, L., Beutheu Youmba, S., Bertiaux-Vandaële, N., Antonietti, M., Lecleire, S., Zalar, A., … Ducrotté, P. (2012). Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. PloS One, 7(8), e42777. http://doi.org/10.1371/journal.pone.0042777

Benson, S., Kattoor, J., Wegner, A., Hammes, F., Reidick, D., Grigoleit, J.-S., … Elsenbruch, S. (2012). Acute experimental endotoxemia induces visceral hypersensitivity and altered pain evaluation in healthy humans. Pain, 153(4), 794–9. http://doi.org/10.1016/j.pain.2011.12.001

Berry, S. H., Elliott, M. N., Suttorp, M., Bogart, L. M., Stoto, M. A., Eggers, P., … Clemens, J. Q. (2011a). Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States. The Journal of Urology, 186(2), 540–4.

Berry, S. H., Elliott, M. N., Suttorp, M., Bogart, L. M., Stoto, M. A., Eggers, P., … Clemens, J. Q. (2011b). Prevalence of symptoms of bladder pain syndrome/interstitial cystitis among adult females in the United States. The Journal of Urology, 186(2), 540–4. http://doi.org/10.1016/j.juro.2011.03.132

Blankley, S., Graham, C. M., Howes, A., Bloom, C. I., Berry, M. P. R., Chaussabel, D., … O'Garra, A. (2014). Identification of the key differential transcriptional responses of human whole blood following TLR2 or TLR4 ligation in-vitro. PloS One, 9(5), e97702. http://doi.org/10.1371/journal.pone.0097702

Buckley, T. M., & Schatzberg, A. F. (2005). On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. The Journal of Clinical Endocrinology and Metabolism, 90(5), 3106–14. http://doi.org/10.1210/jc.2004-1056

Burkman, R. T. (2004). Chronic pelvic pain of bladder origin: epidemiology, pathogenesis and quality of life. The Journal of Reproductive Medicine, 49(3 Suppl), 225–9.

Calippe, B., Douin-Echinard, V., Delpy, L., Laffargue, M., Lelu, K., Krust, A., … Gourdy, P. (2010). 17 - Estradiol Promotes TLR4-Triggered Proinflammatory Mediator Production through Direct Estrogen Receptor Signaling in Macrophages In Vivo. The Journal of Immunology, 185(2), 1169–1176. http://doi.org/10.4049/jimmunol.0902383

Canavan, C., West, J., & Card, T. (2014). The epidemiology of irritable bowel syndrome. Clinical Epidemiology, 6, 71–80.

Chao, C. C., Janoff, E. N., Hu, S. X., Thomas, K., Gallagher, M., Tsang, M., & Peterson, P. K. (1991). Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome. Cytokine, 3(4), 292–8.

Charrua, A., Pinto, R., Taylor, A., Canelas, A., Ribeiro-da-Silva, A., Cruz, C. D., … Cruz, F. (2013). Can the adrenergic system be implicated in the pathophysiology of bladder pain syndrome/interstitial cystitis? A clinical and experimental study. Neurourology and Urodynamics. http://doi.org/10.1002/nau.22542

Chen, H.M., Lin, C.C., Kang, C.S., Lee, C.T., Lin, H.C., & Chung, S.D. (2014). Bladder pain syndrome/interstitial cystitis increase the risk of coronary heart disease. Neurourology and Urodynamics, 33(5), 511–5. http://doi.org/10.1002/nau.22444

Chrousos, G. P. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. The New England Journal of Medicine, 332(20), 1351–62. http://doi.org/10.1056/NEJM199505183322008

Chuang, Y.C., Weng, S.F., Hsu, Y.W., Huang, C. L.C., & Wu, M.P. (2015). Increased risks of healthcare-seeking behaviors of anxiety, depression and insomnia among patients with bladder pain syndrome/interstitial cystitis: a nationwide population-based study. International Urology and Nephrology, 47(2), 275–81. http://doi.org/10.1007/s11255-014-0908-6

Chung, K.H., Liu, S.P., Lin, H.C., & Chung, S.D. (2014). Bladder pain syndrome/interstitial cystitis is associated with anxiety disorder. Neurourology and Urodynamics, 33(1), 101–5. http://doi.org/10.1002/nau.22382

Clauw, D. J. (2014). Fibromyalgia: a clinical review. JAMA : The Journal of the American Medical Association, 311(15), 1547–55.

Clauw, D. J., Schmidt, M., Radulovic, D., Singer, A., Katz, P., & Bresette, J. The relationship between fibromyalgia and interstitial cystitis. Journal of Psychiatric Research, 31(1), 125–31.

Cleeland, C. S., & Ryan, K. M. (1994). Pain assessment: global use of the Brief Pain Inventory. Annals of the Academy of Medicine, Singapore, 23(2), 129–38.

Clemens, J. Q., Brown, S. O., & Calhoun, E. A. (2008). Mental health diagnoses in patients with interstitial cystitis/painful bladder syndrome and chronic prostatitis/chronic pelvic pain syndrome: a case/control study. The Journal of Urology, 180(4), 1378–82. http://doi.org/10.1016/j.juro.2008.06.032

Clemens, J. Q., Calhoun, E. A., Litwin, M. S., McNaughton-Collins, M., Kusek, J. W., Crowley, E. M., & Landis, J. R. (2009). Validation of a modified National Institutes of Health chronic prostatitis symptom index to assess genitourinary pain in both men and women. Urology, 74(5), 983–7, quiz 987.e1–3. http://doi.org/10.1016/j.urology.2009.06.078

Clemens, J. Q., Markossian, T., & Calhoun, E. A. (2009). Comparison of economic impact of chronic prostatitis/chronic pelvic pain syndrome and interstitial cystitis/painful bladder syndrome. Urology, 73(4), 743–6.

Clemens, J. Q., Mullins, C., Kusek, J. W., Kirkali, Z., Mayer, E. A., Rodríguez, L. V, … Clauw, D. J. (2014). The MAPP research network: a novel study of urologic chronic pelvic pain syndromes. BMC Urology, 14, 57. http://doi.org/10.1186/1471-2490-14-57

Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. Nature, 420(6917), 860–7. http://doi.org/10.1038/nature01322

Cox, L., Ayers, S., Nala, K., & Penny, J. (2007). Chronic pelvic pain and quality of life after laparoscopy. European Journal of Obstetrics, Gynecology, and Reproductive Biology, 132(2), 214–9.

