

# Correlation between CCL2, CALCA, and CX3CL1 gene polymorphisms and chronic pain after cesarean section in Chinese Han women

## A case control study

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### Abstract

**Objective:** Postoperative chronic pain is characterized by high incidence, long duration, and complex pathogenesis. The purpose of this study was to investigate the correlation between the single nucleotide polymorphisms of the *CCL2* gene rs4586 (g.5974T>C), *CALCA* rs3781719 (-692T>C), *CX3CL1* rs614230 (2342C>T), and the risk of postoperative chronic pain in Chinese Han women.

**Methods:** We analyzed the *CCL2* gene rs4586, *CALCA* rs3781719, *CX3CL1* rs614230 single nucleotide polymorphism (SNPs) of 350 Chinese Han women with chronic postsurgical pain (CPSP) 6 months after cesarean section and 350 healthy women without chronic pain (HC). The levels of CCL2, CALCA, and CX3CL1 in serum were detected by enzyme-linked immunosorbent assay (ELISA).

**Results:** The *CCL2* rs4586 T allele and the *CX3CL1* gene rs614230C allele were protective factors for CPSP risk (adjusted OR = 0.766, 95% CI: 0.675–0.865 and OR = 0.336, 95% CI: 0.644–0.835). The *CALCA* gene rs3781719C allele was a risk factor for CPSP (adjusted OR = 1.273, 95% CI: 1.125–1.424). *CCL2* rs4586, *CX3CL1* gene rs614230, and *CALCA* gene rs3781719 locus gene polymorphisms were associated with serum CCL2, CX3CL1, and CALCA protein levels.

**Conclusion:** Our results support that *CCL2* gene rs4586, *CALCA* rs3781719, *CX3CL1* rs614230 gene polymorphism are associated with the occurrence of chronic pain after cesarean section in Chinese Han women.

**Abbreviations:** ANOVA = analysis of variance, ASA = American Society of Anesthesiologists, BMI = body mass index, CAD = coronary artery disease, CALCA = calcitonin related polypeptide alpha, CCL2 = C-C motif chemokine ligand 2, CGRP = calcitonin gene-related peptide, CPSP = chronic postsurgical pain, CX3CL1 = C-X3-C motif chemokine ligand 1, DNA = deoxyribonucleic acid, GDM = gestational diabetes mellitus, GMDR = generalized multifactor dimensionality reduction, HC = healthy control, MAF = minor allele frequency, PCR = polymerase chain reaction, SNP = single nucleotide polymorphism.

**Keywords:** CALCA, CCL2, CX3CL1, risk factor, single nucleotide polymorphism

## 1. Introduction

Chronic postsurgical pain (CPSP) refers to the existence of pain associated with surgical-related pain for at least 2 months, except

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GM and JY have contributed equally to this work.

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for other causes (such as chronic infection, recurrence of malignant tumor, etc).<sup>[1]</sup> Open chest surgery, breast cancer (modified) radical operation, cesarean section, amputation, and so on are more likely to lead to CPSP, especially thoracotomy and amputation, the incidence of CPSP can be as high as 50% to 80%.<sup>[2]</sup> Wang et al<sup>[3]</sup> previous follow-up of 466 patients undergoing thoracotomy showed that the incidence of CPSP was as high as 64.5%, which seriously affected the quality of life of the patients after operation.

The mechanism of CPSP is complicated, the nerve injury caused by operation is the necessary premise of CPSP, and the excessive inflammatory counteraction of nervous system and abnormal immunomodulation play a key role in the transition from acute pain to CPSP after operation.<sup>[4–6]</sup> Although the specific mechanism of central inflammatory response to CPSP induced by peripheral tissue and/or nerve injury is unclear, however, many studies have shown that central inflammatory response can enhance the nociceptive reflex arc of the spinal cord and induce central sensitization to nociceptive stimuli.<sup>[7–10]</sup> When the peripheral nociceptors were sensitized by surgical injury, a large number of abnormal excitatory electrical activities were transmitted to the posterior horn of the spinal cord, which resulted in the abnormal opening of calcium channels in the presynaptic membrane and the enhancement of abnormal discharge.

