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Metabotropic Glutamate Receptor 5 Expression Associates with Pain and Inflammatory Pathways in Interstitial Cystitis

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Key words: interstitial cystitis, metabotropic glutamate receptor, bladder pain syndrome

Abstract

Interstitial cystitis (IC)/bladder pain syndrome (BPS) is a chronic condition with severe pelvic pain and urinary symptoms significantly impairing quality of life. This study investigated the clinical relevance of the metabotropic glutamate receptor (mGluR) family in IC/BPS. Bladder biopsy samples were taken from 61 patients, including 42 Hunner-type IC, 11 non-Hunner type IC, and 8 controls without IC. Gene expression analysis revealed that mGluR2, mGluR3, and mGluR5 were significantly elevated in patients with IC/BPS compared to controls. Among these, mGluR5 showed the strongest association with pain severity, fibrosis, and lymphoplasmacytic infiltration. Patients with Hunner-type IC also exhibited increased expression of p65 and interleukin-1β, suggesting activation of inflammatory response modulation in IC/BPS. These findings suggest that mGluR5 may contribute to pain through immune response modulation in IC/BPS. Targeting mGluR5 could represent a promising therapeutic strategy to alleviate symptoms and improve patient quality of life.

Introduction

Interstitial cystitis (IC), also known as bladder pain syndrome (BPS), is a debilitating bladder disease characterized by severe pelvic pain and frequent urination. This condition has been associated with defective bladder mucosal layer, increased mast cell activation, augmented sympathetic innervation, and elevated levels of inflammatory mediators, such as substance P, bradykinin, and interleukins. These factors contribute to the pathogenesis and symptoms of this condition[1,2]. IC/BPS affects millions of people worldwide and causes severe pelvic pain as well as sudden and intense urges to urinate, which significantly impairs the quality of life of patients. However, the mechanisms underlying IC/BPS remain unclear. Considerable research has been conducted on the disease and its pathogenesis, and some studies have identified Hunner's lesions as a distinctive inflammatory response in certain patients. These lesions are characterized by a reddened mucosal area with small blood vessels radiating toward the central ulceration[3]. Following treatment targeting typical inflammatory reactions and Hunner's lesions, prognosis notably improves[4]. Although various attempts have been made to treat IC/BPS, a reliable method for its diagnosis, monitoring, and treating the disease still needs to be identified[5].

Pain is a complex sensory experience with numerous potential causes, including nociceptive signaling, neuroinflammation, and aberrant neuronal plasticity[6,7]. The molecular mechanisms underlying pain perception and modulation need to be elucidated to develop effective treatments for pain-related disorders. Among the numerous genes and signaling pathways that have been identified to play a role in pain, the metabotropic glutamate receptor (mGluR) family has emerged as a particularly promising candidate with significant clinical implications[8-10]. Several studies have demonstrated the relevance of mGluRs in various pain states, including inflammatory, neuropathic, and visceral pain[11-16]. Preclinical studies utilizing animal models have demonstrated altered mGluR expression and activity in bladder tissue, suggesting their potential role in mediating negative signaling and inflammation associated with IC/BPS[17-20]. Elucidating the clinical implications of the mGluR family in the bladder tissues of patients with IC/BPS could advance our knowledge of its pathophysiology and potentially facilitate the development of targeted therapeutic interventions. Although many studies have been conducted on the treatment of IC/BPS, research on the pain control mechanism remains lacking. As the fundamental pathophysiology of IC/BPS has not been elucidated, symptom control is the main treatment goal, and an effective drug treatment for pain control remains undetermined[21]. Therefore, the molecular and cellular mechanisms involved in the pain mechanism of IC/BPS needs to be clarified. The role of mGluRs in IC/BPS is underexplored. In particular, the relationship between mGluR expression and the immune response in Hunner's lesions remains unclear. Therefore, the influence of the mGluR family on pain and neuroinflammatory pathways in IC/BPS needs to be examined[17,22].

This analytical study explored the clinical implications of mGluR family members in the bladder tissue of patients with IC/BPS. The expression patterns of mGluR subtypes in patients were investigated, and their potential contributions to disease progression were discussed. By analyzing the specific mGluR subtypes expressed in the bladder tissue and investigating their genetic variations in IC/BPS patients, this study aimed to investigate the role of mGluRs in IC/BPS and elucidate the mechanisms underlying bladder pain and inflammation in IC/BPS, in order to develop new therapeutic targets. In this study, we analyzed the gene expression of the mGluR family in the bladder tissues of patients with IC/BPS and evaluated the correlation with clinical indicators, especially the severity of pain and the presence of Hunner's lesions. By elucidating how the expression of mGluRs,

especially mGluR5, contributes to pain amplification and the activation of neuroinflammatory pathways, we suggest a new therapeutic target.

Results

Patient biopsy and sample collection

This study analyzed the bladder biopsies and clinical information of 61 patients. An overview of this study is shown in Figure 1. A total of 61 patients (10 men and 51 women) were enrolled in the study following approval from the Institutional Review Board of Konkuk Medical Center (KUMC 2019-07-009 and KUMC 2022-04-003). This study included 11 cases of non-Hunner interstitial cystitis (IC), 42 cases of Hunner interstitial cystitis, and 8 cases of non-interstitial cystitis. Detailed information is presented in Supplementary Table 1. In the table, we recorded the underlying conditions of the non-IC control group. The female patients had stress urinary incontinence, and the male patients were individuals who underwent surgery under general anesthesia for ureteral stones. During the preoperative evaluation, their urinalysis results were normal, and they had no lower urinary tract symptoms (LUTS). The intraoperative cystoscopic inspection revealed no abnormalities within the bladder. The mean age (mean ± standard deviation) of the patients was 62.4 ± 10.0 years, with no significant difference observed between the Hunner type IC and non-Hunner type IC groups (61.2 ± 8.6 years vs. 58.5 ± 9.8 years, p-value = 0.2888). The mean VAS score for all patients was 7.5 ± 2.1, with no significant difference observed between the Hunner IC and non-Hunner IC groups (8.2 ± 1.8 vs. 6.5 ± 2.2, $p = 0.0542$). Additionally, no significant difference was observed in maximum bladder capacity between the two groups. However, a notable difference was evident in symptom duration between the Hunner IC and non-Hunner IC groups (41.3 ± 20.0 months vs. 27.6 ± 7.2 months, p = 0.0311) (Tables 1 and 2).

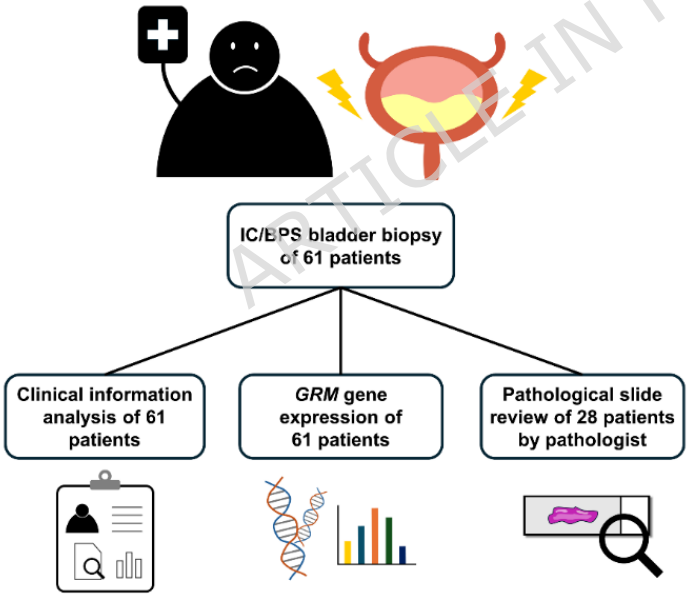

Figure 1. Overview of the study design

This figure illustrates the sequence of participant recruitment, bladder tissue biopsy, clinical information analysis, gene expression analysis, and pathological slide review steps conducted in the present study.

Table 1. Characteristics of the patients included in this research

Patients (n)	61
HIC	42
NHIC	11
Non-IC	8
Sex (n)	□
Male	10
Female	51

HIC, Hunner type interstitial cystitis; NHIC, non-Hunner type interstitial cystitis.

Table 2. Patient characteristics by cohort.

	Total	**HIC**	**NHIC**	***p*-value**
Age (years)	62.4 ± 10.0	61.2 ± 8.6	58.5 ± 9.8	0.2888
VAS	7.5 ± 2.1	8.2 ± 1.8	6.5 ± 2.2	0.0542
Maximum bladder capacity (ml)	436.4 ± 136.8	423.3 ± 131.0	486.4 ± 153.4	0.1573
Duration of symptoms (months)	38.5 ± 19.3	41.3 ± 20.0	27.6 ± 7.2	0.0311

HIC, Hunner-interstitial cystitis; NHIC, non-Hunner type interstitial cystitis.

Statistical analysis of pathological characteristics of IC/BPS tissue samples

A total of 28 patients, including 15 with Hunner-type IC, 9 with non-Hunner-type IC, and 4 with non-IC, were classified according to the histological characteristics of the bladder tissue using pathological slides. Pathological slides were evaluated by a pathologist. This information included mast cells, neutrophils, lymphoplasmacytic cells, fibrosis, and granulation tissue. Mast cells, neutrophils, and lymphoplasmacytic cells were classified into three grades based on the severity of the condition: mild, moderate, and severe. Fibrosis and granulation tissue were classified as present or absent (Table 3 and Figure 2A–E). Differences to mast cell severity were identified among patients with Hunner-type IC. All patients in the non-Hunner type IC and non-IC groups had mild involvement. In the Hunner-type IC group, 20.0% of the patients exhibited severe mast cell involvement, 66.7% demonstrated moderate involvement, and 13.3% displayed mild involvement (Table 3, Figure 2A). The severity of neutrophils was evaluated. Among patients with Hunner-type IC, 66.7% displayed severe involvement, 26.7% had moderate involvement, and 6.7% showed mild involvement. Among the patients with non-Hunner-type IC, 22.2% displayed severe involvement, 55.6% had moderate involvement, and 22.2% showed mild involvement. Among patients without IC, 88.9% exhibited moderate neutrophil involvement, whereas 11.1% had mild involvement. The severity of neutrophil infiltration

was markedly different between the non-IC, non-Hunner-type IC, and Hunner-type IC groups (Table 3 and Figure 2B). Regarding lymphoplasmacytic cell infiltration, 73.3% of the patients with Hunner-type IC exhibited severe involvement, 26.7% demonstrated moderate involvement, and none displayed mild involvement. In the non-Hunner-type IC group, 88.9% of the patients exhibited moderate involvement, 11.1% showed mild involvement, and none presented with severe involvement. In the non-IC group, 75.0% of the patients exhibited moderate involvement, 25.0% showed mild involvement, and none presented with severe involvement (Table 3, Figure 2C). In the Hunner-type IC group, 26.7% demonstrated fibrosis and 33.3% exhibited granulation tissue. By contrast, 11.1% of the patients in the non-Hunner type IC group demonstrated fibrosis, whereas none of the patients exhibited granulation tissue. No cases of fibrosis or granulation tissue were observed among the non-IC group (Table 3 and Figure 2D, E).

Table 3. Pathological slide review of IC/BPS tissue samples.

