in vivo *38*: 205-212 (2024)
doi:10.21873/invivo.13427

CCL2- and Notch2-mediated Central Sensitization in a Rat Chronic Pelvic Pain Model

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Abstract. *Background/Aim: Chronic pelvic pain (CPP) is a common gynecological condition in women with multifactorial etiology. Some studies have revealed that patients with CPP have the same structural and functional changes in the pain matrix in the brain to patients with other types of chronic pain. However, the relationship between localized pelvic pain and changes in the structure and function of the central nervous system is still unclear. Materials and Methods: In this study, a rat model of CPP was established by pelvic nerve ligation and behavioral tests were used to validate the model. Afterwards, we compared the expression of CCL2 in CPP and control rats and observed the changes in their behavioral patterns by blocking the expression of CCL2 in the former group. In addition, we upregulated the expression of CCL2 in human microglia cells (HMC3) to further observe the effect of CCL2 on the Notch2 pathway. Results: Our results showed that the expression of chemokine ligand 2 (CCL2) in the serum exosomes, pelvic vascular endothelial cells, and cerebrospinal fluid was higher in the CPP group than the control group ($p<0.05$). In HMC3 treated with recombinant CCL2 protein, a significant increase in the mRNA and protein expression of Notch2 was observed. Conclusion: CCL2 can activate the Notch2 signaling pathway and plays an important role in the central sensitization of chronic pelvic pain.*

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Key Words: Chronic pelvic pain, chemokine ligand 2, Notch2, signaling pathway.

Pelvic pain is one of common gynecological diseases and can be clinically classified into acute and chronic pelvic pain (CPP), according to the course of disease and the clinical manifestations (1). Its etiology is related to many factors, including gynecology-related conditions (such as endometriosis, inflammation, tumors, and pelvic stasis syndrome) and other unknown primary causes. The clinical definition of CPP syndrome (CPPS) is noncyclical or cyclical episodes of unknown etiology, lasting for more than 3-6 months and related to pelvic structures or pelvic organs. The mechanism underlying the pathogenesis of CPPS is still unclear (2, 3). Patients with CPP often have the same structural and functional changes in the pain matrix of the brain to other patients with chronic pain (4-7). However, the neurobiological mechanism related to the pathogenesis of CCP is poorly understood.

Experimental autoimmune prostatitis (EAP) models have been proven to be reliable for the study of CPP/CPPS. In our study, a CPP model was established in female rats by pelvic nerve ligation, which was confirmed by behavioral test. Our results showed that CPP rats in this model showed a higher positive response on Von Frey pain test at 42 days postoperatively, as compared to control rats.

Chemokine ligand 2 (CCL2) is a member of CC subfamily of chemokines and may cause the accumulation of inflammatory cells in involved tissues, leading to local inflammation (8). CCL2 enhances the sensitivity to pain by directly act on its cognate receptor CCR2 on the nociceptive and/or spinal neurons (9). When CCL2 is overexpressed under the control of glial fibrillary acidic protein promoter, the mice exhibit significantly enhanced behavior in response to both thermal and chemical stimulus modalities (10). In a ttw mice model, *in vivo* depletion of CCL2 in microglia mitigated the severity of chronic spinal compression and related pain (11). In contrast, both INCB3344 and RS504393, specific antagonists of CCR2, improve the CCL-induced nociceptive sensitization (12, 13). Altogether, these studies indicate that CCL2/CCR2 signaling pathway plays an important role in the occurrence and/or persistence of pain. In the present study, the expression of CCL2 increased in the serum exosomes, pelvic

vascular endothelial cells and cerebrospinal fluid (CSF) in the CPP rats, which was consistent with the above findings.

In recent years, the role of Notch signaling in glial cell activation has attracted considerable attention in pain research. The Notch signaling pathway in the excitatory or inhibitory neurons is involved in the pathogenesis of central pain sensitization, but the specific mechanism is still unclear (14). The Notch pathway is highly conserved and involved in the pathogenesis of multiple diseases, including neuropathic pain. The activation of Notch signaling is critical to the occurrence and development of neuropathic pain. In a rat model of neuropathic pain, Notch signaling is activated, which mediates the mechanical allodynia induction and maintenance. Similar findings have been reported in chronic constrictive injury (CCI) rats: the increase of Notch2 induces neuropathic pain behaviors (15-17). These findings suggest that changes in the neuroplasticity related to the Notch signaling pathway are involved in the transmission and modulation of signals in case of neuropathic pain. In our study, the glial cells were treated with CCL2, and results showed the expression of Notch2 increased significantly in these cells.