The expression of CCL2 and its receptor CCR2 in the spinal cord and dorsal root ganglion (DRG) was increased by peripheral or central nerve injury. CCL2/CCR2 mediates glial cell-neuron

interaction in central sensitization and chronic pain regulation during the neuroinflammatory response of peripheral nerves after surgical injury.<sup>[11]</sup> Teler et al<sup>[12]</sup> research shows that *CCL2* rs4586 gene polymorphism is associated with Gestational diabetes mellitus (GDM), the mechanism may be that the polymorphism of this locus affects the expression of *CCL2* protein. Calcitonin-related polypeptide alpha (*CALCA*,  $\alpha$ -CGRP) is a 37 amino acid neuropeptide with extensive biological effects and is one of the mediators of neurogenic inflammation.<sup>[13]</sup> CGRP-related genes are good candidates to screen for an association with migraine susceptibility as several lines of evidence suggest that  $\alpha$ -CGRP is a key player in migraine pathology. Plasma levels of *CALCA* have been found to be elevated during spontaneous and nitroglycerin-induced migraine attacks. However, in the present study we investigated a SNP at position -624(T/C) of the *CALCA* gene promoter (rs3781719), which may influence transcription of  $\alpha$ -CGRP. *CX3CL1* is a membrane-bound CX3C chemokine induced by primary proinflammatory signals in vascular endothelial cells (ECs).<sup>[14]</sup> *CX3CL1* is located at 16q13, it is a member of CX3C chemokines and plays an important role in various diseases.<sup>[15]</sup> In the present study, we have selected *CCL2* rs4586, *CALCA* rs3781719, and *CX3CL1* rs614230 three SNPs, to study whether they contribute to the risk of chronic pain in Chinese Han women.

## 2. Materials and methods

### 2.1. Subjects

A total of 875 Chinese Han women who underwent cesarean section and lumbar-hard anesthesia were enrolled in our hospital during May 2014 and October 2017. Among them, 354 patients directly underwent cesarean section, 454 patients underwent cesarean section after trying delivery per vias naturalis, and 67 patients are first of second cesarean section. Twenty-five patients withdrew from the study, among them, the anesthesia effect of 21 patients could not complete the cesarean section, and it was necessary to assist local anesthesia or general anesthesia, 4 patients voluntarily withdrew from the study without any reason, and 850 patients remained. Among them, 350 women who developed chronic pain at 6 months after surgery were included as CPSP group, while 350 were randomly selected as a healthy control group (HC) among women who did not develop chronic pain at 6 months after surgery. In CPSP group, American Society of Anesthesiologists (ASA) grades I to II, age 20 to 39 years, mean age ( $28.5 \pm 5.2$ ) years old, weight 59 to 88 kg, BMI is ( $28.7 \pm 2.7$ ) kg/m<sup>2</sup>, gestational age 38 to 40 w. Exclusion criteria: maternal congenital cardiopulmonary disease, contraindications for spinal

anesthesia, high blood pressure or gestational hypertension, hyperthyroidism or hypothyroidism, diabetes or gestational diabetes. The study was approved by the Medical Ethics Committee of Shanghai University of Medicine and Health Sciences Affiliated Zhoupu Hospital and The Affiliated Shanghai Pudong Hospital of Fudan University, and all subjects signed informed consent.