De Goeij, M., van Eijk, L. T., Vanelderen, P., Wilder-Smith, O. H., Vissers, K. C., van der Hoeven, J. G., … Pickkers, P. (2013). Systemic inflammation decreases pain threshold in humans in vivo. PloS One, 8(12), e84159. http://doi.org/10.1371/journal.pone.0084159

Deverts, D. J., Cohen, S., DiLillo, V. G., Lewis, C. E., Kiefe, C., Whooley, M., & Matthews, K. A. (2010). Depressive symptoms, race, and circulating C-reactive protein: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Psychosomatic Medicine, 72(8), 734–41. http://doi.org/10.1097/PSY.0b013e3181ec4b98

Díaz Mohedo, E., Wärnberg, J., Barón López, F. J., Mera Velasco, S., & Cabello Burgos, A. (2014). Chronic pelvic pain in Spanish women: prevalence and associated risk factors. A cross-sectional study. Clinical and Experimental Obstetrics & Gynecology, 41(3), 243–8.

Drossman, D. A., & Dumitrascu, D. L. (2006a). Rome III: New standard for functional gastrointestinal disorders. Journal of Gastrointestinal and Liver Diseases : JGLD, 15(3), 237–41.

Drossman, D. A., & Dumitrascu, D. L. (2006b). Rome III: New standard for functional gastrointestinal disorders. Journal of Gastrointestinal and Liver Diseases : JGLD, 15(3), 237–41.

Duffy, D., Rouilly, V., Libri, V., Hasan, M., Beitz, B., David, M., … Albert, M. L. (2014). Functional analysis via standardized whole-blood stimulation systems defines the boundaries of a healthy immune response to complex stimuli. Immunity, 40(3), 436–50. http://doi.org/10.1016/j.immuni.2014.03.002

Dworkin, S. F., Sherman, J., Mancl, L., Ohrbach, R., LeResche, L., & Truelove, E. (2002). Reliability, validity, and clinical utility of the research diagnostic criteria for Temporomandibular Disorders Axis II Scales: depression, non-specific physical symptoms, and graded chronic pain. Journal of Orofacial Pain, 16(3), 207–20.

Ellis, A., Wieseler, J., Favret, J., Johnson, K. W., Rice, K. C., Maier, S. F., … Watkins, L. R. (2014). Systemic administration of propentofylline, ibudilast, and (+)-naltrexone each reverses mechanical allodynia in a novel rat model of central neuropathic pain. The Journal of Pain : Official Journal of the American Pain Society, 15(4), 407–21. http://doi.org/10.1016/j.jpain.2013.12.007

Fernández-de-las-Peñas, C., Galán-del-Río, F., Fernández-Carnero, J., Pesquera, J., Arendt-Nielsen, L., & Svensson, P. (2009). Bilateral widespread mechanical pain sensitivity in women with myofascial temporomandibular disorder: evidence of impairment in central nociceptive processing. The Journal of Pain : Official Journal of the American Pain Society, 10(11), 1170–8.

Ferrucci, L., Harris, T. B., Guralnik, J. M., Tracy, R. P., Corti, M. C., Cohen, H. J., … Havlik, R. J. (1999). Serum IL-6 level and the development of disability in older persons. Journal of the American Geriatrics Society, 47(6), 639–46.

Festa, A., D'Agostino, R., Williams, K., Karter, A. J., Mayer-Davis, E. J., Tracy, R. P., & Haffner, S. M. (2001). The relation of body fat mass and distribution to markers of chronic inflammation. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity, 25(10), 1407–15. http://doi.org/10.1038/sj.ijo.0801792

Ford, A. C., Bercik, P., Morgan, D. G., Bolino, C., Pintos–Sanchez, M. I., & Moayyedi, P. (2013). Validation of the Rome III Criteria for the Diagnosis of Irritable Bowel Syndrome in Secondary Care. Gastroenterology, 145(6), 1262–1270.e1. http://doi.org/10.1053/j.gastro.2013.08.048

Foster, R., Jung, J., Farooq, A., McClung, C., Ripsch, M. S., Fitzgerald, M. P., & White, F. A. (2011). Sciatic nerve injury induces functional pro-nociceptive chemokine receptors in bladder-associated primary afferent neurons in the rat. Neuroscience, 183, 230–7. http://doi.org/10.1016/j.neuroscience.2011.03.035

Fukuda, K., Straus, S. E., Hickie, I., Sharpe, M. C., Dobbins, J. G., & Komaroff, A. (1994). The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Annals of Internal Medicine, 121(12), 953–9.

Gaab, J., Rohleder, N., Heitz, V., Engert, V., Schad, T., Schürmeyer, T. H., & Ehlert, U. (2005). Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. Psychoneuroendocrinology, 30(2), 188–98. http://doi.org/10.1016/j.psyneuen.2004.06.008

Ganzel, B. L., Eckenrode, J. J., Kim, P., Wethington, E., Horowitz, E., & Temple, E. (2007). Salivary cortisol levels and mood vary by lifetime trauma exposure in a sample of healthy women. Journal of Traumatic Stress, 20(5), 689–99. http://doi.org/10.1002/jts.20287

Gaskin, D. J., & Richard, P. (2012). The economic costs of pain in the United States. The Journal of Pain : Official Journal of the American Pain Society, 13(8), 715–24.

Giesecke, J., Reed, B. D., Haefner, H. K., Giesecke, T., Clauw, D. J., & Gracely, R. H. (2004). Quantitative sensory testing in vulvodynia patients and increased peripheral pressure pain sensitivity. Obstetrics and Gynecology, 104(1), 126–33. http://doi.org/10.1097/01.AOG.0000129238.49397.4e

Gomborone, J. E., Gorard, D. A., Dewsnap, P. A., Libby, G. W., & Farthing, M. J. (1996). Prevalence of irritable bowel syndrome in chronic fatigue. Journal of the Royal College of Physicians of London, 30(6), 512–3.