	Grade	Non-IC	NHIC	HIC
Mast cell	Mild	4	9	2
	Moderate	0	0	10
	Severe	0	0	3
Neutrophil	Mild	1	2	0
	Moderate	3	5	5
	Severe	0	2	10
Lymphoplasmacytic cell	Mild	1	1	0
	Moderate	3	8	4
	Severe	0	0	11
Fibrosis	Absent	4	8	11
	Present	0	1	4
Granulation tissue	Absent	4	9	10
	Present	0	0	5

IC/BPS, interstitial cystitis/bladder pain syndrome; non-IC, non-interstitial cystitis; NHIC, non-Hunner type interstitial cystitis.

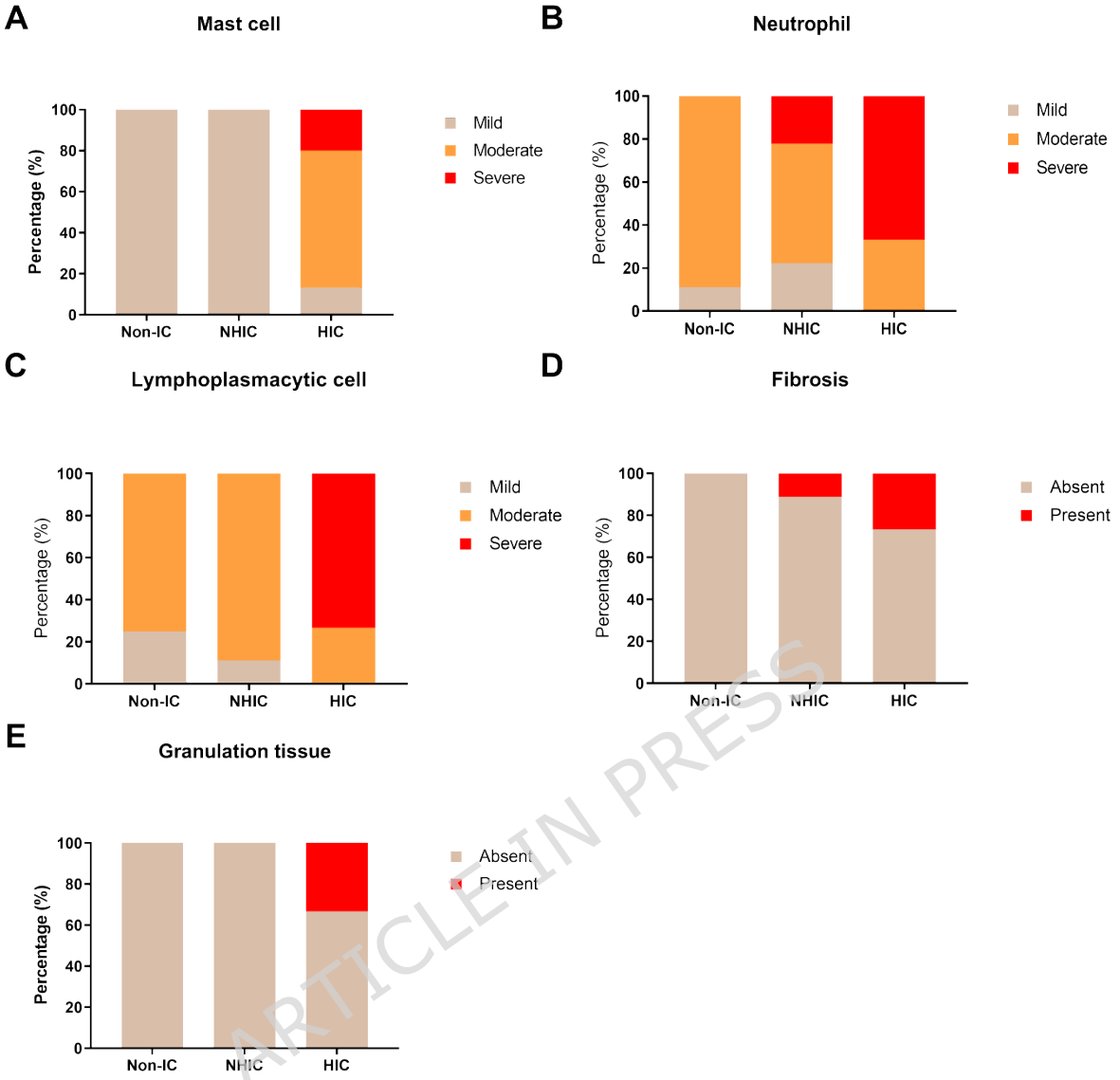

Figure 2. Statistical graphs of patient pathological features

(A) Comparative analysis of the severity of mast cells in the HIC, NHIC, and non-IC groups. (B) Comparative analysis of the severity of neutrophils in the HIC, NHIC, and non-IC groups. (C) Comparative analysis of the severity of lymphoplasmacytic cells in the HIC, NHIC, and non-IC groups. (D) Comparative analysis of bladder tissue from patients in the HIC, NHIC group, and non-IC groups, with and without fibrosis. (E) Comparative analysis of bladder tissue from patients in the HIC, NHIC, and non-IC groups, with and without granulation tissue.

Non-IC, non-interstitial cystitis; HIC, Hunner type interstitial cystitis; NHIC, non-Hunner type interstitial cystitis.

Analysis of mGluR family gene expression in IC/BPS

To assess the gene expression levels of the mGluR family (mGluR1–mGluR8), total RNA

was extracted from homogenized bladder tissues obtained from all 61 patients. RT-qPCR analysis demonstrated notable overexpression of mGluR2, mGluR3, and mGluR5 in IC/BPS tissue samples (n=53) compared to non-IC/BPS samples (n=8). The statistical significance of the observed differences was determined using a non-parametric two-tailed unpaired t-test, followed by the Mann–Whitney test (Figure 3A). Subsequently, the mGluR gene family was further analyzed by dividing the IC/BPS group into HIC and non-HIC based on the presence or absence of Hunner lesions. The HIC group had significantly higher mGluR5 expression levels than the non-IC group. The statistical significance of this finding was evaluated using a non-parametric two-tailed unpaired one-way ANOVA, followed by the Kruskal–Wallis test (Figure 3B).

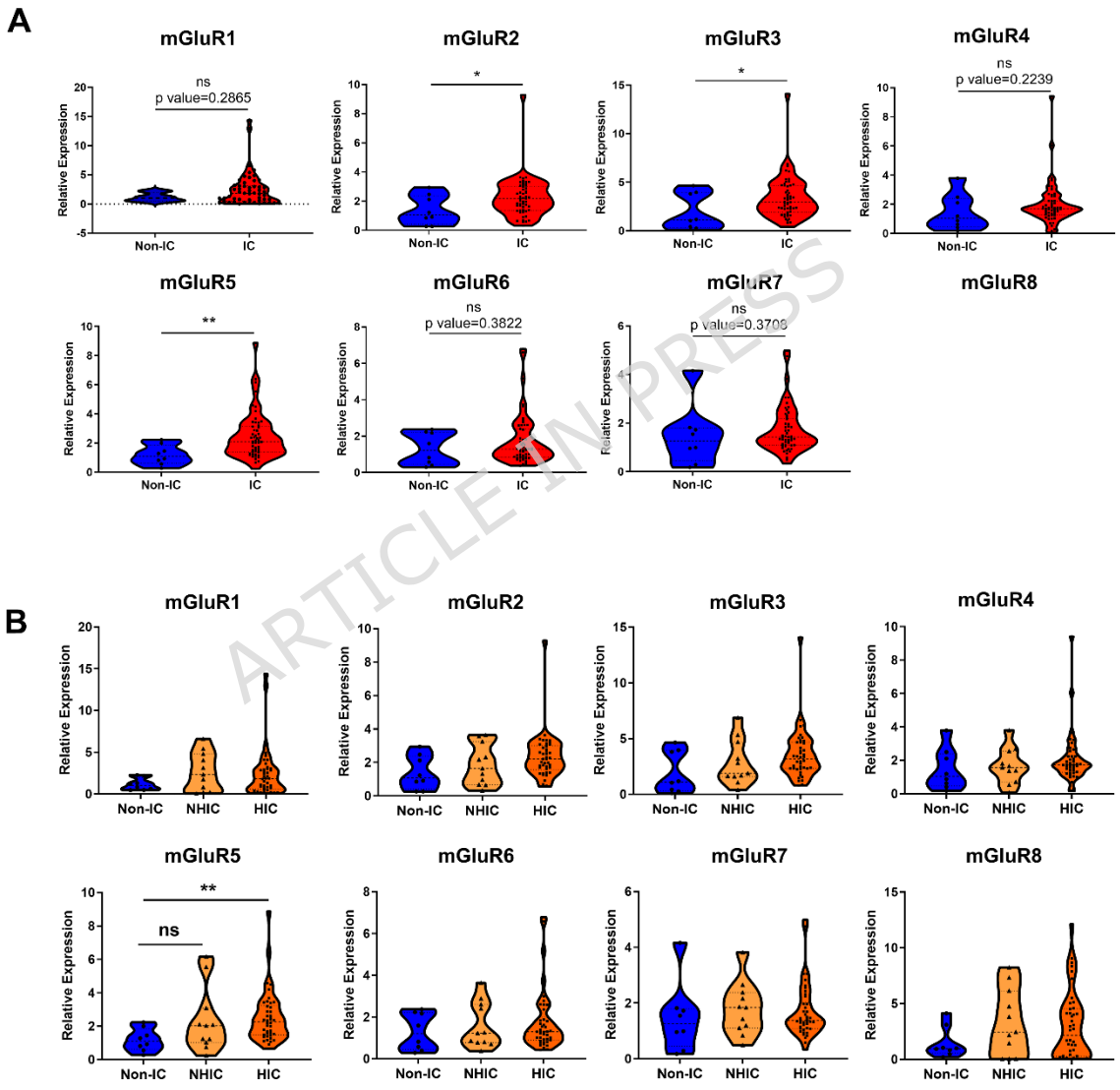

Figure 3. Comparative analysis of mGluR subtype gene expression in tissues from patients with IC/BPS.

(A) Comparative analysis of the gene expression of mGluR family (mGluR1-mGluR8) in the bladder tissues of the IC (n=53) and non-IC (n=8) groups. (B) Comparative analysis of the

gene expression of mGluR family in each subtype of IC (HIC, n=42; NHIC, n=11) and non-IC (n=8). *$p \leq 0.05$, **$p \leq 0.01$, and ns; not significant.

Association of mGluR family gene expression with VAS scores in patients with IC/BPS

Gene expression levels of mGluR family were dichotomized based on the VAS scores of all patients (n=61). The VAS scores were dichotomized into two groups: VAS 2–6 (n=28) and VAS 7–10 (n=25). Significant mGluR5 overexpression was observed across all VAS groups ($p < 0.05$). The statistical significance of the observed differences was determined using a non-parametric two-tailed unpaired one-way ANOVA, followed by the Kruskal–Wallis test (Figure 4A). The correlation between mGluR5 expression levels and VAS scores was further evaluated using bladder tissue samples from 53 patients with IC/BPS and eight controls. The correlation between mGluR5 expression levels and VAS scores was further evaluated. While mGluR5 expression was significantly elevated in IC/BPS patients compared to non-IC controls (Figure 4B), no significant linear correlation was observed between mGluR5 expression and VAS scores within the IC/BPS patient group ($R = 0.0767$, $p = 0.5850$; Figure 4B). This suggests that while mGluR5 is a potent diagnostic marker for identifying IC/BPS, its expression levels do not proportionally reflect the subjective pain intensity within the diseased population.