### 2.2. Anesthesia and postoperative analgesia

Anesthesia method was intrathecal injection of 10 mg ropivacaine and 4  $\mu$ g of dexmedetomidine hydrochloride. The epidural analgesia was performed at L2–3 interspace by an 18-gauge Tuohy needle using the method of loss of resistance to air in left lateral position. After the epidural puncture was successful, the epidural puncture needle was used as a guide. A 25G lumbar puncture needle was inserted into the needle, and the arachnoid was pierced through the epidural puncture needle. The needle core was withdrawn and the cerebrospinal fluid was discharged from the needle. Injection of 0.5% ropivacaine plus 0.5  $\mu$ g/mL dexmedetomidine 2 to 3 mL via spinal anesthesia, exit the lumbar puncture needle, and place the epidural catheter through the epidural needle (3 cm through the needle). Exit the epidural needle, the puncture point covers the sterile gauze, and the catheter was fixed. The spinal anesthesia block plane was measured after helping patients turn over and lie on his back. The epidural catheter was injected with 3 to 5 mL of local anesthetic, and after 5 min, there was no obstructed plane hyperactivity, and surgery can be started. During the operation, an appropriate amount of local anesthetic is injected through the epidural catheter as needed to maintain anesthesia. Analgesic method was 0.1% ropivacaine hydrochloride and 0.5  $\mu$ g/kg dexmedetomidine hydrochloride were added to 0.9 mL physiological saline to 100 mL, and epidural continuous controlled analgesia was used. Maternal mean arterial pressure <60 mmHg intravenous injection of ephedrine 10 mg, heart rate <50 bpm intravenous injection of atropine sulfate 0.2 mg. Epidural 10 mL of 1% lidocaine was administered to rescue if VSA >3 after 30 min of epidural injection.

### 2.3. Genomic DNA isolation and PCR

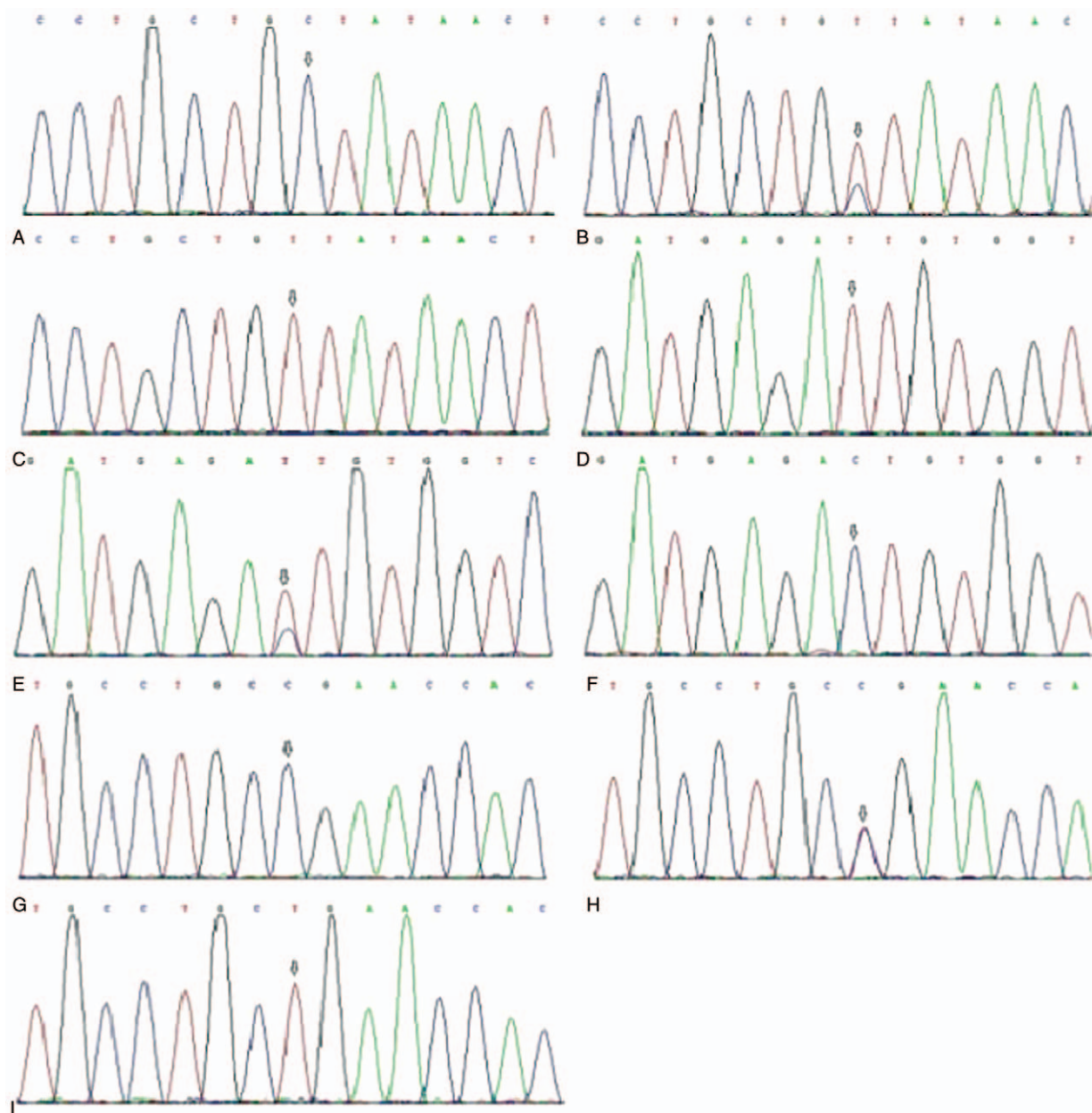
We collected approximately 5 mL of the venous blood of each subject before the cesarean section, and then used the QIAamp DNA Blood Mini Kit (Qiagen, Germany) to obtain blood for genomic DNA analysis. After quantified by Nanodrop2000, DNA sequence was amplified using specific primers (Table 1). The PCR reaction system was as follows: 3  $\mu$ L 10X rTaq buffer, 1  $\mu$ L Forward primer, 1  $\mu$ L Reverse primer, 2  $\mu$ L dNTP, 2  $\mu$ L gDNA, 0.2  $\mu$ L rTaq, 20.8  $\mu$ L