Gormsen, L., Rosenberg, R., Bach, F. W., & Jensen, T. S. (2010). Depression, anxiety, health-related quality of life and pain in patients with chronic fibromyalgia and neuropathic pain. European Journal of Pain (London, England), 14(2), 127.e1–8. http://doi.org/10.1016/j.ejpain.2009.03.010

Gottsch, H. P., Miller, J. L., Yang, C. C., & Berger, R. E. (2011). A pilot study of botulinum toxin for interstitial cystitis/painful bladder syndrome. Neurourology and Urodynamics, 30(1), 93–6.

Grace, P. M., Hutchinson, M. R., Maier, S. F., & Watkins, L. R. (2014). Pathological pain and the neuroimmune interface. Nature Reviews. Immunology, 14(4), 217–31. http://doi.org/10.1038/nri3621

Hains, L. E., Loram, L. C., Taylor, F. R., Strand, K. A., Wieseler, J. L., Barrientos, R. M., … Watkins, L. R. (2011). Prior laparotomy or corticosterone potentiates lipopolysaccharide-induced fever and sickness behaviors. Journal of Neuroimmunology, 239(1-2), 53–60. http://doi.org/10.1016/j.jneuroim.2011.08.011

Hains, L. E., Loram, L. C., Weiseler, J. L., Frank, M. G., Bloss, E. B., Sholar, P., … Watkins, L. R. (2010). Pain intensity and duration can be enhanced by prior challenge: initial evidence suggestive of a role of microglial priming. The Journal of Pain : Official Journal of the American Pain Society, 11(10), 1004–14. http://doi.org/10.1016/j.jpain.2010.01.271

Hammoud, A., Gago, L. A., & Diamond, M. P. (2004). Adhesions in patients with chronic pelvic pain: a role for adhesiolysis? Fertility and Sterility, 82(6), 1483–91.

Hanno, P. M., Burks, D. A., Clemens, J. Q., Dmochowski, R. R., Erickson, D., Fitzgerald, M. P., … Faraday, M. M. (2011). AUA guideline for the diagnosis and treatment of interstitial cystitis/bladder pain syndrome. The Journal of Urology, 185(6), 2162–70.

Harte, S. E., Mitra, M., Ichesco, E. A., Halvorson, M. E., Clauw, D. J., Shih, A. J., & Kruger, G. H. (2013). Development and validation of a pressure-type automated quantitative sensory testing system for point-of-care pain assessment. Medical & Biological Engineering & Computing, 51(6), 633–44. http://doi.org/10.1007/s11517-013-1033-x

Hawkes, N. D., Rhodes, J., Evans, B. K., Rhodes, P., Hawthorne, A. B., & Thomas, G. A. O. (2002). Naloxone treatment for irritable bowel syndrome--a randomized controlled trial with an oral formulation. Alimentary Pharmacology & Therapeutics, 16(9), 1649–54.

Heim, C., Ehlert, U., Rexhausen, J., Hanker, J. P., & Hellhammer, D. H. (1997). Psychoendocrinological Observations in Women with Chronic Pelvic Pain. Annals of the New York Academy of Sciences, 821(1 Psychobiology), 456–458. http://doi.org/10.1111/j.1749-6632.1997.tb48304.x

Henningsen, P., Zimmermann, T., & Sattel, H. (2003). Medically unexplained physical symptoms, anxiety, and depression: a meta-analytic review. Psychosomatic Medicine, 65(4), 528–33.

Hill, J. R., Isom-Batz, G., Panagopoulos, G., Zakariasen, K., & Kavaler, E. (2008). Patient perceived outcomes of treatments used for interstitial cystitis. Urology, 71(1), 62–6.

Hungin, A. P. S., Chang, L., Locke, G. R., Dennis, E. H., & Barghout, V. (2005). Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. Alimentary Pharmacology & Therapeutics, 21(11), 1365–75.

Hutchinson, M. R., Bland, S. T., Johnson, K. W., Rice, K. C., Maier, S. F., & Watkins, L. R. (2007). Opioid-Induced Glial Activation: Mechanisms of Activation and Implications for Opioid Analgesia, Dependence, and Reward. The Scientific World JOURNAL, 7, 98–111. http://doi.org/10.1100/tsw.2007.230

Hutchinson, M. R., La Vincente, S. F., & Somogyi, A. A. (2004). In vitro opioid induced proliferation of peripheral blood immune cells correlates with in vivo cold pressor pain tolerance in humans: a biological marker of pain tolerance. Pain, 110(3), 751–5. http://doi.org/10.1016/j.pain.2004.05.017

Hutchinson, M. R., Ramos, K. M., Loram, L. C., Wieseler, J., Sholar, P. W., Kearney, J. J., … Watkins, L. R. (2009). Evidence for a role of heat shock protein-90 in toll like receptor 4 mediated pain enhancement in rats. Neuroscience, 164(4), 1821–32. http://doi.org/10.1016/j.neuroscience.2009.09.046

Hutchinson, M. R., Zhang, Y., Brown, K., Coats, B. D., Shridhar, M., Sholar, P. W., … Watkins, L. R. (2008). Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). European Journal of Neuroscience, 28(1), 20–29. http://doi.org/10.1111/j.1460-9568.2008.06321.x

Jarcho, M. R., Slavich, G. M., Tylova-Stein, H., Wolkowitz, O. M., & Burke, H. M. (2013). Dysregulated diurnal cortisol pattern is associated with glucocorticoid resistance in women with major depressive disorder. Biological Psychology, 93(1), 150–8. http://doi.org/10.1016/j.biopsycho.2013.01.018

Kannampalli, P., Pochiraju, S., Bruckert, M., Shaker, R., Banerjee, B., & Sengupta, J. N. (2014). Analgesic effect of minocycline in rat model of inflammation-induced visceral pain. European Journal of Pharmacology, 727, 87–98. http://doi.org/10.1016/j.ejphar.2014.01.026

Kato, K., Sullivan, P. F., Evengård, B., & Pedersen, N. L. (2009). A population-based twin study of functional somatic syndromes. Psychological Medicine, 39(3), 497–505. http://doi.org/10.1017/S0033291708003784

Kersh, B. C., Bradley, L. A., Alarcón, G. S., Alberts, K. R., Sotolongo, A., Martin, M. Y., … Triana-Alexander, M. (2001). Psychosocial and health status variables independently predict health care seeking in fibromyalgia. Arthritis and Rheumatism, 45(4), 362–71.