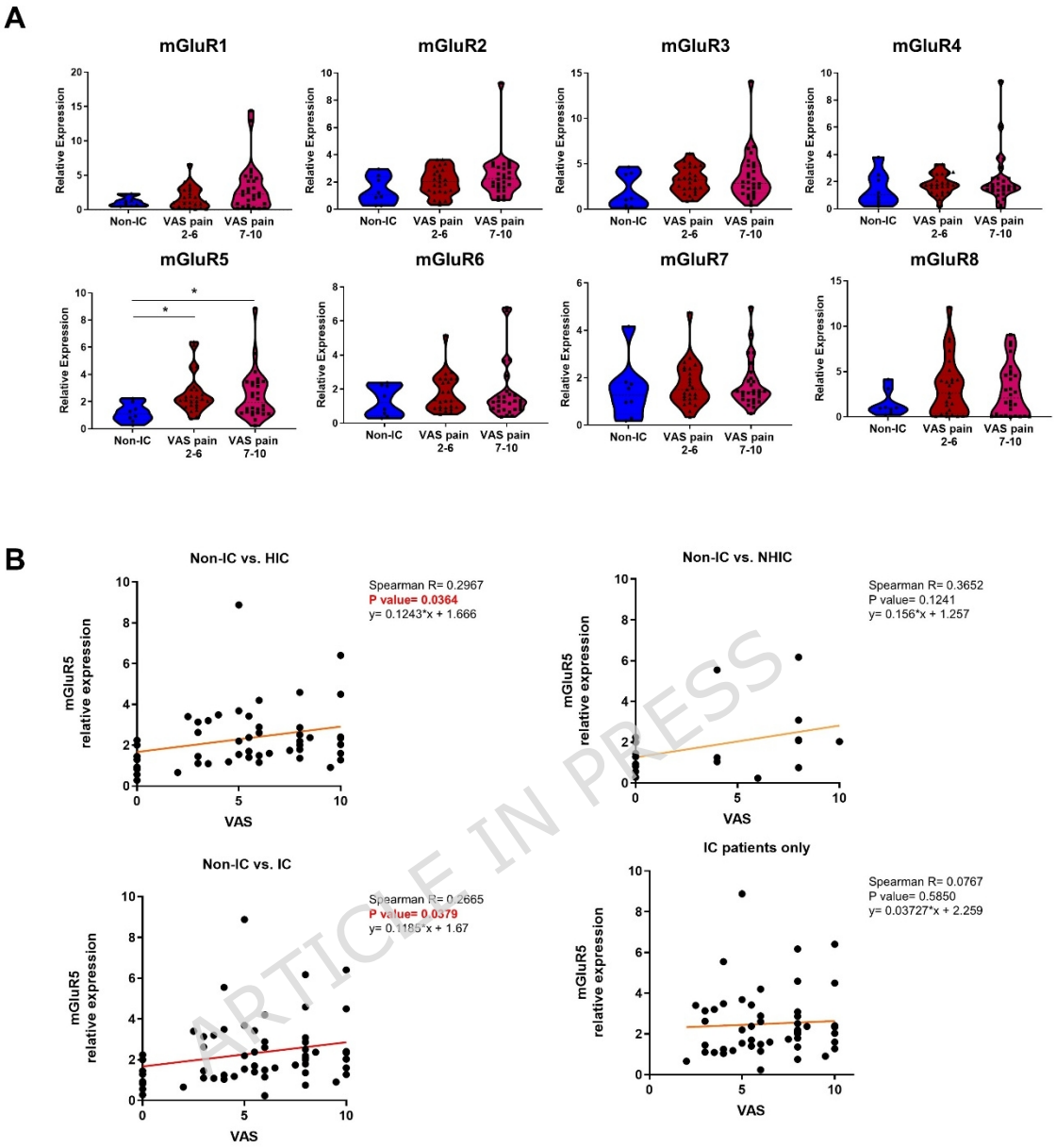

Figure 4. Comparative analysis of the correlation between VAS scores and gene expressions of mGluR subtypes in patients with IC/BPS.

(A) Comparative analysis of the gene expression of mGluR family by VAS score compared to the non-IC group. VAS scores were dichotomized into two groups: VAS 2–6 (n=28) and VAS 7–10 (n=25). (B) Correlation between mGluR5 gene expression and VAS score in the non-IC (n=8), HIC (n=42), and NHIC (n=11) groups. *$p \leq 0.05$.

VAS, visual analog scale; mGluR, metabotropic glutamate receptor; IC/BPS, interstitial cystitis/bladder pain syndrome; NHIC, non-Hunner type interstitial cystitis; HIC, Hunner-type interstitial cystitis; non-IC, non-interstitial cystitis.

Analysis of the correlation between mGluR family gene expression and histopathological characteristics in patients with IC/BPS

We observed whether the gene expression levels of the mGluR family (mGluR1–mGluR8) changed according to the histopathological characteristics revealed on the pathological slides of the patients. Pathological slides for histopathological analysis and bladder tissue RNA for gene expression observation were extracted from the bladder tissues of 28 patients. The histopathological characteristics of the bladder slides were analyzed through the bladder tissue staining, with the slides divided into three categories based on the presence or absence of IC: non-IC, NHIC, and HIC (Figure 5A). HIC patients exhibited significant fibrosis and lymphoplasmacytic inflammation (Figure 5B, C). We compared and analyzed the correlation between the histopathological characteristics of the patients and gene expression of mGluR subtypes. The mGluR5 expression level in patient tissue slides was significantly higher in the group with fibrosis than in the group without fibrosis. No significant differences in gene expression changes were observed according to the presence or absence of fibrosis in the other mGluR subtypes (Figure 6A). Thus, fibrosis developed in the bladder wall of patients with high mGluR5 expression levels owing to excessive collagen accumulation caused by an increased immune response. We investigated the changes in mGluR family gene expression according to lymphoplasmacytic cell infiltration levels. We confirmed that mGluR5 expression significantly increased in patients with severe lymphoplasmacytic cell infiltration. In the other mGluR subtypes, no correlation was observed between changes in gene expression and degree of infiltration (Figure 6B). Thus, increased mGluR5 expression in patients with IC increased immune cell infiltration. Additionally, we investigated the expression of mGluR family genes according to histopathological characteristics, including granulation tissue, mast cells, and neutrophils. HIC patients exhibited significant granulation tissue formation, mast cell, and neutrophilic margination levels (Supplementary Figure 1). When comparing mGluR gene expression according to the presence or absence of granulation tissue, mGluR5 and mGluR7 expressions were significantly increased in the patient group with granulation tissue (Supplementary Figure 2A). No change in mGluR family gene expression according to mast cell levels was observed (Supplementary Figure 2B). mGluR2 expression increased as neutrophil levels increased (Supplementary Figure 2C). The statistical significance of the observed differences was determined using a non-parametric two-tailed unpaired t-test, followed by the Mann–Whitney U test, and a non-parametric two-tailed unpaired one-way ANOVA, followed by the Kruskal–Wallis test.

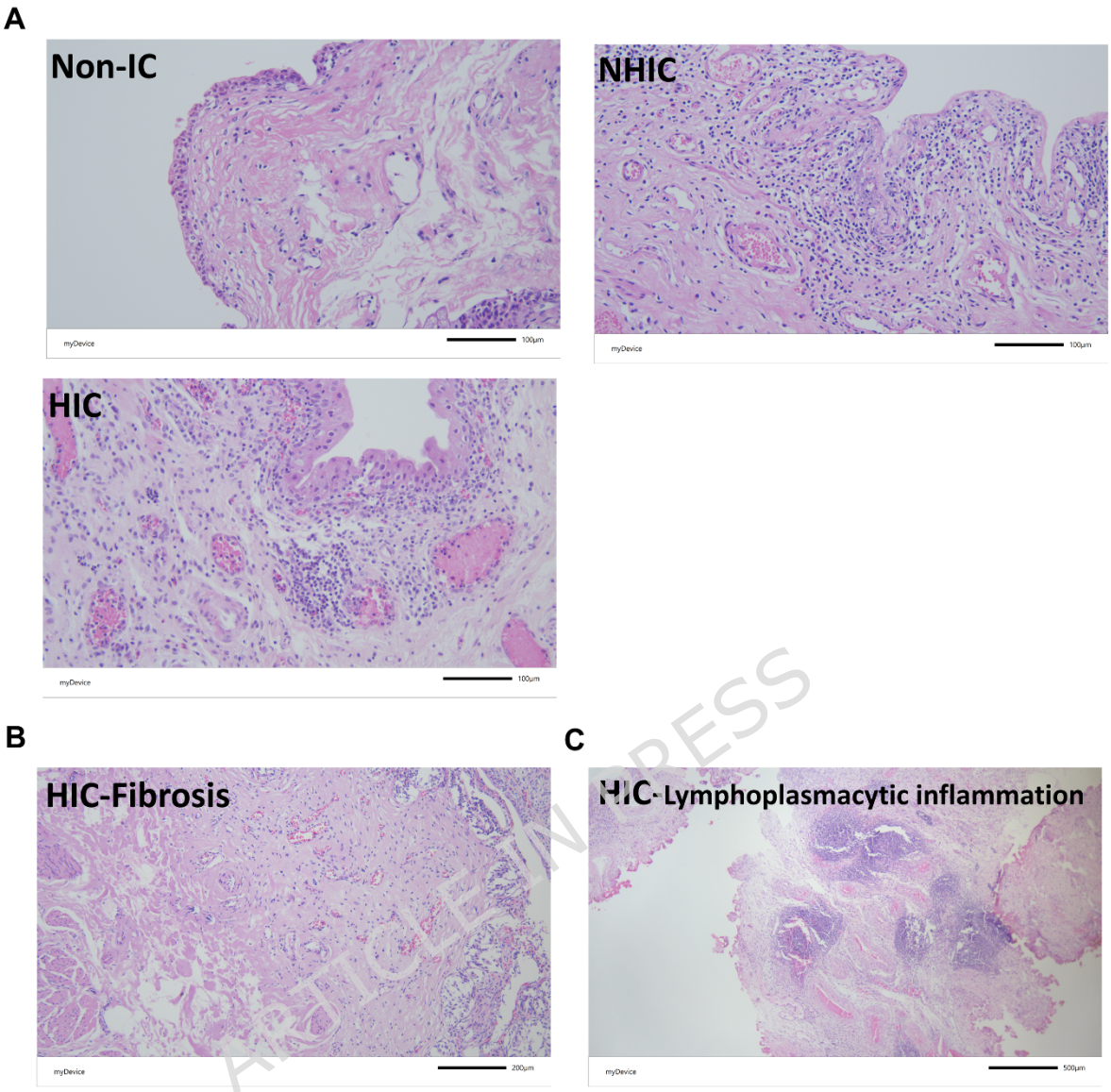

Figure 5. Histopathological characteristics of bladder tissue in Non-IC, NHIC, and HIC

(A) Representative images of H&E staining of bladder tissues are shown. Compared to non-IC, NHIC, and HIC, NHIC and HIC exhibit increased inflammatory cell infiltration. (B) Marked fibrosis is observed in HIC patients, characterized by thickened fibrous tissue and decreased cellular density. (C) Histological findings showing marked lymphoplasmacytic inflammation in HIC patients, with lymphocytes and plasma cell clusters in the lamina propria and submucosa. Scale bars are indicated in each panel.