In the present study, a CPP rat model was established, and CCL2 was found to be highly expressed in the pelvic vascular endothelial cells, serum exosomes, and CSF. In addition, glial cells were treated with CCL2, and results showed that the expression of Notch2 increased significantly in these cells. Thus, we speculate that CCL2 was involved in the central sensitization by activating Notch2 signaling pathway in the case of CPP.

Materials and Methods

Animal model establishment. This study was approved by the Ethics Committee of Tongji Hospital (KYSB-2018-[088]). Adult female Sprague–Dawley rats, aged 8 weeks and weighing 280-320 g, were purchased from Shanghai Siple-Bikai Laboratory Animal Co (Shanghai, PR China). All rats were housed and maintained under specific pathogen-free conditions and allowed to accommodate to the environment for 1 week. The animal model of CPP was established by pelvic nerve ligation. In brief, 30 rats were randomly assigned to the CPP group and the control group at a ratio of 1:1. On day 0, rats were intraperitoneally anesthetized with volatile isoflurane, and the pelvic nerve was exposed through a large sciatic foramen incision in the buttock. In the CPP group, the pelvic nerve was ligated at four sites with nonabsorbable surgical sutures at an interval of 3-5 mm. In the control group, the left pelvic nerve was exposed without ligation. On day 42, behavioral tests were performed. Behavioral tests were performed again after the rats were injected with recombinant CCL2 (control group) or a CCR2 antagonist (CPP group). On day 44, the serum, vascular endothelial cells and CSF were collected for further tests. The rats were euthanized immediately at pre-designated time points.

Chronic pelvic pain assessment by behavioral testing. Rats were subjected to behavioral test 1 day before and 42 days after operation. Tests were performed in an individual transparent plastic box with a stainless-steel wire grid floor. They were allowed to accommodate to the environment for 20 min before testing. The hyperalgesia and tactile allodynia were tested using von Frey filaments with forces of 1, 4, 8, 15 and 26 g (18). Each filament was applied for 1-2 seconds with an interstimulus interval of 5 s, testing was done a total of 10 times, and the filaments were tested in ascending order of force. Stimulation was confined to the lower abdominal area in the general vicinity of the pelvic cavity, and care was taken to stimulate different areas within this region to avoid desensitization or "windup" effects. An investigator blind to the grouping assessed the behavioral responses of animals. The behaviors were graded as positive responses to filament stimulation: 1) sharp retraction of the abdomen, 2) immediate licking or scratching the area of filament stimulation, or 3) jumping (19).

Extraction and detection of exosomes. Rats were anaesthetized with 1% pentobarbital (35 mg/kg) intraperitoneally. After laparotomy, blood was collected *via* the inferior vena cava. Blood samples were allowed to stay for 15 min at room temperature and then centrifuged at 3,500 r/min for 15 min. The serum was harvested for exosome extraction. It was centrifuged at 4˚C for 5 min at 2,000 *g*. The supernatant was removed, followed by centrifugation at 4˚C for 15-20 min. The supernatant was removed again, followed by centrifugation at 4˚C for 30-40 min at 10,000 *g*. The resultant sediment was collected, transferred to an ultra-high-speed centrifuge tube, and was re-suspended in 1× phosphate-buffered saline (PBS). After centrifugation, the supernatant was removed, and the precipitate was resuspended in 1× PBS, which was filtered through a 0.22-μm sterile filter. The filtrate was transferred to a centrifuge tube, followed by centrifugation at 120,000×*g* for 70 min at 4˚C. After centrifugation, the supernatant was removed and the sediment at the bottom were the exosomes. Then, 100 μl of 1×PBS was added, which was then transferred to a centrifuge tube (0.5 ml). For the assay, 10 μl of exosome suspension was collected, stained with 2% uranium dioxyacetate solution, fixed for 1 min, blotted, and air dried at room temperature. The morphology of the exosomes was examined under a transmission electron microscope (TEM). The size and distribution of the exosomes were measured using the NanoSight NS300 nanoparticle tracking analysis (NTA). The expression of CD63 and TSG101, the characteristic proteins of exosomes, was measured by Western blotting.