Killinger, K. A., Boura, J. A., & Peters, K. M. (2013). Pain in interstitial cystitis/bladder pain syndrome: do characteristics differ in ulcerative and non-ulcerative subtypes? International Urogynecology Journal, 24(8), 1295–301.

Kilpatrick, L. A., Kutch, J. J., Tillisch, K., Naliboff, B. D., Labus, J. S., Jiang, Z., … Mayer, E. A. (2014). Alterations in resting state oscillations and connectivity in sensory and motor networks in women with interstitial cystitis/painful bladder syndrome. The Journal of Urology, 192(3), 947–55. http://doi.org/10.1016/j.juro.2014.03.093

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. Psychoneuroendocrinology, 19(4), 313–333. http://doi.org/10.1016/0306-4530(94)90013-2

Komaroff, A. L., Fagioli, L. R., Geiger, A. M., Doolittle, T. H., Lee, J., Kornish, R. J., … Guerriero, R. T. (1996). An examination of the working case definition of chronic fatigue syndrome. The American Journal of Medicine, 100(1), 56–64.

Kovarik, J. J., Tillinger, W., Hofer, J., Hölzl, M. A., Heinzl, H., Saemann, M. D., & Zlabinger, G. J. (2011). Impaired anti-inflammatory efficacy of n-butyrate in patients with IBD. European Journal of Clinical Investigation, 41(3), 291–8. http://doi.org/10.1111/j.1365-2362.2010.02407.x

Kowalski, M. L., Wolska, A., Grzegorczyk, J., Hilt, J., Jarzebska, M., Drobniewski, M., … Kurowski, M. (2008). Increased Responsiveness to Toll-Like Receptor 4 Stimulation in Peripheral Blood Mononuclear Cells from Patients with Recent Onset Rheumatoid Arthritis. Mediators of Inflammation, 2008, 1–7. http://doi.org/10.1155/2008/132732

Kraemer, H. C., Giese-Davis, J., Yutsis, M., O'Hara, R., Neri, E., Gallagher-Thompson, D., … Spiegel, D. (2006). Design decisions to optimize reliability of daytime cortisol slopes in an older population. The American Journal of Geriatric Psychiatry : Official Journal of the American Association for Geriatric Psychiatry, 14(4), 325–33. http://doi.org/10.1097/01.JGP.0000201816.26786.5b

Kroenke, K., & Price, R. K. (1993). Symptoms in the community. Prevalence, classification, and psychiatric comorbidity. Archives of Internal Medicine, 153(21), 2474–80.

Kupper, N., de Geus, E. J. C., van den Berg, M., Kirschbaum, C., Boomsma, D. I., & Willemsen, G. (2005). Familial influences on basal salivary cortisol in an adult population. Psychoneuroendocrinology, 30(9), 857–68. http://doi.org/10.1016/j.psyneuen.2005.04.003

Kwok, Y. H., Hutchinson, M. R., Gentgall, M. G., & Rolan, P. E. (2012). Increased responsiveness of peripheral blood mononuclear cells to in vitro TLR 2, 4 and 7 ligand stimulation in chronic pain patients. PloS One, 7(8), e44232. http://doi.org/10.1371/journal.pone.0044232

Kwok, Y. H., Tuke, J., Nicotra, L. L., Grace, P. M., Rolan, P. E., & Hutchinson, M. R. (2013). TLR 2 and 4 responsiveness from isolated peripheral blood mononuclear cells from rats and humans as potential chronic pain biomarkers. PloS One, 8(10), e77799. http://doi.org/10.1371/journal.pone.0077799

Lacourt, T. E., Houtveen, J. H., Veldhuijzen van Zanten, J. J. C. S., Bosch, J. A., Drayson, M. T., & Van Doornen, L. J. P. (2015). Negative affectivity predicts decreased pain tolerance during low-grade inflammation in healthy women. Brain, Behavior, and Immunity, 44, 32–36. http://doi.org/10.1016/j.bbi.2014.10.003

Lai, H. H., Gardner, V., Ness, T. J., & Gereau, R. W. (2014). Segmental hyperalgesia to mechanical stimulus in interstitial cystitis/bladder pain syndrome: evidence of central sensitization. The Journal of Urology, 191(5), 1294–9.

Landis, J. R., Williams, D. A., Lucia, M. S., Clauw, D. J., Naliboff, B. D., Robinson, N. A., … Clemens, J. Q. (2014). The MAPP research network: design, patient characterization and operations. BMC Urology, 14(1), 58. http://doi.org/10.1186/1471-2490-14-58

Lee, Y. C., Lu, B., Bathon, J. M., Haythornthwaite, J. A., Smith, M. T., Page, G. G., & Edwards, R. R. (2011). Pain sensitivity and pain reactivity in osteoarthritis. Arthritis Care & Research, 63(3), 320–7. http://doi.org/10.1002/acr.20373

Lewis, S. S., Loram, L. C., Hutchinson, M. R., Li, C.-M., Zhang, Y., Maier, S. F., … Watkins, L. R. (2012). (+)-naloxone, an opioid-inactive toll-like receptor 4 signaling inhibitor, reverses multiple models of chronic neuropathic pain in rats. The Journal of Pain : Official Journal of the American Pain Society, 13(5), 498–506. http://doi.org/10.1016/j.jpain.2012.02.005

Li, G., Zhang, N., Du, P., Yang, Y., Wu, S., Xiao, Y., … Dai, Y. (2010). Risk factors for interstitial cystitis/painful bladder syndrome in patients with lower urinary tract symptoms: a Chinese multi-center study. Chinese Medical Journal, 123(20), 2842–6.

Loram, L. C., Taylor, F. R., Strand, K. A., Frank, M. G., Sholar, P., Harrison, J. A., … Watkins, L. R. (2011). Prior exposure to glucocorticoids potentiates lipopolysaccharide induced mechanical allodynia and spinal neuroinflammation. Brain, Behavior, and Immunity, 25(7), 1408–15. http://doi.org/10.1016/j.bbi.2011.04.013

Lutgendorf, S. K., Kreder, K. J., Rothrock, N. E., Hoffman, A., Kirschbaum, C., Sternberg, E. M., … Ratliff, T. L. (2002). Diurnal cortisol variations and symptoms in patients with interstitial cystitis. The Journal of Urology, 167(3), 1338–43.