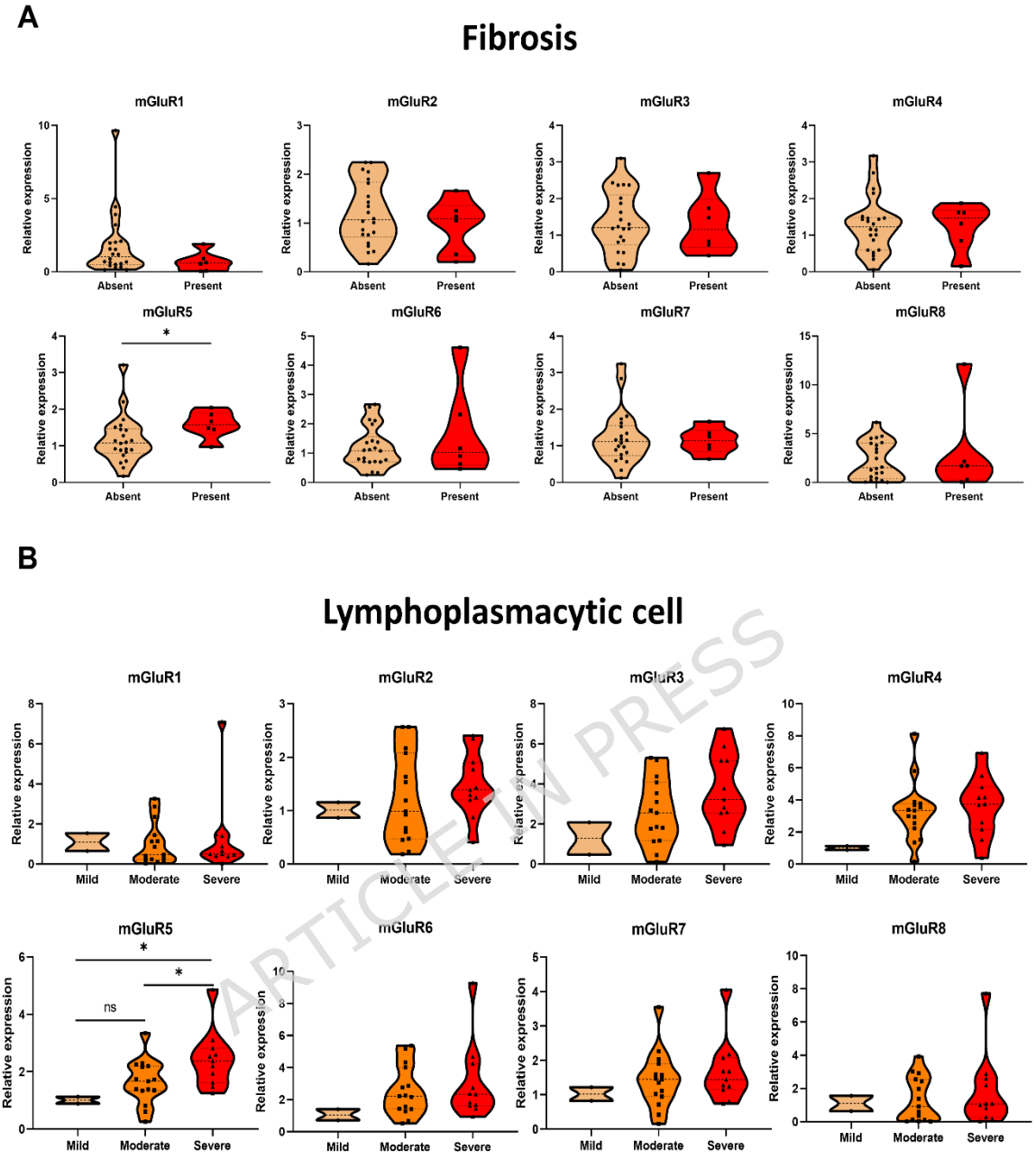

Figure 6. Comparative analysis of the correlation between patient histopathological features (fibrosis and lymphoplasmacytic cell) and gene expression of mGluR subtypes

(A) Comparative analysis of the gene expression of mGluR family by presence or absence of fibrosis on patient tissue samples (n=28). The patient tissue samples were dichotomized into absent (n=23) and present (n=5). (B) Correlation between mGluR family gene expression and lymphoplasmacytic cell infiltration levels. The patient tissue samples were categorized as mild (n=2), moderate (n=15), or severe (n=11). *$p \leq 0.05$.

Analysis of p65 and pro-inflammatory cytokine IL-1β expression in patients with

IC/BPS

mGluR5 is associated with NF-kB in neuronal cells[23,24]. We examined p65 gene expression in a patient group to determine whether the high mGluR5 expression in patients with IC/BPS was associated with NF-kB signaling. A total of 28 patients were analyzed for gene expression, including 4 patients with non-IC, 9 with NHIC, and 15 with HIC. p65 expression was significantly increased in patients with HIC. In addition, the expression of IL-1β, a pro-inflammatory cytokine, was significantly increased in patients with HIC (Figure 7A). To confirm whether mGluR5 expression induced inflammation by regulating NF-kB expression in patients with IC/BPS, IL-1β expression was confirmed when mGluR5 activation was regulated by treating human urothelial cells with mGluR5 agonists and antagonists. SV-HUC-1 cells were treated with CHPG (mGluR5 selective agonist) and MPEP (a potent mGluR5 antagonist) for 2 h to confirm the changes in cellular gene expression. When CHPG was treated, IL-1β expression significantly increased compared to the control cells, and when MPEP was additionally treated, expression decreased again (Figure 7B). The same result was observed when cells were treated with CHPG and MPEP for an extended period, and changes in expression were observed after 4 h (Supplementary Figure 3). Thus, when mGluR5 expression increased, the expression of inflammatory cytokines, such as IL-1β, increased through NF-kB signaling, which could induce an immune response (Figure 7C).

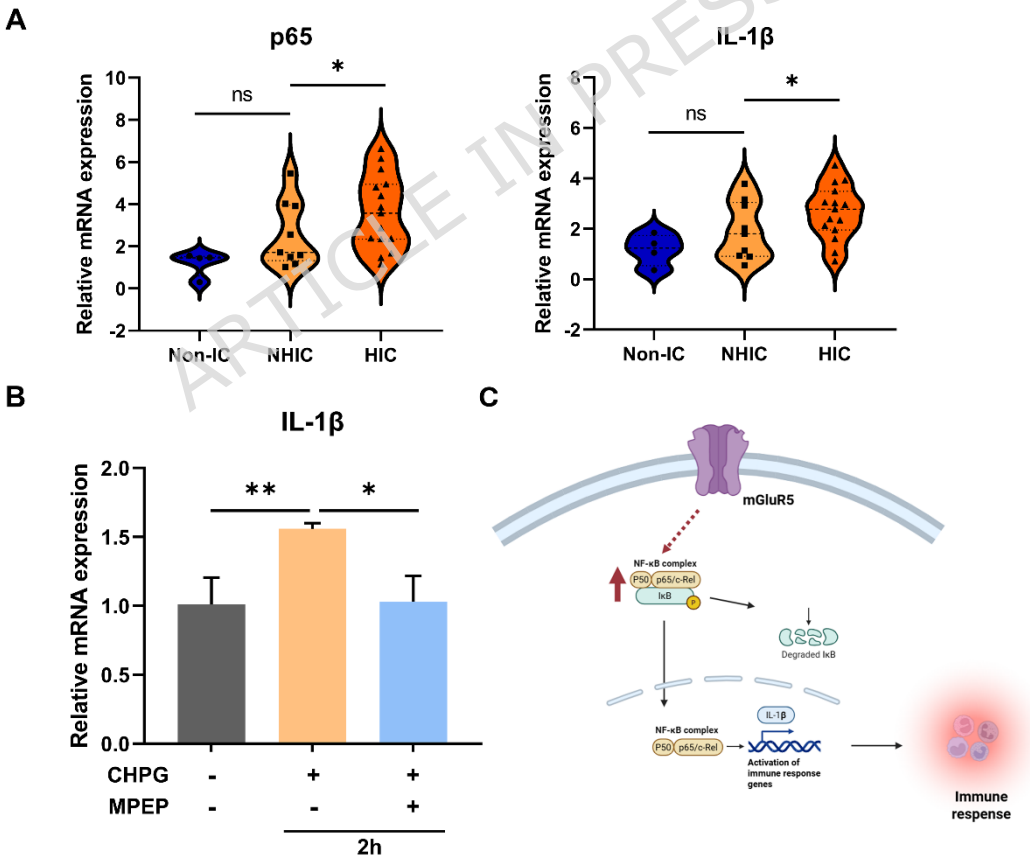

Figure 7. Analysis of inflammatory cytokine expression changes according to NF-kB level in patients with IC

(A) Comparative analysis of the gene expression of part of NF-kB signaling in tissue

samples of patients with IC (n=28). The patient tissue samples were categorized as non-IC (n=4), NHIC (n=9), or HIC (n=15). (B) Analysis of IL-1β expression according to mGluR5 activation/inhibition for 2 h in human urothelial cells. (CHPG, mGluR5 selective agonist; MPEP, potent mGluR5 antagonist) (Created with BioRender.com) *p ≤0.05 and **p ≤0.01. (C) Schematic diagram of the pathway predicted to elicit an immune response in patients with IC.

Discussion

This study aimed to investigate the clinical implications of the mGluR family in the bladder tissue of patients with IC/BPS. By conducting gene expression and correlation analyses with clinical parameters, we elucidated the potential influences and pathogenesis of patients with IC/BPS. This study confirmed that the mGluR2, mGluR3, and mGluR5 genes in the mGluR family were significantly overexpressed in patients with IC/BPS compared to those in patients without IC/BPS. In addition, we observed a significant upregulation of Group II mGluRs (mGluR2 and mGluR3) alongside mGluR5. Unlike mGluR5, which is typically associated with pro-nociceptive and excitatory signaling, Group II mGluRs are known to exert inhibitory modulatory roles by suppressing excessive glutamate release. The concurrent increase of mGluR2 and mGluR3 in the IC/BPS bladder may represent a compensatory homeostatic response aimed at mitigating chronic neuroinflammation and pain amplification. However, the predominance of pain symptoms in our patients suggests that this inhibitory potential might be insufficient to overcome the pathological effects of mGluR5-mediated pathways. Further functional studies are needed to elucidate the precise interplay between these receptor groups in the context of IC/BPS. Moreover, mGluR5 expression was significantly increased in patients with HIC. mGluR5 overexpression was significantly associated with lymphoplasmacytic infiltration, fibrosis, and pain scores (VAS). Thus, mGluR5 may promote immune cell influx and a chronic inflammatory response, which may contribute to structural changes in the bladder wall and pain aggravation. The positive correlation between mGluR5 expression and the pain score (VAS) suggests the involvement of mGluR5 in pain signal amplification and nervous system hypersensitivity. In fact, we confirmed that inflammatory cytokine (IL-1β) expression was reduced when treated with mGluR5 selective antagonist (MPEP), indicating that mGluR5 is correlated with inflammatory response through NF-κB pathway. This trend was particularly evident only in Hunner lesions, emphasizing the need for customized treatment for each patient subtype. Therefore, mGluR5 expression may be useful in distinguishing between Hunner types and non-Hunner types, and may be utilized as an auxiliary diagnostic tool for invasive cystoscopy. In addition to conventional cystoscopic lesion resection, neuroimmune inflammation control via administration of an mGluR5 antagonist may be a new treatment option. Combination therapy through simultaneous regulation of inflammatory cytokines, such as IL-1β, together with mGluR5 may have a synergistic effect on improving fibrosis and pain.