Detection of CCL-2 expression in exosomes by quantitative polymerase chain reaction. Quantitative real-time polymerase chain reaction (PCR) was used to detect the mRNA expression of CCL2 in serum exosomes. In brief, RNA was extracted from serum exosomes using an Exosome RNA Isolation Kit (Norgen Biotek, #NGB-58000, AmyJet Scientific Inc, Wuhan, PR China). The cDNA was reverse transcribed using a Titan One Tube RT-PCR kit (Roche, #11939823001, Yubo Biological, Shanghai, PR China) according to the manufacturer's instructions. PCR was performed as follows: 95˚C for 5 min, 95˚C for 10 s, 60˚C for 30 s, 72˚C for 10 s (for a total of 40 cycles). The experiment was done in triplicate. The mRNA expression of CCL2 in the exosomes was analyzed using the $2^{-\Delta\Delta Ct}$ method and GAPDH was used as an internal control.

Detection of CCL2 expression in pelvic vascular endothelial cell supernatants by enzyme-linked immunosorbent assay (ELISA). An incision was made bilaterally at the groin (about 1-2 cm in length).

Table I. *Behavioral test in the control and CPP groups (n=10).*

Group	Test time/Force	1 g	4 g	8 g	15 g	26 g
Control	1 day before modeling	0.40±0.52	0.90±0.32	1.00±0.47	1.20±0.42	1.40±0.52
	42 days after modeling	0.60±0.52	1.40±0.52	1.70±0.48	1.80±0.42	2.10±0.32
CPP	1 day before modeling	0.50±0.53	0.90±0.32	1.00±0.00	1.10±0.32	1.30±0.48
	42 days after modeling	3.00±0.67*#	4.00±0.67*#	4.50±0.53*#	5.50±0.71*#	6.50±0.85*#

*$p<0.01$ *vs*. 1 day before operation; #$p<0.01$ *vs*. control group.

The femoral vein was separated, collected and stored in sterile PBS. The samples were washed three times with PBS and then incubated in the media containing penicillin, streptomycin, and amphotericin B solution for 10 min. A blunt syringe needle was inserted into the end of the vein, and the vein was washed with 1% PBS until transparent fluid was observed. Then, 1% type I collagenase was injected to the vascular cavity while clamping an end of the vein. The vein was incubated with 1% type I collagenase for 5, 7, and 10 min, and the fluid was harvested. Finally, the vessel was flushed with cell culture medium (M199 containing 20% fetal bovine serum). After centrifugation, the supernatant was removed, 2 ml of culture medium was added, and the cells were re-suspended, followed by staining with *Trypanosoma cruzi* blue. Cells were observed under an inverted phase-contrast microscope. The supernatants of vascular endothelial cells in both groups were collected and the CCL-2 content was detected using a CCL2 assay kit (ABclonal, RK00206) following the manufacturer's instructions. The A450 was measured, and a standard curve was delineated. The content of CCL-2 was determined according to the standard curve.

Detection of CCL2 expression in CSF by ELISA. Rats were intraperitoneally anesthetized with volatile isoflurane, and the CSF was collected according to the method previously reported (19). The rat's head was fixed in the brain fixation apparatus, and a 1-ml syringe was inserted at an angle of approximately 135˚. The needle was inserted into the medullary pool of the cerebellum at a depth of approximately 0.5 cm. Then, 80-100 μl of CSF was slowly collected, and the needle was quickly withdrawn. Subsequently, 1.5 ml of CSF was transferred into a 1.5-ml EP tube and stored at –40˚C. The CCL-2 content was detected using the CCL2 Assay Kit (RK00206, ABclonal, Wuhan, PR China) according to the manufacturer's instructions. The A450 was measured, and a standard curve was delineated. The CCL-2 content was calculated according to the standard curve.

Intrathecal injections of CCL2 or CCR2 antagonist and behavioral testing. Recombinant CCL2 (Recombinant Human CCL2/MCP-1 Protein, RP00316, ABclonal) or CCR2 antagonist (INCB3344, Shanghai Yuanye Bio-Technology Co, Shanghai, PR China) were injected for the control and CPP group, respectively, 43 days after the operation Rats were anesthetized with volatile isoflurane and an intrathecal catheter (PE-10 tube) was inserted through the gap between L4 and L5. 10 ng of recombinant CCL2 were injected into the control group and 14.43 μg (1 mM) of INCB3344 were injected into the CPP group rats, in a total volume of 25 μl. Behavioral testing was measured again after 2 h in the control group and 24 h in the CPP group by using von Frey filaments, as described previously and compared with the previous responses.

