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Platinum Priority – Pelvic Pain

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The Expression of Inflammatory Mediators in Bladder Pain Syndrome

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Abstract

Background: Bladder pain syndrome (BPS) pathology is poorly understood. Treatment strategies are empirical, with limited efficacy, and affected patients have diminished quality of life.
Objective: We examined the hypothesis that inflammatory mediators within the bladder contribute to BPS pathology.
Design, setting, and participants: Fifteen women with BPS and 15 women with stress urinary incontinence without bladder pain were recruited from Cork University Maternity Hospital from October 2011 to October 2012. During cystoscopy, 5-mm bladder biopsies were taken and processed for gene expression analysis. The effect of the identified genes was tested in laboratory animals.
Outcome measures and statistical analysis: We studied the expression of 96 inflammation-related genes in diseased and healthy bladders. We measured the correlation between genes and patient clinical profiles using the Pearson correlation coefficient.
Results and limitations: Analysis revealed 15 differentially expressed genes, confirmed in a replication study. *FGF7* and *CCL21* correlated significantly with clinical outcomes. Intravesical *CCL21* instillation in rats caused increased bladder excitability and increased *c-fos* activity in spinal cord neurons. *CCL21* atypical receptor knockout mice showed significantly more *c-fos* upon bladder stimulation with *CCL21* than wild-type littermates. There was no change in *FGF7*-treated animals. The variability in patient samples presented as the main limitation. We used principal component analysis to identify similarities within the patient group.
Conclusions: Our study identified two biologically relevant inflammatory mediators in BPS and demonstrated an increase in nociceptive signalling with *CCL21*. Manipulation of this ligand is a potential new therapeutic strategy for BPS.
Patient summary: We compared gene expression in bladder biopsies of patients with bladder pain syndrome (BPS) and controls without pain and identified two genes that were increased in BPS patients and correlated with clinical profiles. We tested the effect of these genes in laboratory animals, confirming their role in bladder pain. Manipulating these genes in BPS is a potential treatment strategy.

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1. Introduction

Bladder pain syndrome (BPS) is pain related to the urinary bladder accompanied by frequency, urgency, or nocturia, with the exclusion of any other diseases of the lower urinary tract [1,2]. Although the disease affects both sexes, women are more commonly affected than men by 5:1 [3]. Some patients display a mild form of the disease, with treatment generally orchestrated in the outpatient setting; in other cases, the disease is debilitating and requires prolonged hospitalisation and, often, repeated surgical intervention.

The aetiology of BPS is unknown. Multiple theories exist, including epithelial disruption and mast cell and vascular abnormalities [4,5]. The contributions of peripheral neuronal mechanisms remain unclear. We explore the hypothesis that many patients have a peripheral inflammatory disorder and that the expression of inflammatory mediators in the bladder wall activates and sensitises the bladder sensory afferents, driving BPS symptoms. Because there are currently no disease-modifying treatments for BPS, we postulated that the identification of novel inflammatory mediators associated with the disease might be manipulated to alter the disease's course.

We used quantitative gene expression analysis of 96 inflammatory mediators to measure gene expression levels in BPS and control samples. We then tested the activity of the identified mediators in an animal model of BPS using the enzymes chondroitinase and heparanase to digest the proteoglycan barrier [6,7].

2. Materials and methods

We performed a prospective observational study of 15 women with BPS and 15 age-matched female controls between October 2011 and October 2012 at Cork University Maternity Hospital. BPS participants had bladder pain for at least 3 mo, with urodynamic and cystoscopic evidence of disease. Controls were patients undergoing tension-free vaginal tape surgery. Patients who had systemic disease such as malignancy, coagulopathies, or other forms of cystitis (eg, infective, chemical, or radiation cystitis) were excluded.