Our study integrated clinical, pathological, and molecular biology findings with the clinical implications of the mGluR family in IC/BPS. The differential expression of mGluRs, particularly mGluR5, suggests their involvement in the development, symptoms, and pain modulation in IC/BPS. These findings provide a foundation for further investigation into the underlying mechanisms and potential therapeutic targets of mGluRs in IC/BPS. However, this study has some limitations. The sample of patients with pathological findings was relatively small and comprised 28 individuals with IC/BPS. Despite efforts to ensure the

representativeness of the sample, a larger cohort would enhance the generalizability of the findings and provide more robust statistical power. Further research with a larger sample size is required to investigate the association between mGluRs and IC/BPS, particularly in relation to Hunner's lesions. Moreover, our study primarily focused on the expression patterns of mGluR subtypes in bladder tissue and their correlation with clinical parameters. Although this approach yielded valuable insights into the potential clinical impact of mGluRs in IC/BPS, analyzing gene expression alone could not comprehensively elucidate the functional consequences and downstream signaling pathways. Moreover, the causal relationship between elevated mGluR expression and pain in patients with IC/BPS requires further investigation. Further studies, including functional analyses and animal models, will facilitate the demonstration of the specific role of mGluRs in the pathophysiology of IC/BPS. Additional analyses, such as immunohistochemical analyses based on the intracellular localization of mGluR5 in neurons or immune cells, are necessary. Moreover, tracking the changes in mGluR5 expression before and after treatment to evaluate its potential as a prognostic factor would be helpful in future research.

Nevertheless, this study makes a valuable contribution to research on the clinical implications of the mGluR family in IC/BPS. By identifying mGluRs as potential therapeutic targets and highlighting their association with clinical parameters, this study provides a foundation for further research and development of targeted interventions to enhance the management of IC/BPS and related pain conditions.

Conclusions

The present study provides evidence for the clinical implications of the mGluR family in the bladder tissue of patients with IC/BPS. The overexpression of mGluR2, mGluR3, and especially mGluR5 suggests their involvement in the pathogenesis, symptoms, and pain modulation of IC/BPS. Furthermore, this study identified new biomarkers and potential targets for IC/BPS, highlighting the potential of pain-modulating drugs targeting mGluR5. The mGluR5 overexpression observed in the IC/BPS bladder tissue and the correlation between mGluR5 expression levels and pain scores provide a rationale for further investigation of the therapeutic potential of mGluR5 modulation in the management of IC-related pain. Precision therapeutic strategies targeting the mGluR5 signaling pathway could is potentially linked to a paradigm shift from conventional symptom alleviation-focused treatments to pathogenesis-based treatments.

Materials and methods

Clinical information

IC/BPS was diagnosed according to the American Urological Association criteria. Patient symptoms were assessed using the Pelvic Pain and Urgency/Frequency Patient Symptom Scale, O’Leary–Sant Symptom and Problem Index, and visual analog score pain questionnaire (VAS). IC/BPS subtypes were categorized according to physical examination, urine culture, cytology, and cystoscopy. Patients with an overactive bladder, urinary tract infection, urinary tuberculosis, bladder cancer, or other neurological diseases were excluded. Urinary frequency and maximal bladder capacity were performed in 3- day voiding diaries.

Tissue acquisition

This study was approved by the Institutional Review Board of Konkuk Medical Center (KUMC 2020-12-052 and KUMC 2022-04-003) and was performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all enrolled patients. Patients were classified as having Hunner-type IC or non-Hunner-type IC based on the cystoscopic findings. Hunner's lesions are characterized by a reddened mucosal area with small blood vessels radiating toward the central ulceration (Figure 8). Bladder tissue was obtained by isolating a portion (0.5–1 g) of the tissue specimen obtained following the cystoscopic removal of Hunner's lesions. During the procedure, resection was performed using TUR. Therefore, we were able to obtain tissue samples up to the superficial portion of the muscle layer for analysis. The selection criteria for the study participants were as follows: patients hospitalized for surgery due to IC/BPS were selected as the experimental group, and patients hospitalized for surgery due to urolithiasis or urinary incontinence and confirmed as not having IC/BPS by cystoscopic examination comprised the control group (non-IC). Patients who were not diagnosed with bladder cancer after endoscopic surgery owing to suspicious findings of bladder cancer were excluded. A pathologist examined and graded the patient population using a blinded method.

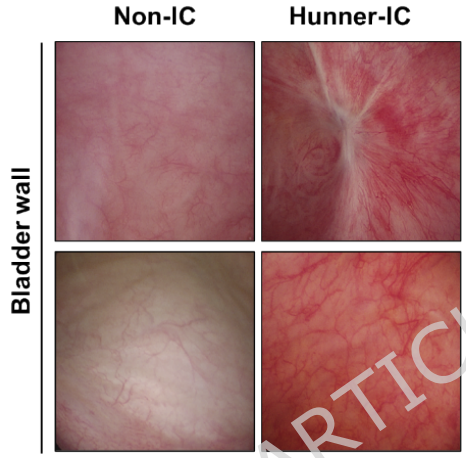

Figure 8. Cystoscopic images of bladder wall in patients with non-IC and Hunner-type IC.

Patients with IC/BPS have reddened mucosal areas with small blood vessels radiating toward the central ulceration. IC, interstitial cystitis.

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of bladder tissue

Freshly obtained total bladder tissue samples from all the 61 patients were homogenized using an MT-13K mini handheld homogenizer (Hangzhou Miu Instruments Co., Ltd., Hangzhou City, China). Homogenized tissues were lysed using LaboPass™ Labozol reagent (Cosmo Genetech, Seoul, Republic of Korea). Total RNA was isolated using LaboZol according to the manufacturer's instructions. The total RNA concentration was determined using a nanophotometer (IMPLEN, Munich, Germany). Subsequently, 1 µg of total RNA was reverse-transcribed into complementary DNA (cDNA) using an oligo dT primer and a LaboPass™ M-MuLV reverse transcriptase kit (Cosmo Genetech, Seoul, Republic of Korea),

following the manufacturer's instructions. The relative mRNA expression levels of target genes between the control and IC groups were quantified using the Real-Time PCR 2× Master Mix (SYBR green, ROX) (ELPISBIO, Daejeon, Republic of Korea) and a Quantstudio3 Real-Time PCR instrument (Applied Biosystems, Waltham, Massachusetts, MA, US). Human-specific primers used in the present study are listed in Table 4.

Table 4. Real-time polymerase chain reaction primers

Species	Target	Forward primer	Reverse primer
Human	*GRM1*	5′-CAGCCGATTCGCTTTAGCC-3′	5′-GGGATCGCGGTACTGAAGTTG-3′
	GRM2	5′-CTATGGCGAGACAGGCATTGA-3′	5′-CATCCTCAGAACGGGTGAACA-3′
	GRM3	5′-GCACCTCAACAGGTTCAGTGT-3′	5′-TGGTGGAGTCGAGGACTTCC-3′
	GRM4	5′-GACAACAGCCGCTACGACTT-3′	5′-GAGGCCACTGTGGACACAT-3′
	GRM5	5′-AATCTCCCGATGTCAAGTGGT-3′	5′-AGGGTTTCGGTGGTTTGTTTC-3′
	GRM6	5′-CCACACAGCGTGATTGACTAT-3′	5′-GCAGCCGATGAGAGACAGAT-3′
	GRM7	5′-GGCTGGAAGCGATGCTCTAC-3′	5′-TGTTCGAGCGCGTAAGTGTC-3′
	GRM8	5′-CCAGAGCTAAGTGATAACACCAG-3′	5′-TCTGTGACTGAGCAATGCAAA-3′
	RELA(p65)	5′-ACG ATC TGT TTC CCC TCA TCT-3′	5′-TGG GTG CGT CTT AGT GGT ATC-3′
	IL-1β	5′-CCA CAG ACC TTC CAG GAG AAT G-3′	5′-GTG CAG TTC AGT GAT CGT ACA GG-3′
	GAPDH	5′-GTCTCCTCTGACTTCAACAGCG-3′	5′-ACCACCCTGTTGCTGTAGCCAA-3′

Pathological analysis

Immediately after the collection of bladder biopsy samples from the patients, specimens were prepared for pathological analysis. Those designated for this purpose were fixed in 10% formalin buffer, embedded in paraffin, and sectioned into 4-μm slices. Subsequently, Hematoxylin and eosin (H&E) staining procedures were performed in a sequential manner. Inflammation, ulceration, fibrosis, mast cell counts were evaluated using H&E-stained slides. The presence or absence of ulceration, granulation tissue, and fibrosis in the subepithelial connective tissue was also noted. The degree of inflammatory cell infiltration (mast cells, neutrophils, and lymphoplasmacytic cells) was semi-quantitatively graded as mild, moderate, or severe. First, the entire tissue slide was scanned at low magnification to identify 'hot spots' representing the areas of highest cellular density. Subsequently, the infiltration intensity was evaluated under high-power field (HPF; ×400 magnification) within these selected areas. The grading criteria were defined as follows: 'mild' for scattered or focal infiltration; 'moderate' for intermediate density without aggregation; and 'severe' for diffuse, dense infiltration or the presence of distinct cell clusters. The grading criteria in the present study is listed in Table 5.

Table 5. Histological grading criteria for inflammatory cell infiltration in bladder tissue

	Mast cells	Neutrophils	Lymphoplasmacytic cells
Mild	<5/HPF	<10/HPF	<5/HPF
Moderate	5~30/HPF	10~50/HPF	5~30/HPF
Severe	>30/HPF	>50/HPF	>30/HPF

*HPF: high-power field (400x)

Cell culture

SV-HUC-1 human urothelial cells (CRL-9520, ATCC) were obtained from the American Type Culture Collection (Manassas, Virginia, VA, USA). The cells were cultured in F-12 K nutrient mixture medium (21127-022, Gibco™, Thermo Fisher Scientific, Waltham, Massachusetts, MA, USA) supplemented with 10% fetal bovine serum (Gibco™) and 1% penicillin–streptomycin (Gibco™), respectively, in a humidified incubator with an atmosphere of 5% CO_2 at 37 ºC.

Drug treatments on cells

To observe changes in response to stimulation or blocking of mGluR5 in SV-HUC-1 cells, the following reagents were used: (RS)-2-Chloro-5-hydroxyphenylglycine (CHPG, Tocris Biosciences, Ellisville, MO, USA) or 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP) hydrochloride (Tocris Biosciences, Ellisville, MO, USA). SV-HUC-1 cells were seeded in 6-well plates at a density of 1×10^5 cells/well. Once cell confluency reached 80%, each group was treated with phosphate-buffered saline, 200 μM CHPG or 5 μM MPEP. The cells were harvested and analyzed after 2 and 4 h.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software, version 9.5.0 (GraphPad Software, LLC, https://www.graphpad.com). Data are presented as means and standard errors of the mean. To determine two statistically significant groups, *p*-values were calculated using the non-parametric t-test, followed by the Mann–Whitney U test. To determine three statistically significant groups, *p*-values were calculated using a non-parametric one-way analysis of variance (ANOVA), followed by the Kruskal–Wallis test. Significance was set at p = 0.05 (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ns; not significant).