**Table 1**  
SNPs site primer sequence information.

Gene	SNPs	MAF*	Location	Sequence (5' > 3')
<i>CCL2</i>	rs4586	T: 0.3641	17:34256250	F: TCCTTTGTGGTTTATTTCCAGATGC; R: CGAGCCTCTGCACTGAGAT.
<i>CALCA</i>	rs3781719	C: 0.1456	11:14972978	F: CTCTGATCCAAGCCACCTCC; R: GATGCTGAGAGCCAGGAAGG.
<i>CX3CL1</i>	rs614230	C: 0.3883	16:57385374	F: 5'-TGGGATATGGTACTGAC-3'; R: 5'-AGCTAGAAGCTGGTACTGAC-3'.

\* Southern Han Chinese 1000 genomes data.

MAF = Minor allele frequency, SNP = single nucleotide polymorphism.



**Figure 1.** Sequencing chromatogram of mutations in CCL2 rs4586, CALCA rs3781719, and CX3CL1 rs614230. (A–C) are the result of genotyping of CCL2 gene rs4586 locus, A is the CC genotype, B is the CT genotype, and C is the TT genotype. (D–F) are the result of CALCA rs3781719 site sequencing, D is the TT genotype, E is the TC genotype, and F is the CC genotype. (G–I) are the result of genotype sequencing of CX3CL1 rs614230 locus, where G is the CC genotype, H is the CT genotype, and I is the TT genotype.

ddH<sub>2</sub>O. PCR reaction conditions: pre-denaturation for 5 min at 95°C, denaturation for 30s at 95°C, annealing for 45s at 60°C, extension for 1 min at 72°C, 30 cycles, then another 10 min at 72°C. The PCR products were sent to complete the sequencing (Sunny, Shanghai), results were shown in Fig. 1.