Detection of Notch2 mRNA expression in human microglia treated with recombinant CCL2. Human microglia (HMC3) were incubated with 20 ng/ml recombinant CCL2 (Recombinant Human CCL2/MCP-1 Protein, ABclonal, RP00316) for 24 h. The cDNA was reverse transcribed according to the Titan One Tube RT-PCR kit instructions, followed by qPCR. 40 cycles of 95˚C for 5 min, 95˚C for 10 s, 60˚C for 30 s and 72˚C for 10 s were performed. Three replicate wells were set up for each experiment. qPCR data were collected using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal control.

Notch2 protein expression in human microglia treated with recombinant CCL2. Human microglia (HMC3) were incubated with 20 ng/ml recombinant CCL2 protein (ABclonal, #RP00316) for 24 h, and total protein was collected. The protein concentration was determined using a BCA kit (Beyotime, #P0012, YuanXin Bio, Shanghai, PR China). Proteins (10 μg) in each sample were mixed with loading buffer, separated by electrophoresis on a 12% SDS-PAGE gel, and transferred onto a poly(vinylidene fluoride) membrane. The membrane was then blocked with 5% non-fat milk for 1 h and incubated with primary β-actin (1:2,000) antibody (KGDN16, KeyGen BioTECH, Jiangsu, PR China) overnight at 4˚C. After rinsing with Tris-buffered saline containing Tween 20, the membrane was incubated with secondary antibody (KGP1201, KeyGen BioTECH) at room temperature for 1 h. The membrane was rinsed with TBST, and then visualized with a ChemiDoc XRS chemiluminescence imaging system (Bio-Rad, Hercules, CA, USA). β-actin served as an internal reference.

Statistical analysis. The SPSS 27.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. Quantitative data are expressed as mean±standard deviation (SD) and compared with *t*-test between two groups or one-way analysis of variance among multiple groups followed by SNK-q test between two groups. A value of $p<0.05$ was considered statistically significant.

Results

CPP rat model. In the present study, a CPP rat model was established by ligating the pelvic nerve. Behavioral tests were performed using von Frey filament, 1 day before and 42 days after the surgery. Results showed the number of positive responses was significantly higher in the CPP group compared to the control group at 42 days after surgery ($p<0.05$) (Table I).

Serum exosomes and CCL2 expression. TEM and NTA showed that the exosomes from peripheral serum of both