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Author’s contributions

Conception and design: Yeonjoo Kwak, Moonjung Lee, and Aram Kim

Data analysis and interpretation: Yeonjoo Kwak, Bohyn Kim, Jaekwon Seok, and Moonjung Lee

Data acquisition: Yeonjoo Kwak, Bohyun Kim, Jaekwon Seok, Ssang-Goo Cho, Sehwan Kim, and Hana Yoon

Drafting the manuscript: Yeonjoo Kwak, Jaekwon Seok, Moonjung Lee, and Aram Kim

Statistical analysis: Yeonjoo Kwak, Bohyun Kim, and Moonjung Lee

Data Processing: Yeonjoo Kwak, Bohyun Kim, Jaekwon Seok, and Aram Kim

Supervision: Aram Kim

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Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Konkuk Medical Center (KUMC 2020-12-052 and KUMC 2022-04-003). Informed consent was obtained from all participants prior to sample acquisition and retrieval of their health information.

Competing interests

The authors declare that they have no competing interests.

Data Availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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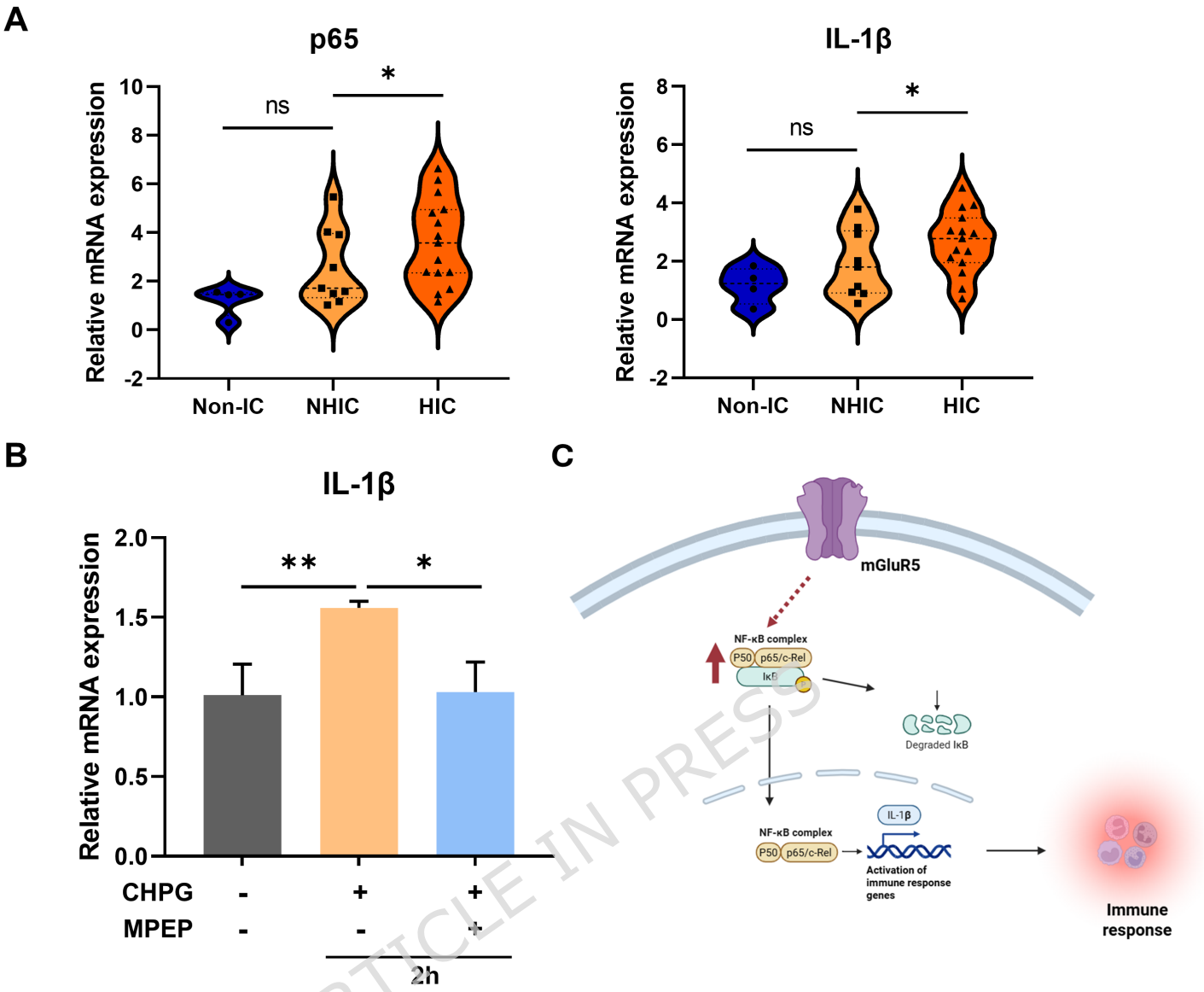