**2.4. Quantitative determination of CCL2, CALCA, and CX3CL1 in serum**

The serum CCL2, CALCA, and CX3CL1 levels were detected by ELISA kit respectively (EKbio, Magden, Switzerland) strictly according to the manufacturer’s instructions. Three replicate wells were tested at one time.

**2.5. Statistical analysis**

SPSS version 20.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The descriptive statistics for quantitative variables were given as means standard deviation, comparison between the two groups were analyzed by *t* test. Differences between different genotypes were evaluated using one-way ANOVA. The allele frequencies and the distribution of genotypes were expressed as percentages, statistical analysis among multi-groups were performed by Chi-square test. Genotype frequency of two groups was checked by Chi-square test for Hardy-Weinberg equilibrium. The correlation between genotype and Chronic pain was analyzed by odds ratio (OR) and 95% confidence interval (CI), and factors such as age, BMI, gestational

**Table 2**  
Basal characteristics of CPSP group and of HC group.

	CPSP (n = 350)	HC (n = 350)	P value
Age (years, mean ± SD)	28.5 ± 5.2	29.1 ± 6.1	.16
BMI (kg/m <sup>2</sup> , mean ± SD)	28.7 ± 2.7	28.9 ± 3.1	.36
Gestational age (week, mean ± SD)	38.2 ± 0.9	38.1 ± 0.8	.12
Operation time (mean, mean ± SD)	60.5 ± 7.9	61.0 ± 0.9	.44
Apgar score (1 min)	8 (8–10)	8 (8–10)	.85
Birth weight (g, mean ± SD)	3109.1 ± 286.5	3104.5 ± 291.3	.83

BMI=body mass index, CPSP=chronic postsurgical pain, HC=healthy controls.

age, operation time, apgar score, and birth weight were corrected by multiple factor Logistic regression analysis. Logistic regression was performed to investigate association between SNP and CPSP. Generalized multifactor dimensionality reduction (GMDR)<sup>[16]</sup> was used to analyze the interaction among three SNPs, and cross-validation consistency, the testing balanced accuracy, and the sign test, to assess each selected interaction, were calculated. Results were considered statistically significant when *P* values <.05.

### 3. Results

#### 3.1. Genotype and allele distribution of mutations in CCL2 gene

There was no significant difference between the two groups in age, BMI, gestational age, operation time, apgar score, and birth

weight (Table 2). The comparison of genotype and allele frequencies of the *CCL2* gene rs4586 locus between the CPSP group and HC group was presented in Table 3. The genotype and allele distribution of *CCL2* gene rs4586 locus mutations in the control group were in Hardy–Weinberg equilibrium (*P* >.05). When wild-type CC was a reference, heterozygous (CT) and homozygous (TT) were protective factors for CPSP (adjusted OR=0.812, 95%CI: 0.691–0.953, *P* =.010; adjusted OR =.584, 95%CI: 0.420–0.782, *P* <.001). CPSP risk were significantly reduced under both recessive and dominant model of *CCL2* gene rs4586 locus (adjusted OR=0.755, 95%CI: 0.650–0.880, *P* <.001; adjusted OR=0.641, 95%CI: 0.462–0.852, *P* =.001). The risk of CPSP in T allele carriers was significantly lower than that of C allele carriers (adjusted OR=0.766, 95%CI: 0.675–0.865, *P* <.001) (Table 3).

#### 3.2. Genotype and allele distribution of mutations in CALCA

The genotype and allele distribution of *CALCA* gene rs3781719 locus mutations in the control group were in Hardy–Weinberg equilibrium (*P* >.05) (Table 3). In the case of wild type TT as a reference, homozygous (CC) was risk factors for CPSP (adjusted OR=1.697, 95%CI: 1.345–1.950, *P* <.001), there is no significant change in the risk of CPSP in heterozygotes (adjusted OR=1.091, 95%CI: 0.905–1.296, *P* =.377). CPSP risk were significantly increased under recessive and dominant model of *CALCA* gene rs3781719 locus (adjusted OR=1.211, 95%CI:

**Table 3**  
Genotype and allele frequency of CCL2 rs4586, CALCA rs3781719, and CX3CL1 rs614230 and the risk of CPSP analysis.