Malt, E., Olafsson, S., Lund, A., & Ursin, H. (2002). Factors explaining variance in perceived pain in women with fibromyalgia. BMC Musculoskeletal Disorders, 3(1), 12.

Mattioli, T. A., Leduc-Pessah, H., Skelhorne-Gross, G., Nicol, C. J. B., Milne, B., Trang, T., & Cahill, C. M. (2014). Toll-like receptor 4 mutant and null mice retain morphine-induced tolerance, hyperalgesia, and physical dependence. PloS One, 9(5), e97361. http://doi.org/10.1371/journal.pone.0097361

McKernan, D. P., Gaszner, G., Quigley, E. M., Cryan, J. F., & Dinan, T. G. (2011). Altered peripheral toll-like receptor responses in the irritable bowel syndrome. Alimentary Pharmacology & Therapeutics, 33(9), 1045–52. http://doi.org/10.1111/j.1365-2036.2011.04624.x

Medzhitov, R. (2010). Inflammation 2010: new adventures of an old flame. Cell, 140(6), 771–6. http://doi.org/10.1016/j.cell.2010.03.006

Melzack, R., & Wall, P. D. (1965). Pain mechanisms: a new theory. Science (New York, N.Y.), 150(3699), 971–9.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychological Bulletin, 133(1), 25–45. http://doi.org/10.1037/0033-2909.133.1.25

Milligan, E. D., & Watkins, L. R. (2009). Pathological and protective roles of glia in chronic pain. Nature Reviews. Neuroscience, 10(1), 23–36. http://doi.org/10.1038/nrn2533

Morris, G., & Maes, M. (2013). Case definitions and diagnostic criteria for Myalgic Encephalomyelitis and Chronic fatigue Syndrome: from clinical-consensus to evidence-based case definitions. Neuro Endocrinology Letters, 34(3), 185–99.

Nellesen, D., Yee, K., Chawla, A., Lewis, B. E., & Carson, R. T. (2013). A systematic review of the economic and humanistic burden of illness in irritable bowel syndrome and chronic constipation. Journal of Managed Care Pharmacy : JMCP, 19(9), 755–64.

Ness, T. J., Lloyd, L. K., & Fillingim, R. B. (2014). An endogenous pain control system is altered in subjects with interstitial cystitis. The Journal of Urology, 191(2), 364–70.

Nickel, J. C., Herschorn, S., Whitmore, K. E., Forrest, J. B., Hu, P., Friedman, A. J., & Baseman, A. S. (2014). Pentosan Polysulfate Sodium for Treatment of Interstitial Cystitis/Bladder Pain Syndrome: Insights from a Randomized, Double-Blind, Placebo-Controlled Study. The Journal of Urology.

Nickel, J. C., Shoskes, D., & Irvine-Bird, K. (2009). Clinical phenotyping of women with interstitial cystitis/painful bladder syndrome: a key to classification and potentially improved management. The Journal of Urology, 182(1), 155–60. http://doi.org/10.1016/j.juro.2009.02.122

Nickel, J. C., Tripp, D. A., Pontari, M., Moldwin, R., Mayer, R., Carr, L. K., … Nordling, J. (2010). Interstitial cystitis/painful bladder syndrome and associated medical conditions with an emphasis on irritable bowel syndrome, fibromyalgia and chronic fatigue syndrome. The Journal of Urology, 184(4), 1358–63.

Nickel, J. C., Tripp, D. A., Pontari, M., Moldwin, R., Mayer, R., Carr, L. K., … Nordling, J. (2011). Childhood sexual trauma in women with interstitial cystitis/bladder pain syndrome: a case control study. Canadian Urological Association Journal = Journal de l'Association Des Urologues Du Canada, 5(6), 410–5.

Nicotra, L., Loram, L. C., Watkins, L. R., & Hutchinson, M. R. (2012). Toll-like receptors in chronic pain. Experimental Neurology, 234(2), 316–329. http://doi.org/10.1016/j.expneurol.2011.09.038

Nimnuan, C., Rabe-Hesketh, S., Wessely, S., & Hotopf, M. (2001). How many functional somatic syndromes? Journal of Psychosomatic Research, 51(4), 549–557. http://doi.org/10.1016/S0022-3999(01)00224-0

Novi, J. M., Jeronis, S., Srinivas, S., Srinivasan, R., Morgan, M. A., & Arya, L. A. (2005). Risk of irritable bowel syndrome and depression in women with interstitial cystitis: a case-control study. The Journal of Urology, 174(3), 937–40.

O'Leary, M. P., Sant, G. R., Fowler, F. J., Whitmore, K. E., & Spolarich-Kroll, J. (1997). The interstitial cystitis symptom index and problem index. Urology, 49(5A Suppl), 58–63.

Offiah, I., McMahon, S. B., & O'Reilly, B. A. (2013). Interstitial cystitis/bladder pain syndrome: diagnosis and management. International Urogynecology Journal, 24(8), 1243–56.

Ohman, L., Lindmark, A.-C., Isaksson, S., Posserud, I., Strid, H., Sjövall, H., & Simrén, M. (2012). Increased TLR2 expression on blood monocytes in irritable bowel syndrome patients. European Journal of Gastroenterology & Hepatology, 24(4), 398–405. http://doi.org/10.1097/MEG.0b013e3283503f39

Ortiz-Lucas, M., Saz-Peiró, P., & Sebastián-Domingo, J. J. (2010). Irritable bowel syndrome immune hypothesis. Part two: the role of cytokines. Revista Española de Enfermedades Digestivas : Organo Oficial de La Sociedad Española de Patología Digestiva, 102(12), 711–7.

Paras, M. L., Murad, M. H., Chen, L. P., Goranson, E. N., Sattler, A. L., Colbenson, K. M., … Zirakzadeh, A. (2009). Sexual abuse and lifetime diagnosis of somatic disorders: a systematic review and meta-analysis. JAMA, 302(5), 550–61.

Patarca, R. (2001). Cytokines and chronic fatigue syndrome. Annals of the New York Academy of Sciences, 933, 185–200.