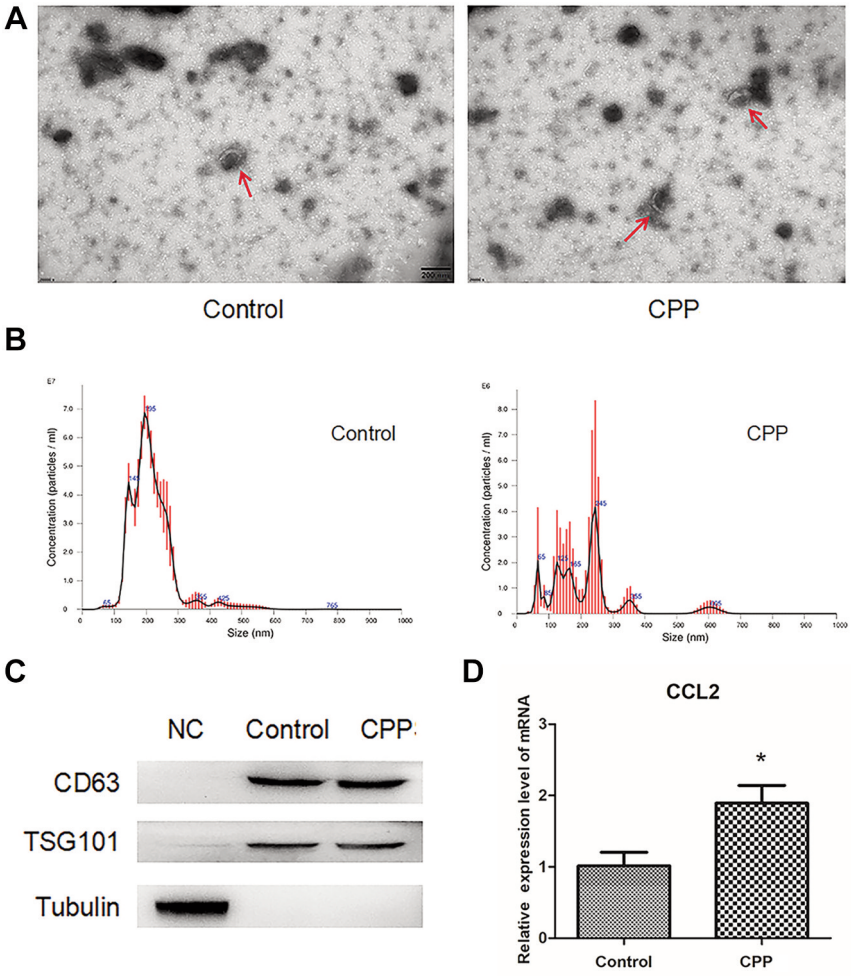

Figure 1. *High expression of chemokine ligand 2 (CCL2) in the peripheral serum exosomes in the chronic pelvic pain (CPP) group. (A) Morphology of exosomes on transmission electron microscope (TEM). (B) Size and density of exosomes on nanoparticle tracking analysis (NTA). (C) Western blotting was performed to detect the expression of CD63 and TSG101 and the exosome-negative molecule tubulin. (D) qPCR was employed to detect the content of CCL2 in exosomes. *p<0.05 using t-test.*

groups were round with a particle size of 215.8±6.0 nm in the control group and 130.6±44.8 nm in the model group (Figure 1A and B). In addition, Western blotting showed that the exosomal samples from both groups had high expression of CD63 and TGS101 but did not express tubulin (Figure 1C). qPCR showed the levels of CCL2 in exosomes significantly increased in the CPP group as compared to the control group (p<0.05) (Figure 1D).

Expression of CCL2 in pelvic vascular endothelial cells and CSF. The morphology of vascular endothelial cells was similar in both groups under an inverted phase-contrast microscope: cells in both groups were short shuttle-shaped (Figure 2A). ELISA showed the CCL2 expression in the vascular endothelial cells of the CPP model group to be significantly higher than the control group (p<0.05, Figure 2B). In addition, ELISA showed that the CCL2 expression in the CSF of the CPP group was significantly higher compared to the control group (Figure 3).

Behavioral testing after Intrathecal Injections of CCL2 or CCR2 Antagonist and effect of CCL2 on Notch2 expression. In the control group, the number of responses was found to be significantly higher after recombinant CCL2 injection than before. The number of reactions was found to be significantly lower 24 h after the injection of CCR2 antagonist compared to the pre-injection period (Figure 4). Other than that, the Notch2 expression in HMC3 cells treated with recombinant CCL2 was detected by qPCR and Western blotting. Results showed the mRNA and protein expression of Notch2 in the HMC3 cells treated with recombinant CCL2 significantly increased as compared to untreated HMC3 cells (Figure 5).

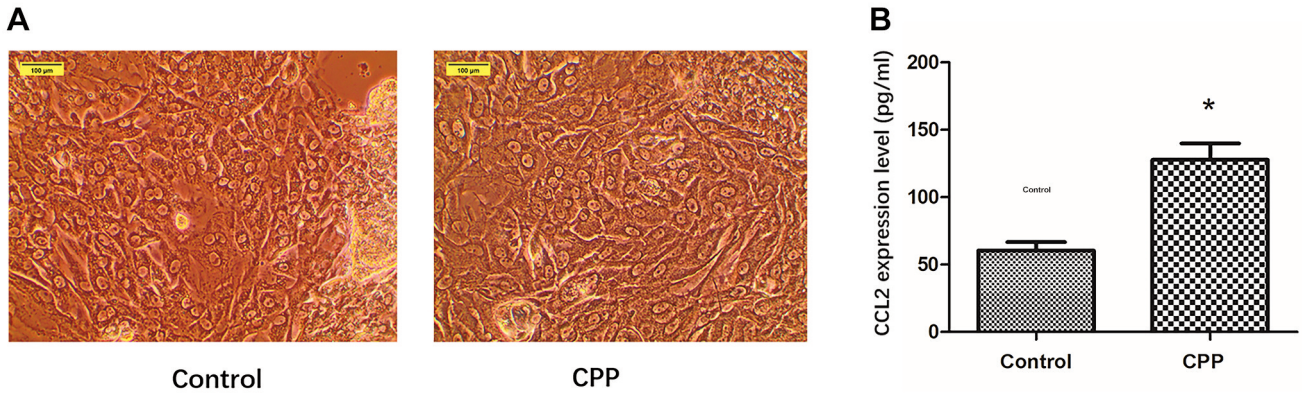

Figure 2. *(A) The morphology of vascular endothelial cells was similar in both groups under an inverted phase-contrast microscope: cells in both groups were short shuttle-shaped. (B) ELISA showed the CCL2 expression in the vascular endothelial cells of the CPP model group to be significantly higher than the control group. *p<0.05 using t-test.*