SNP	CPSP (n = 350)	HC (n = 350)	Crude OR (95%CI)	P value	Adjusted OR (95%CI)	P value
CCL2						
rs4586						
CC	184(52.6%)	135(38.6%)	1.00(reference)			
CT	134(38.3%)	152(43.4%)	0.647(0.463–0.904)	.008	0.812(0.691–0.953)	.010
TT	32 (9.1%)	63 (18.0%)	0.373 (0.224–0.618)	<.001	0.584 (0.420–0.782)	<.001
CT/TT	166 (47.4%)	215 (61.4%)	0.566 (0.415–0.774)	<.001	0.755 (0.650–0.880)	<.001
CC/CT	318 (90.9%)	287 (82.0%)	1.00 (reference)			
TT	32 (9.1%)	63 (18.0%)	0.458 (0.284–0.739)	<.001	0.641 (0.462–0.852)	.001
C	502 (71.7%)	422 (60.3%)	1.00 (reference)			
T	198 (28.3%)	278 (39.7%)	0.599 (0.476–0.753)	<.001	0.766 (0.675–0.865)	<.001
CALCA						
rs3781719						
TT	224 (64.0%)	254 (72.6%)	1.00 (reference)			
TC	91 (26.0%)	87 (24.9%)	1.186 (0.828–1.699)	.331	1.091 (0.905–1.296)	.377
CC	35 (10.0%)	9 (2.6%)	4.410 (1.983–10.098)	<.001	1.697 (1.345–1.950)	<.001
TC/CC	126 (36.0%)	96 (27.4%)	1.488 (1.066–2.078)	.015	1.211 (1.032–1.406)	.019
TT/TC	315 (90.0%)	341 (97.4%)	1.00 (reference)			
CC	35 (10.0%)	9 (2.6%)	4.210 (1.907–9.575)	<.001	1.657 (1.320–1.892)	<.001
T	539 (77.0%)	595 (85.0%)	1.00 (reference)			
C	161 (23.0%)	105 (15.0%)	1.693 (1.278–2.243)	<.001	1.273 (1.125–1.424)	<.001
CX3CL1						
rs614230						
TT	213 (60.9%)	139 (39.7%)	1.00 (reference)			
TC	94 (26.9%)	154 (44.0%)	0.398 (0.281–0.564)	<.001	0.626 (0.520–0.751)	<.001
CC	43 (12.3%)	57 (16.3%)	0.492 (0.306–0.791)	.002	0.711 (0.544–0.900)	.003
TC/CC	137 (39.1%)	211 (60.3%)	0.424 (0.309–0.580)	<.001	0.651 (0.555–0.762)	<.001
TT/TC	307 (87.7%)	293 (83.7%)	1.00 (reference)			
CC	43 (12.3%)	57 (16.3%)	0.720 (0.459–1.127)	.130	0.840 (0.642–1.060)	.160
T	520 (74.3%)	432 (61.7%)	1.00 (reference)			
C	180 (25.7%)	268 (38.3%)	0.558 (0.441–0.705)	<.001	0.736 (0.644–0.835)	<.001

CI=confidential interval, CPSP=chronic postsurgical pain, HC=healthy controls, OR=odds ratio.