Pepys, M. B., & Hirschfield, G. M. (2003). C-reactive protein: a critical update. The Journal of Clinical Investigation, 111(12), 1805–12. http://doi.org/10.1172/JCI18921

Petzke, F., Khine, A., Williams, D., Groner, K., Clauw, D. J., & Gracely, R. H. (2001). Dolorimetry performed at 3 paired tender points highly predicts overall tenderness. The Journal of Rheumatology, 28(11), 2568–9.

Phillips, K., & Clauw, D. J. (2011). Central pain mechanisms in chronic pain states--maybe it is all in their head. Best Practice & Research. Clinical Rheumatology, 25(2), 141–54. http://doi.org/10.1016/j.berh.2011.02.005

Piché, M., Arsenault, M., Poitras, P., Rainville, P., & Bouin, M. (2010). Widespread hypersensitivity is related to altered pain inhibition processes in irritable bowel syndrome. Pain, 148(1), 49–58.

Potts, J. M., & Payne, C. K. (2012). Urologic chronic pelvic pain. Pain, 153(4), 755–8. http://doi.org/10.1016/j.pain.2011.10.005

Powell-Boone, T., Ness, T. J., Cannon, R., Lloyd, L. K., Weigent, D. A., & Fillingim, R. B. (2005). Menstrual cycle affects bladder pain sensation in subjects with interstitial cystitis. The Journal of Urology, 174(5), 1832–6. http://doi.org/10.1097/01.ju.0000176747.40242.3d

Propert, K. J., Schaeffer, A. J., Brensinger, C. M., Kusek, J. W., Nyberg, L. M., & Landis, J. R. (2000). A prospective study of interstitial cystitis: results of longitudinal followup of the interstitial cystitis data base cohort. The Interstitial Cystitis Data Base Study Group. The Journal of Urology, 163(5), 1434–9.

Pu, S., Xu, Y., Du, D., Yang, M., Zhang, X., Wu, J., & Jiang, W. (2013). Minocycline attenuates mechanical allodynia and expression of spinal NMDA receptor 1 subunit in rat neuropathic pain model. Journal of Physiology and Biochemistry, 69(3), 349–57. http://doi.org/10.1007/s13105-012-0217-4

Queiroz, L. P. (2013). Worldwide epidemiology of fibromyalgia. Current Pain and Headache Reports, 17(8), 356.

Ridker, P. M. (2003). Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention. Circulation, 107(3), 363–369. http://doi.org/10.1161/01.CIR.0000053730.47739.3C

Rosen, R., Brown, C., Heiman, J., Leiblum, S., Meston, C., Shabsigh, R., … D'Agostino, R. (2000). The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. Journal of Sex & Marital Therapy, 26(2), 191–208. http://doi.org/10.1080/009262300278597

Rothrock, N. E., Lutgendorf, S. K., Hoffman, A., & Kreder, K. J. (2002). Depressive Symptoms And Quality Of Life In Patients With Interstitial Cystitis. The Journal of Urology, 167(4), 1763–1767.

Rothrock, N. E., Lutgendorf, S. K., Kreder, K. J., Ratliff, T., & Zimmerman, B. (2001). Stress and symptoms in patients with interstitial cystitis: a life stress model. Urology, 57(3), 422–7.

Rudick, C. N., Billips, B. K., Pavlov, V. I., Yaggie, R. E., Schaeffer, A. J., & Klumpp, D. J. (2010). Host-pathogen interactions mediating pain of urinary tract infection. The Journal of Infectious Diseases, 201(8), 1240–9. http://doi.org/10.1086/651275

Scheff, J. D., Calvano, S. E., Lowry, S. F., & Androulakis, I. P. (2012). Transcriptional implications of ultradian glucocorticoid secretion in homeostasis and in the acute stress response. Physiological Genomics, 44(2), 121–9. http://doi.org/10.1152/physiolgenomics.00128.2011

Schrepf, A., Bradley, C. S., O'Donnell, M., Luo, Y., Harte, S. E., Kreder, K., & Lutgendorf, S. (2015). Toll-like Receptor 4 and comorbid pain in Interstitial Cystitis/Bladder Pain Syndrome: A Multidisciplinary Approach to the Study of Chronic Pelvic Pain research network study. Brain, Behavior, and Immunity. http://doi.org/10.1016/j.bbi.2015.03.003

Schrepf, A., O'Donnell, M., Luo, Y., Bradley, C. S., Kreder, K., & Lutgendorf, S. (2014). Inflammation and inflammatory control in interstitial cystitis/bladder pain syndrome: Associations with painful symptoms. Pain, 155(9), 1755–61. http://doi.org/10.1016/j.pain.2014.05.029

Schuh-Hofer, S., Wodarski, R., Pfau, D. B., Caspani, O., Magerl, W., Kennedy, J. D., & Treede, R.-D. (2013). One night of total sleep deprivation promotes a state of generalized hyperalgesia: a surrogate pain model to study the relationship of insomnia and pain. Pain, 154(9), 1613–21. http://doi.org/10.1016/j.pain.2013.04.046

Schweitzer, R., Kelly, B., Foran, A., Terry, D., & Whiting, J. (1995). Quality of life in chronic fatigue syndrome. Social Science & Medicine, 41(10), 1367–1372.

Silverman, M. N., & Sternberg, E. M. (2012). Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Annals of the New York Academy of Sciences, 1261, 55–63. http://doi.org/10.1111/j.1749-6632.2012.06633.x

Simon, L. J., Landis, J. R., Erickson, D. R., & Nyberg, L. M. (1997). The Interstitial Cystitis Data Base Study: concepts and preliminary baseline descriptive statistics. Urology, 49(5A Suppl), 64–75.

Sinaii, N., Plumb, K., Cotton, L., Lambert, A., Kennedy, S., Zondervan, K., & Stratton, P. (2008). Differences in characteristics among 1,000 women with endometriosis based on extent of disease. Fertility and Sterility, 89(3), 538–45.

Skapinakis, P., Lewis, G., & Meltzer, H. (2003). Clarifying the relationship between unexplained chronic fatigue and psychiatric morbidity: results from a community survey in Great Britain. International Review of Psychiatry (Abingdon, England), 15(1-2), 57–64.