Discussion

Some factors have been found to be related to the pathogenesis of CPP, including endometriosis, irritable bowel syndrome, interstitial cystitis and painful bladder syndrome, among others. In addition, the central nervous system (CNS) is involved in the experience of pain (20). Patients with CPP have the same structural and functional changes in the CNS seen in patients with other types of chronic pain (5, 6). The structural association of sensory sensitivity and visceral pain sensitivity with the pain matrix network in the brain has been reported; the brain areas associated with chronic pain include the thalamus, insula, and basal ganglia (21). However, the mechanism by which the changes in the structure and function of CNS affect localized pelvic pain is still unclear.

Exosomes are extracellular vesicles produced by cells with the size ranging between 30 and 100 nm. Their biological function and heterogeneity depend on the type of cells from which they originate and the state of cells from which they are released. Exosomes can carry proteins, lipids, RNA and DNA, and may influence the physiological and pathological processes of various diseases, including cancer, infections, neurodegenerative and autoimmune diseases (22). They can cross the blood-brain barrier and maintain the stability of molecules in the exosomes, thus bridging an effective exchange of substances between CNS and blood (23). CSF is directly connected to the subarachnoid space and the ventricular system, and exosomes may reflect the physiological and pathological changes in the brain. A number of studies have investigated the role of blood exosomes in the diagnosis, staging, and even treatment of traumatic brain injury (24). In this study, the serum exosomes were harvested for further analysis in a rat model of CPP, aiming to investigate the relationship between local pelvic pain and CNS.

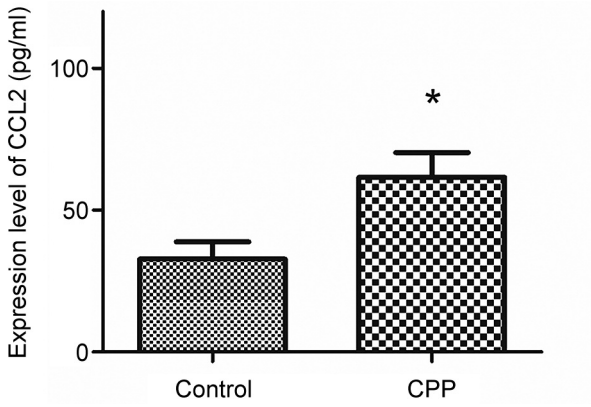

Figure 3. *ELISA showed that the CCL2 expression in the CSF of the CPP group was significantly higher compared to the control group. *p<0.05 using t-test.*

Studies in recent years have confirmed that CCL2 and its receptor CCR2 can act in the central and peripheral nervous system and are involved in nociceptive sensory processes following inflammation or nerve injury (25). CCL2 is involved in neuropathic pain at different levels in peripheral sensory afferent nerves, dorsal root ganglia, spinal cord, and medulla oblongata, and may facilitate nociceptive transmission (26). Qucik *et al*. (27) found the expression of CCL2 increased in mouse prostate tissues in an EAP mouse model, which was related to the pain in these rats. Furthermore, anti-CCL2 antibody and its cognate receptor significantly attenuated the pain symptoms in CCR2-deficient rats, suggesting that CCL2 is involved in the pathogenesis of CPP and pain. In addition, chemokines not only modulate inflammation in the immune system, but also mediate neuroinflammation in the peripheral and central nervous systems, contributing to the development and maintenance of