**Table 4**  
Summary of gene–gene interaction result in CPSP by GMDR.

Model	Training balance accuracy	Testing balance accuracy	Cross-validation consistency	$\chi^2$ (P)	OR (95% CI)
CX3CL1/rs614230	0.6057	0.6057	10/10	28.16 (<.001)	2.36 (1.71–3.25)
CALCA/rs3781719, CX3CL1/rs614230	0.6271	0.6271	10/10	41.48 (<.001)	2.89 (2.08–4.01)
CCL2/rs4586, CALCA/rs3781719, CX3CL1/rs614230	0.6413	0.5886	10/10	50.30 (<.001)	3.20 (2.31–4.43)

P value was adjusted for age, Apgar score, birth weight, CALCA = Calcitonin-related polypeptide alpha, CCL2 = C-C motif chemokine ligand 2, CI = Confidence interval, CX3CL1 = C-X3-C motif chemokine ligand 1, OR = odd ratio.

1.032–1.406,  $P=0.019$ ; adjusted OR = 1.657, 95%CI: 1.320–1.892,  $P<.001$ ). The risk of CPSP in C allele carriers was significantly higher than that of T allele carriers (adjusted OR = 1.273, 95%CI: 1.125–1.424,  $P<.001$ ) (Table 3).

**3.3. Genotype and allele distribution of mutations in CX3CL1**

The genotype and allele distribution of CX3CL1 gene rs614230 locus mutations in the control group were in Hardy–Weinberg equilibrium ( $P>.05$ ) (Table 3). In the case of wild type TT as a reference, heterozygous (TC) and homozygous (CC) were protective factors for CPSP (adjusted OR = 0.626, 95%CI: 0.520–0.751,  $P<.001$ ; adjusted OR = 0.711, 95%CI: 0.544–0.900,  $P=.003$ ). CPSP risk were significantly decreased under recessive model of CX3CL1 gene rs614230 locus (adjusted OR = 0.651, 95%CI: 0.555–0.762,  $P<.001$ ). There was no significant change in the risk of CPSP under the recessive model (adjusted OR = 0.840, 95%CI: 0.642–1.060,  $P=.160$ ). The risk of CPSP in C allele carriers was significantly lower than that of T allele carriers (adjusted OR = 0.736, 95%CI: 0.644–0.835,  $P<.001$ ) (Table 3).

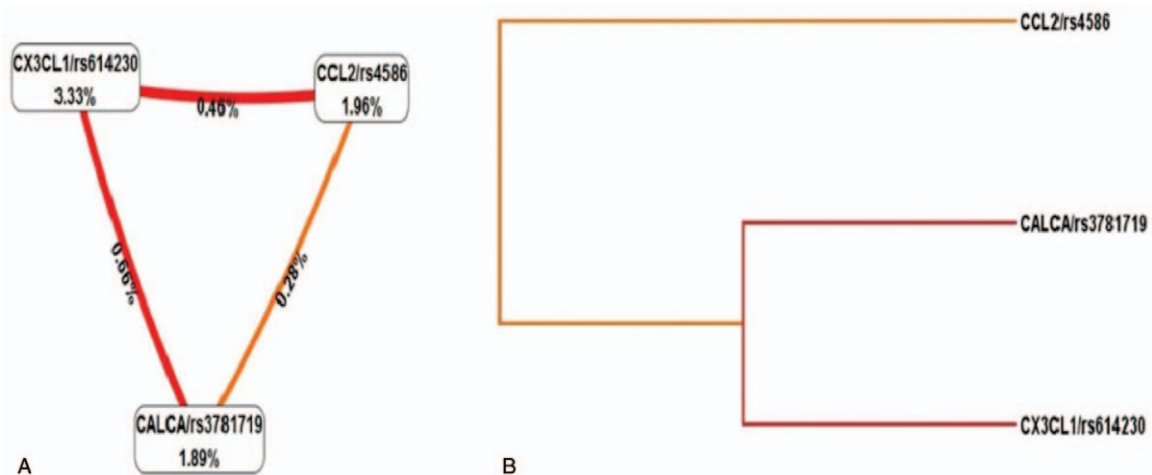
**3.4. CCL2, CALCA, and CX3CL1 gene–gene interaction**