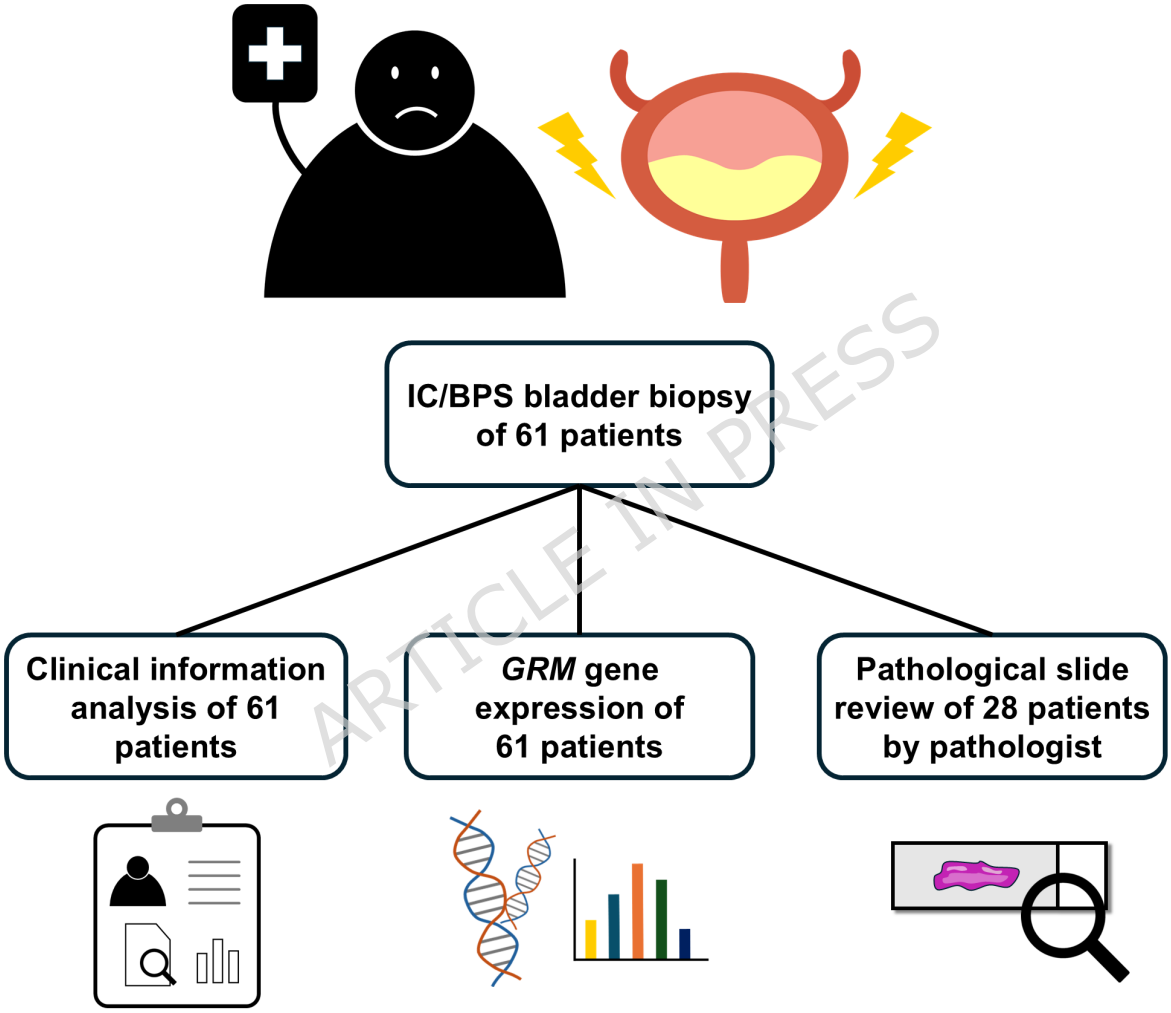

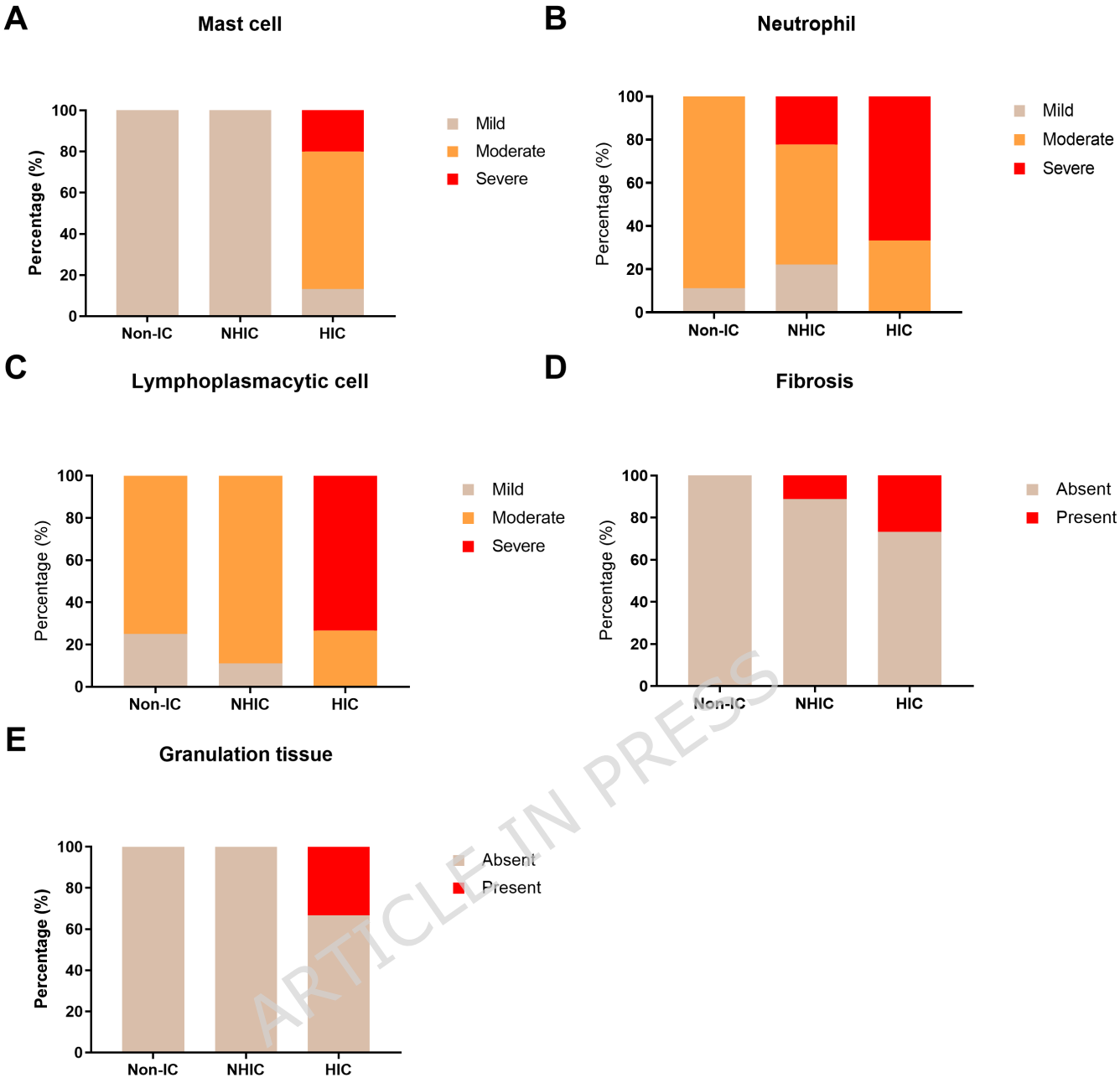

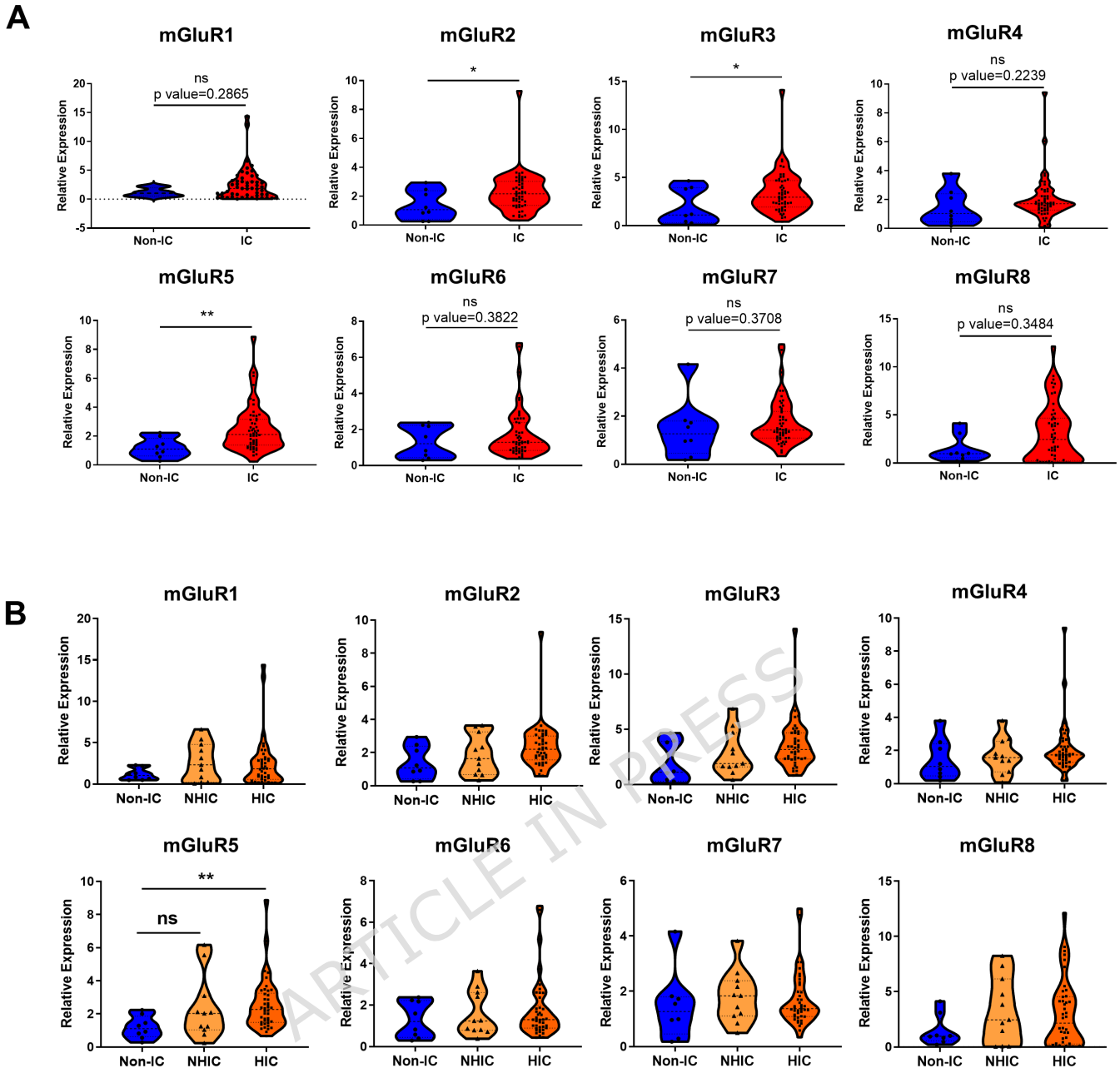

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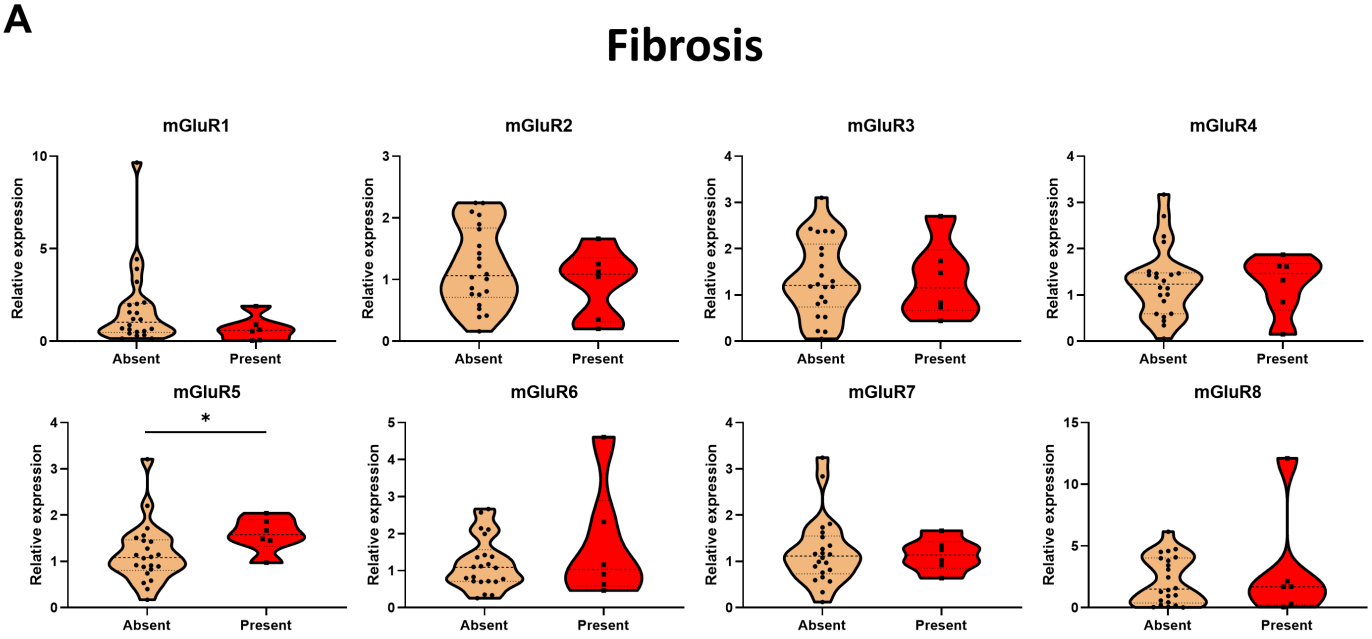

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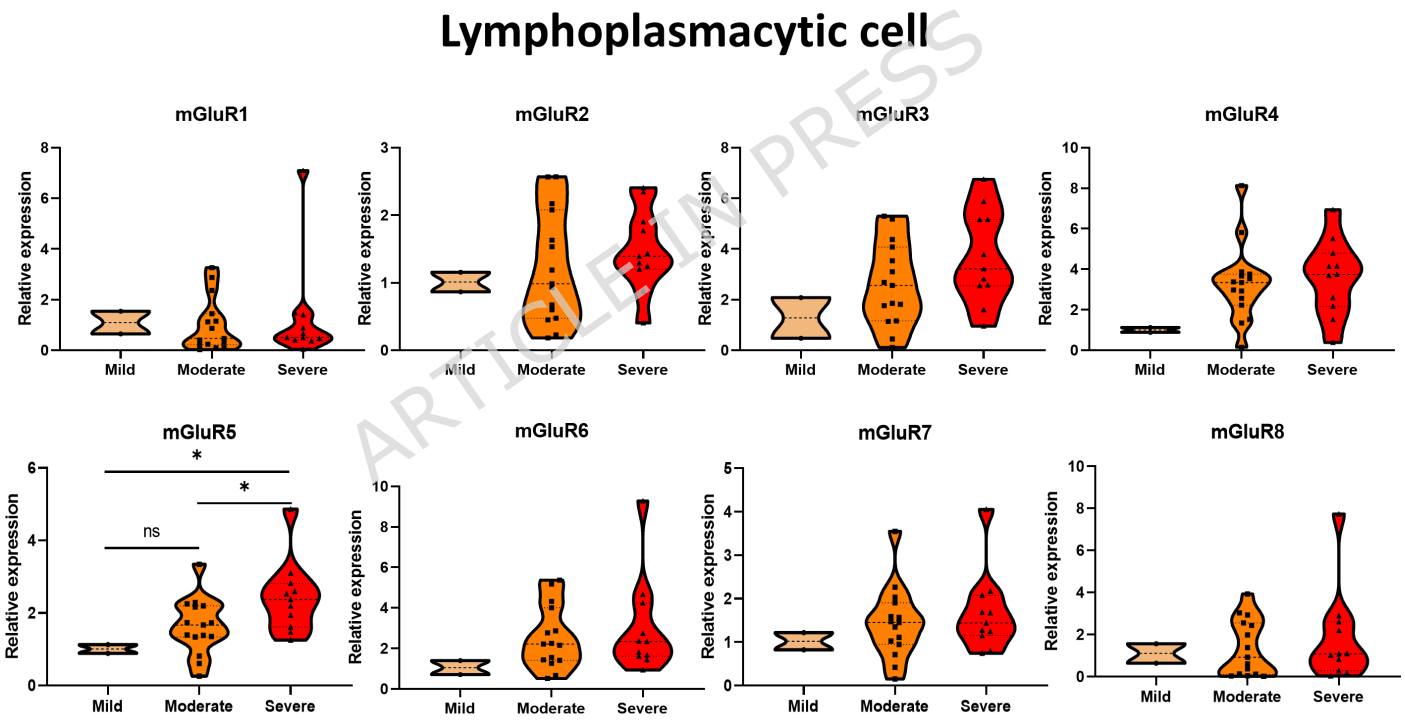