Smith, B. W., & Zautra, A. J. (2008). The effects of anxiety and depression on weekly pain in women with arthritis. Pain, 138(2), 354–61. http://doi.org/10.1016/j.pain.2008.01.008

Soares, R. L. (2014). Irritable bowel syndrome: A clinical review. World Journal of Gastroenterology, 20(34), 12144.

Song, J., & Abraham, S. N. (2008). TLR-mediated immune responses in the urinary tract. Current Opinion in Microbiology, 11(1), 66–73. http://doi.org/10.1016/j.mib.2007.12.001

Sorge, R. E., LaCroix-Fralish, M. L., Tuttle, A. H., Sotocinal, S. G., Austin, J.-S., Ritchie, J., … Mogil, J. S. (2011). Spinal Cord Toll-Like Receptor 4 Mediates Inflammatory and Neuropathic Hypersensitivity in Male But Not Female Mice. Journal of Neuroscience, 31(43), 15450–15454. http://doi.org/10.1523/JNEUROSCI.3859-11.2011

Stabell, N., Stubhaug, A., Flægstad, T., Mayer, E., Naliboff, B. D., & Nielsen, C. S. (2014). Widespread hyperalgesia in adolescents with symptoms of irritable bowel syndrome: results from a large population-based study. The Journal of Pain : Official Journal of the American Pain Society, 15(9), 898–906.

Stokes, J. A., Cheung, J., Eddinger, K., Corr, M., & Yaksh, T. L. (2013). Toll-like receptor signaling adapter proteins govern spread of neuropathic pain and recovery following nerve injury in male mice. Journal of Neuroinflammation, 10, 148. http://doi.org/10.1186/1742-2094-10-148

Suárez-Hitz, K. A., Otto, B., Bidlingmaier, M., Schwizer, W., Fried, M., & Ehlert, U. Altered psychobiological responsiveness in women with irritable bowel syndrome. Psychosomatic Medicine, 74(2), 221–31. http://doi.org/10.1097/PSY.0b013e318244fb82

Sugaya, K., Nishijima, S., Yamada, T., Miyazato, M., Hatano, T., & Ogawa, Y. (2002). Molecular analysis of adrenergic receptor genes and interleukin-4/interleukin-4 receptor genes in patients with interstitial cystitis. The Journal of Urology, 168(6), 2668–71. http://doi.org/10.1097/01.ju.0000028441.81066.7f

Suskind, A. M., Berry, S. H., Suttorp, M. J., Elliott, M. N., & Clemens, J. Q. (2014). Symptom persistence in a community cohort of women with interstitial cystitis/bladder pain syndrome (IC/BPS): 3-, 6-, 9-, and 12-month follow-up from the RICE cohort. International Urogynecology Journal, 25(12), 1639–43. http://doi.org/10.1007/s00192-014-2420-z

Sutcliffe, S., Colditz, G. A., Goodman, M. S., Pakpahan, R., Vetter, J., Ness, T. J., … Lai, H. H. (2014). Urological chronic pelvic pain syndrome symptom flares: characterisation of the full range of flares at two sites in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. BJU International. http://doi.org/10.1111/bju.12778

Takeda, K., Kaisho, T., & Akira, S. (2003). Toll-like receptors. Annual Review of Immunology, 21, 335–76.

Tan, G., Jensen, M. P., Thornby, J. I., & Shanti, B. F. (2004). Validation of the brief pain inventory for chronic nonmalignant pain. The Journal of Pain, 5(2), 133–137. http://doi.org/10.1016/j.jpain.2003.12.005

Tang, N. K. Y., Salkovskis, P. M., Hodges, A., Wright, K. J., Hanna, M., & Hester, J. (2008). Effects of mood on pain responses and pain tolerance: an experimental study in chronic back pain patients. Pain, 138(2), 392–401. http://doi.org/10.1016/j.pain.2008.01.018

Tanga, F. Y., Nutile-McMenemy, N., & DeLeo, J. A. (2005). The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proceedings of the National Academy of Sciences of the United States of America, 102(16), 5856–61. http://doi.org/10.1073/pnas.0501634102

Ten Oever, J., Kox, M., van de Veerdonk, F. L., Mothapo, K. M., Slavcovici, A., Jansen, T. L., … Netea, M. G. (2014). The discriminative capacity of soluble Toll-like receptor (sTLR)2 and sTLR4 in inflammatory diseases. BMC Immunology, 15, 55. http://doi.org/10.1186/s12865-014-0055-y

Thornton, J. G., Morley, S., Lilleyman, J., Onwude, J. L., Currie, I., & Crompton, A. C. (1997). The relationship between laparoscopic disease, pelvic pain and infertility; an unbiased assessment. European Journal of Obstetrics, Gynecology, and Reproductive Biology, 74(1), 57–62.

Tilg, H., Trehu, E., Atkins, M. B., Dinarello, C. A., & Mier, J. W. (1994). Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood, 83(1), 113–8.

Tomoda, A., Joudoi, T., Rabab, E.-M., Matsumoto, T., Park, T. H., & Miike, T. (2005). Cytokine production and modulation: comparison of patients with chronic fatigue syndrome and normal controls. Psychiatry Research, 134(1), 101–4. http://doi.org/10.1016/j.psychres.2005.01.002

Tramullas, M., Finger, B. C., Moloney, R. D., Golubeva, A. V, Moloney, G., Dinan, T. G., & Cryan, J. F. (2014). Toll-like receptor 4 regulates chronic stress-induced visceral pain in mice. Biological Psychiatry, 76(4), 340–8. http://doi.org/10.1016/j.biopsych.2013.11.004

Troxel, W. M., Booth, M., Buysse, D. J., Elliott, M. N., Suskind, A. M., Clemens, J. Q., & Berry, S. H. (2014). Sleep disturbances and nocturnal symptoms: relationships with quality of life in a population-based sample of women with interstitial cystitis/bladder pain syndrome. Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine, 10(12), 1331–7. http://doi.org/10.5664/jcsm.4292

Uçeyler, N., Häuser, W., & Sommer, C. (2011). Systematic review with meta-analysis: cytokines in fibromyalgia syndrome. BMC Musculoskeletal Disorders, 12, 245. http://doi.org/10.1186/1471-2474-12-245

Underhill, D. M., Ozinsky, A., Hajjar, A. M., Stevens, A., Wilson, C. B., Bassetti, M., & Aderem, A. (n.d.). The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens.