Participants completed the O'Leary-Sant Interstitial Cystitis Symptom and Problem Index (ICS/PI) questionnaire. While under general anaesthesia, a rigid cystoscopy (30° lens) was performed, and three 5-mm biopsies were taken from above the bladder trigone by cold-cup biopsy technique from each participant [7]. Biopsies were also taken from healthy-looking bladder away from lesion sites. RNA was extracted from the tissue using a combination of phenol extraction and column purification. RNA integrity was determined using an Agilent RNA 6000 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

We performed reverse transcription reactions using a complementary DNA reverse transcription kit (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA). Custom-made TaqMan microfluidic cards (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) were used to measure expression levels of 96 inflammatory mediators in disease versus control tissue calculated using the ΔCT (cycle time) method [8] and normalised against the geometric mean of three housekeeping genes using the ReadqPCR and NormqPCR packages [9].

2.1. Patient clustering

We used principal component analysis with eigenvalue decomposition to visualise biologic variability within the patient group [10]. We identified patient clusters based on their gene expression levels and used the first two components for repeat gene expression analysis. Pearson correlation coefficient was used to visualise the hierarchical clustering of each group of patients for significantly dysregulated genes.

2.2. Replication study

Confirmatory gene expression analysis was replicated in an independent cohort of 23 patients with bladder pain and 15 controls without pain. BPS was diagnosed based on history of bladder pain, and control participants were patients who had lower urinary tract disorders such as stress urinary incontinence or overactive bladder without pain. Biopsies were taken under general anaesthesia from the bladder dome away from lesion sites using the cold-cup biopsy technique.

2.3. Statistical analysis

Data analysis was performed using the ΔCT method [8]. We applied the Benjamini-Hochberg false discovery rate (FDR) algorithm (5%) to the data. We used a volcano plot to illustrate variation within the data, thus visually highlighting differentially regulated genes (*t* test p value = 0.01; twofold difference). Spearman rank correlation was used to determine the correlation of gene expression levels against BPS clinical phenotypes derived from the ICS/PI questionnaire.

2.4. Animal experiments

All experiments were conducted using adult female Wistar rats (approximate weight: 200–250 g, Harlan, UK) in accordance with the UK Home Office Regulations. All rats were housed in the licensed biological services unit of King's College London with a 12-h day/night cycle. Food and water were available at all times. Each animal was randomly assigned to treatment protocols, and assessors were blinded to the treatments the rats received.

2.5. Cystometry

Fifteen animals were anaesthetised with 1 mg/kg urethane; 20-gauge catheters were inserted transurethrally and attached to a syringe pump and pressure transducer. Bladders were distended with 0.9% saline, with 50 μl/min and baseline cystometric analysis recorded. Ten animals had 200 μl of 0.25 IU chondroitinase ABC and heparanase III (Sigma-Aldrich, St. Louis, MO, USA) bladder instillation. Five control animals had 200 μl phosphate-buffered saline (PBS) instilled, and solutions were allowed to remain in situ for 2 h. Then, five animals each had 10 μl of 250 ng/ml of either *CCL21* or *FGF7* instilled for 2 h. Cystometric analysis was repeated, and total contraction time was measured.

2.6. Behavioural assessment

Pelvic pain response was assessed using calibrated Von Frey monofilaments. Von Frey withdrawal is typically performed on the hind paw of laboratory animals. We performed mechanical withdrawal assessment on the suprapubic region, which is reported as a valid method for the assessment of referred hyperalgesia and mechanical allodynia in animal models of bladder hypersensitivity [11]. Tactile sensitivity of the suprapubic region was assessed using the Chaplan method [12]. A positive behavioural response was recorded as licking or scratching of the stimulated area, sharp withdrawal, or jumping. Rats were anaesthetised with isoflurane and transurethrally catheterised; 10 rat bladders were permeabilised with 200 μl of 0.25 IU chondroitinase ABC and heparanase III. Experimental rats received 10 μl of 250 ng/ml *CCL21* or *FGF7* postdigestion (five per group), while five controls received PBS. Von Frey assessment was repeated, and 50% threshold values were calculated.