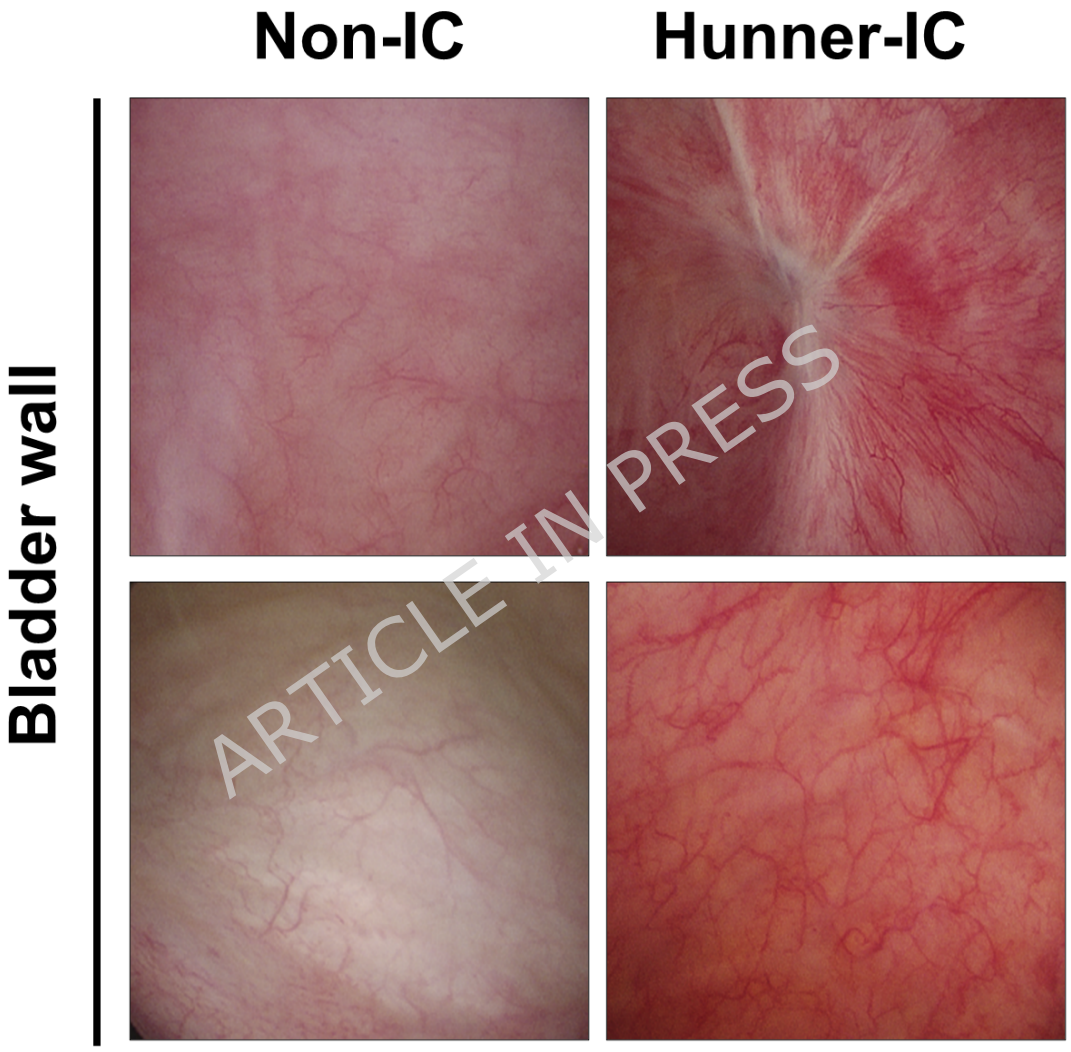

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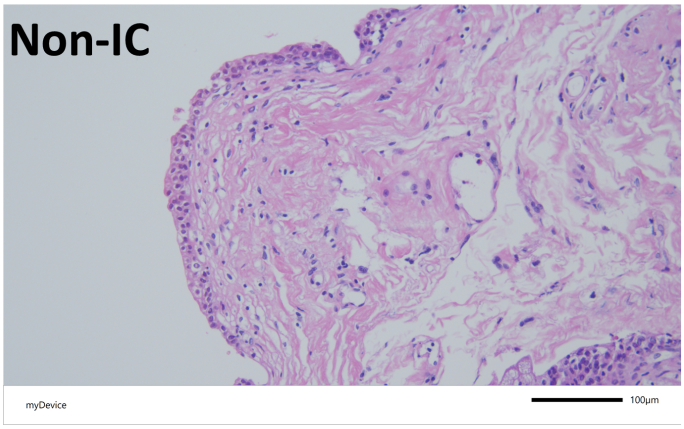

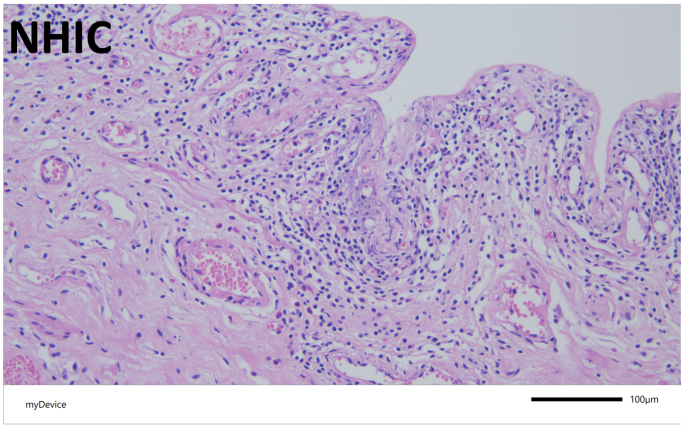

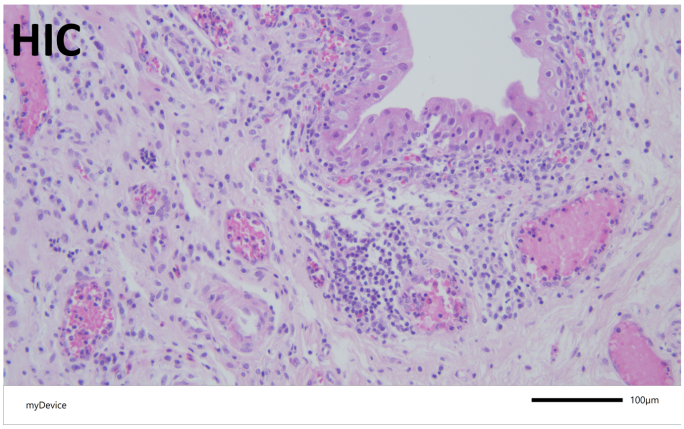

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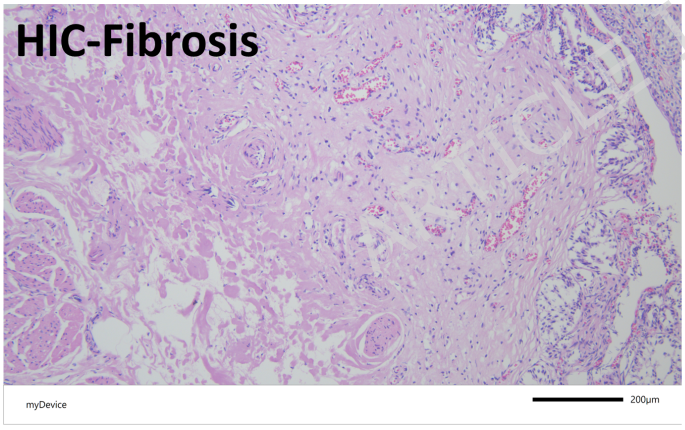

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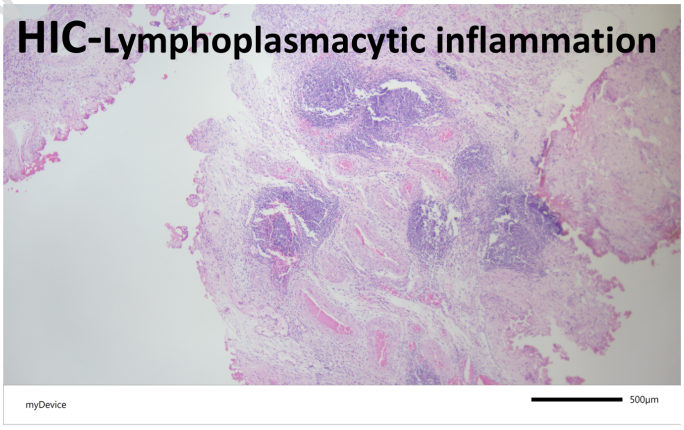

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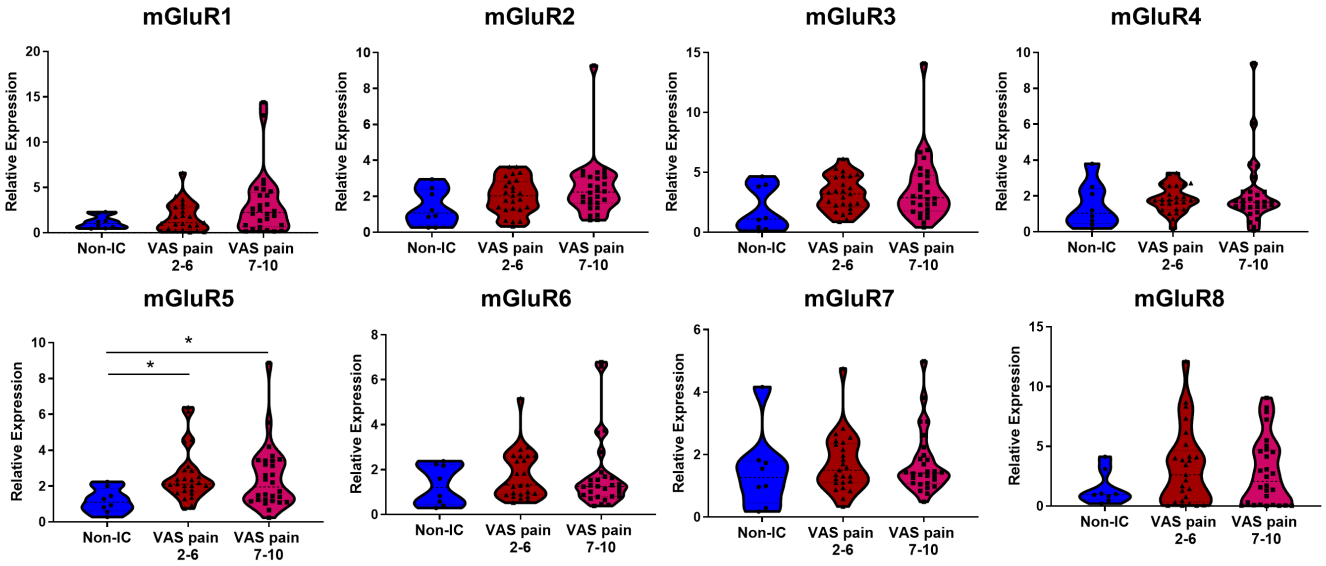

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