Van Tilburg, M. A. L., Palsson, O. S., & Whitehead, W. E. (2013). Which psychological factors exacerbate irritable bowel syndrome? Development of a comprehensive model. Journal of Psychosomatic Research, 74(6), 486–92.

Vehof, J., Zavos, H. M. S., Lachance, G., Hammond, C. J., & Williams, F. M. K. (2014). Shared genetic factors underlie chronic pain syndromes. Pain, 155(8), 1562–8. http://doi.org/10.1016/j.pain.2014.05.002

Vercellini, P., Fedele, L., Aimi, G., Pietropaolo, G., Consonni, D., & Crosignani, P. G. (2007). Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. Human Reproduction (Oxford, England), 22(1), 266–71.

Walker, E. A., Katon, W. J., Hansom, J., Harrop-Griffiths, J., Holm, L., Jones, M. L., … Russo, J. (1995). Psychiatric diagnoses and sexual victimization in women with chronic pelvic pain. Psychosomatics, 36(6), 531–40.

Warren, J. W. (2014). Bladder pain syndrome/interstitial cystitis as a functional somatic syndrome. Journal of Psychosomatic Research.

Warren, J. W., Howard, F. M., Cross, R. K., Good, J. L., Weissman, M. M., Wesselmann, U., … Clauw, D. J. (2009). Antecedent nonbladder syndromes in case-control study of interstitial cystitis/painful bladder syndrome. Urology, 73(1), 52–7.

Warren, J. W., Langenberg, P., & Clauw, D. J. (2013). The number of existing functional somatic syndromes (FSSs) is an important risk factor for new, different FSSs. Journal of Psychosomatic Research, 74(1), 12–7.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of Personality and Social Psychology, 54(6), 1063–1070.

Waylonis, G. W., & Heck, W. (1992). Fibromyalgia syndrome. New associations. American Journal of Physical Medicine & Rehabilitation / Association of Academic Physiatrists, 71(6), 343–8.

Wegner, A., Elsenbruch, S., Maluck, J., Grigoleit, J.-S., Engler, H., Jäger, M., … Benson, S. (2014). Inflammation-induced hyperalgesia: Effects of timing, dosage, and negative affect on somatic pain sensitivity in human experimental endotoxemia. Brain, Behavior, and Immunity, 41, 46–54. http://doi.org/10.1016/j.bbi.2014.05.001

Weissman, M. M., Gross, R., Fyer, A., Heiman, G. A., Gameroff, M. J., Hodge, S. E., … Wickramaratne, P. J. (2004). Interstitial cystitis and panic disorder: a potential genetic syndrome. Archives of General Psychiatry, 61(3), 273–9. http://doi.org/10.1001/archpsyc.61.3.273

Wellen, K. E., & Hotamisligil, G. S. (2005). Inflammation, stress, and diabetes. The Journal of Clinical Investigation, 115(5), 1111–9. http://doi.org/10.1172/JCI25102

Wessely, S., Nimnuan, C., & Sharpe, M. (1999). Functional somatic syndromes: one or many? The Lancet, 354(9182), 936–939. http://doi.org/10.1016/S0140-6736(98)08320-2

Whitehead, W. E., Burnett, C. K., Cook, E. W., & Taub, E. (1996). Impact of irritable bowel syndrome on quality of life. Digestive Diseases and Sciences, 41(11), 2248–2253.

Whiteside, S. A., Razvi, H., Dave, S., Reid, G., & Burton, J. P. (2015). The microbiome of the urinary tract-a role beyond infection. Nature Reviews. Urology, 12(2), 81–90. http://doi.org/10.1038/nrurol.2014.361

Wieseler-Frank, J., Maier, S. F., & Watkins, L. R. (2005). Central proinflammatory cytokines and pain enhancement. Neuro-Signals, 14(4), 166–74. http://doi.org/10.1159/000087655

Willis, W. D. (1985). Central nervous system mechanisms for pain modulation. Applied Neurophysiology, 48(1-6), 153–65.

Wingenfeld, K., Heim, C., Schmidt, I., Wagner, D., Meinlschmidt, G., & Hellhammer, D. H. (2008). HPA axis reactivity and lymphocyte glucocorticoid sensitivity in fibromyalgia syndrome and chronic pelvic pain. Psychosomatic Medicine, 70(1), 65–72. http://doi.org/10.1097/PSY.0b013e31815ff3ce

Wirtz, P. H., Ehlert, U., Emini, L., & Suter, T. (2008). Higher body mass index (BMI) is associated with reduced glucocorticoid inhibition of inflammatory cytokine production following acute psychosocial stress in men. Psychoneuroendocrinology, 33(8), 1102–1110. http://doi.org/10.1016/j.psyneuen.2008.05.002

Wolfe, F., Clauw, D. J., Fitzcharles, M.-A., Goldenberg, D. L., Katz, R. S., Mease, P., … Yunus, M. B. (2010). The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care & Research, 62(5), 600–10.

Woolf, C. J. (2011). Central sensitization: implications for the diagnosis and treatment of pain. Pain, 152(3 Suppl), S2–15.

Xing, Z., Gauldie, J., Cox, G., Baumann, H., Jordana, M., Lei, X. F., & Achong, M. K. (1998). IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. Journal of Clinical Investigation, 101(2), 311–320. http://doi.org/10.1172/JCI1368

You, S., Yang, W., Anger, J. T., Freeman, M. R., & Kim, J. (2012). "Omics" approaches to understanding interstitial cystitis/painful bladder syndrome/bladder pain syndrome. International Neurourology Journal, 16(4), 159–68. http://doi.org/10.5213/inj.2012.16.4.159

Younger, J., Parkitny, L., & McLain, D. (2014). The use of low-dose naltrexone (LDN) as a novel anti-inflammatory treatment for chronic pain. Clinical Rheumatology, 33(4), 451–9. http://doi.org/10.1007/s10067-014-2517-2

Zhang, X., Xu, J., Ke, X., Zhang, S., Huang, P., Xu, T., … Pan, S. (2015). Expression and function of Toll-like receptors in peripheral blood mononuclear cells from patients with ovarian cancer. Cancer Immunology, Immunotherapy : CII, 64(3), 275–86. http://doi.org/10.1007/s00262-014-1632-x