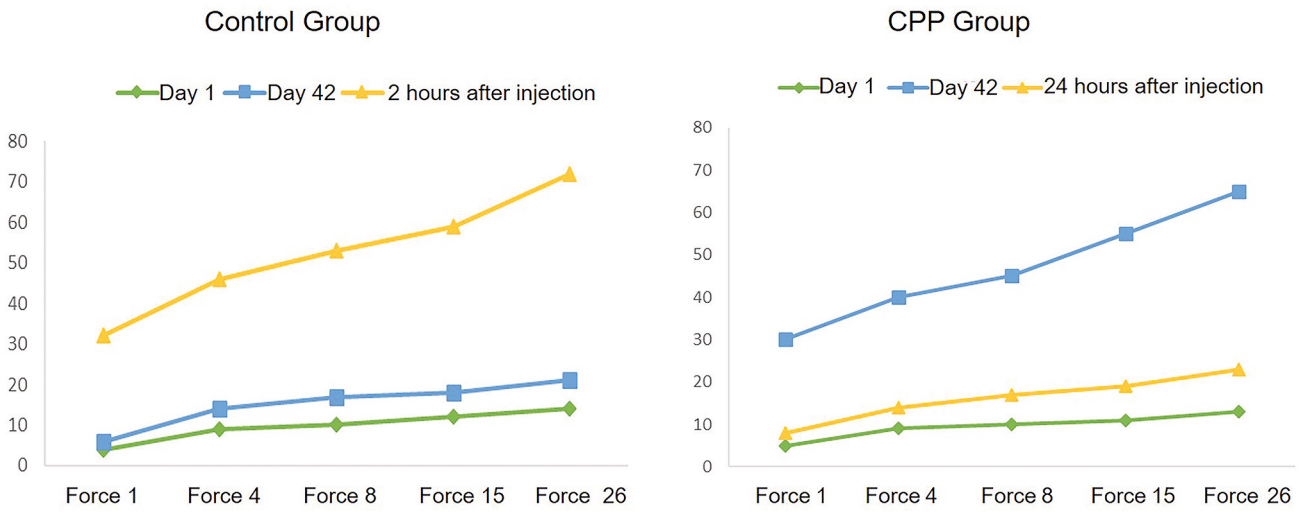

Figure 4. *Behavioral testing of rats in the control and CPP groups. Changes in the number of responses to abnormal nociceptive tests in the two groups using different forces of von Frey filaments were depicted 1 day before the surgery, 42 days after surgery, 2 h after CCL2 injection and 24 h after CCR2 antagonist injection. In the CPP group, the number of responses increased significantly at 42 days postoperatively compared to preoperatively and decreased significantly 24 h after intrathecal injection of CCR2 antagonist compared to pre-injection ($p<0.01$). In the control group, the number of responses did not change significantly at 42 days postoperatively compared to preoperatively but increased significantly after 2 h of intrathecal CCL2 injection compared to pre-injection ($p<0.01$).*

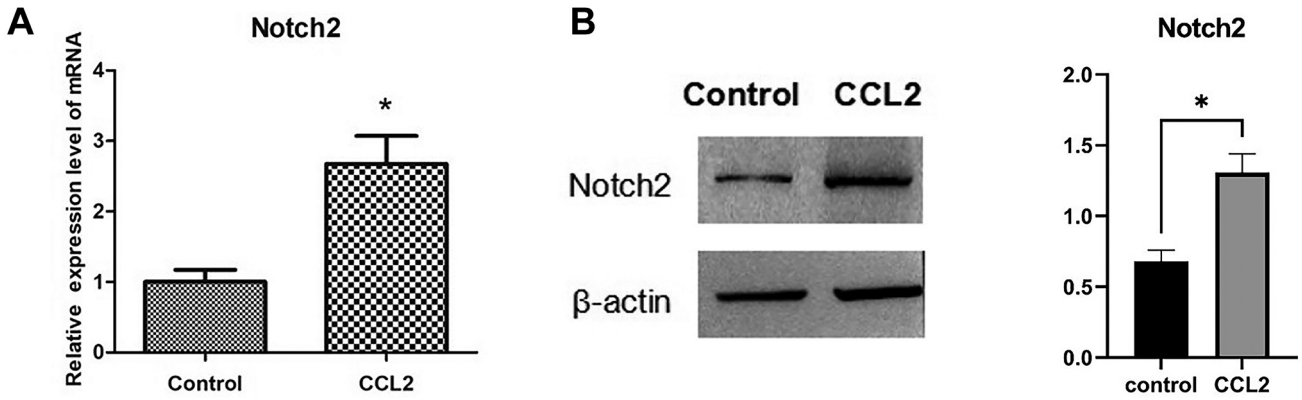

Figure 5. *The Notch2 expression in HMC3 cells treated with recombinant CCL2 was detected by qPCR and Western blotting. (A) The mRNA expression of Notch2 in the HMC3 cells treated with recombinant CCL2 significantly increased as compared to untreated HMC3 cells (qPCR). (B) Notch2 protein expression significantly increased in the CCL2 group compared to the control group (western blotting). *$p<0.05$ using t-test.*

various types of chronic pain. An increasing body of evidence suggests that several chemokines and their receptors (*e.g.*, CCL2/CCR2, CXCL1/CSCR2, CXCL12/CXCR4, *etc.*) are expressed in the peripheral and central nervous systems and play important roles in the pathogenesis of chronic pain by mediating neuronal and non-neuronal cell interactions and enhancing neuroinflammation (28). In the present study, high expression of CCL2 was indicated in the peripheral blood, vascular endothelial cells, and CSF of CPP rats. CCR2, as a kind of neuromodulators, is involved in neuropathic pain through different signaling pathways and multiple targets; it may act on the peripheral and central nervous system at different levels. CCR2 increases the excitability of the nervous system by modulating ion channels and receptors associated with nerve excitability, leading to the sensitization of peripheral and central nerves. It has been reported that CCL2/CCR2 plays a pivotal role in the establishment and maintenance of pain and that CCL2-neutralising antibodies and CCR2 specific antagonists may exert favorable analgesic effects. For example, intrathecal injection of anti-CCL2-neutralising antibodies can reduce the mechanically abnormal pain

caused by bone cancer (29). In the present study, the expression of CCL2 was significantly higher in both local pelvic tissues and CSF of CPP rats as compared to control rats, suggesting that CCL2 is involved in the nociceptive process of CPP, in which exosomes serve as a carrier, although the transmission process of exosomes needs to be confirmed by further fluorescence conjugation tests. Our study found that the blockade of CCL2 significantly attenuated the established mechanical allodynia in CPP rats. Furthermore, intrathecal exogenous CCL2 induced pain behavior. These findings indicate that CCL2 and its receptor CCR2 play an important role in the central sensitization of chronic pelvic pain. However, the mechanism by which chemokines regulate the interaction between neurons and glial cells is still poorly understood.

In the present study, we found that the mRNA and protein expression of Notch2 in the HMC3 cells treated with recombinant CCL2 was significantly increased. This suggests that CCL2 can activate the Notch2 signaling pathway. Notch signaling is an evolutionarily highly conserved signaling pathway, and closely related to intercellular communication, cell differentiation and proliferation, as well as embryonic development and cell fate. Four Notch receptors (Notch1–4) and multiple ligands (Jagged1 and 2 and Delta1, 3, and 4) have been identified in the mammalian nervous system, all of which are single transmembrane proteins. The role of Notch signaling in glial cell activation has gained much attention in research on pain in recent years. Notch signaling pathways are involved in central sensitization by exciting or inhibiting neurons, but the underlying mechanisms are unclear (14).

Woolf *et al*. (29) proposed a mechanism for the classic CPP. During the physiological pain response, peripheral injury information is transmitted to the cerebral cortex to produce normal nociception. During the early pathological pain, the receptors and channels are phosphorylated and the intracellular signaling pathways are activated at the post-translational level. During chronic pain, the main changes are long-lasting and persistent changes in receptors, channels, and intracellular signaling pathways occur, which involve various aspects of transmitter expression, synaptic connectivity, and neuronal structure and survival at the transcriptional and translational levels (30). The activation of these signaling pathways, which in turn affect the changes at transcription and translation levels, is related to the alteration of neuroplasticity. Notch in human tissues is involved in the proliferation and differentiation of neural stem cells as well as learning and memory in the embryonic period (31). However, their expression significantly increases in adult mammals after neural injury. Thus, they participate in glial cell activation and neural remodeling; the inhibition of Notch signaling may exert significant analgesic effects (32, 33).

Conclusion

In conclusion, the present study demonstrates that CCL2 is highly expressed in the endothelium of pelvic vessels, peripheral blood, exosomes, and CSF in CPP rats. CCL2 plays an important role in the pathogenesis of CPP. Furthermore, the present study also indicates that CCL2 can activate the Notch2 signaling pathway in human brain glial cells, but the specific mechanism is not well understood. In the future, we can further study how the Notch signaling pathway affects the occurrence and development of neuropathic pain and central sensitization of chronic pelvic pain, and explore new ways to alleviate chronic pelvic pain.

Conflicts of Interest

The Authors declare that there are no conflicts of interest.

Authors' Contributions

Conceptualization: HFL; Data curation: JD; Formal analysis: JHL; Funding acquisition: HFL; Investigation: JD; Methodology: JD and JHL; Project administration: HFL and XWT; Resources: HFL; Software: JD; Supervision: HFL and XWT; Validation: JD; Visualization: JD; Writing - original draft: JD; Writing - review and editing: JD and YGC.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (NSFC, 81873827).

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Received August 1, 2023
Revised September 19, 2023
Accepted September 25, 2023