We employed the GMDR analysis to investigate the impact of the interaction among three SNPs after adjustment for covariates. Table 4 summarizes the results obtained from GMDR analysis,

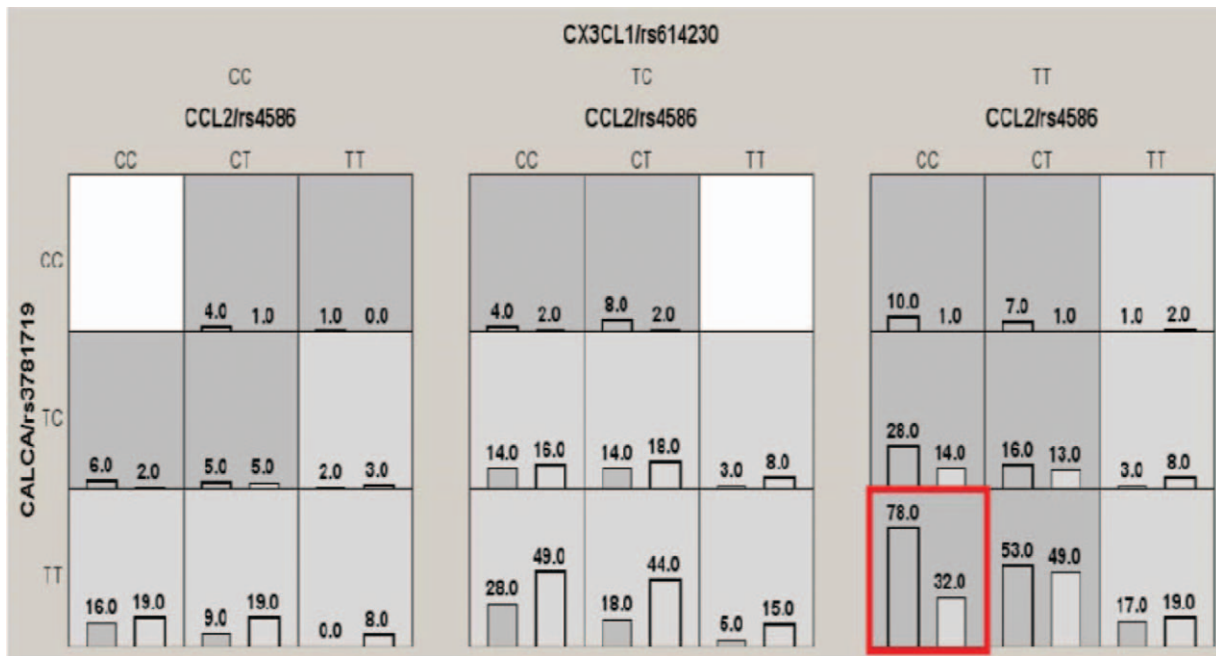
and we found that there was a significant two-locus model ( $P<.001$ ) involving CALCA gene rs3781719 locus and CX3CL1 gene rs614230 locus. Indicating a potential gene–gene interaction between CALCA gene rs3781719 locus and CX3CL1 gene rs614230 locus. Overall, the cross-validation consistency of this two-locus model was 10/10, and the testing accuracy was 62.71%. Hierarchical interaction graphs showed that the entropy of CALCA gene rs3781719 locus and CX3CL1 gene rs614230 locus was 0.66%, which was higher other two interaction (Fig. 2A). Interaction dendrogram showed CALCA gene rs3781719 locus and CX3CL1 gene rs614230 locus had a strong interaction (Fig. 2B). The best model of distribution of CCL2, CALCA, and CX3CL1 gene–gene interaction was CALCA gene rs3781719 TT, CCL2 gene rs4586 CC, and CX3CL1 gene rs614230 TT genotype (Fig. 3).

**3.5. The correlation between CCL2, CALCA, CX3CL1 protein levels and CALCA, CCL2, and CX3CL1 gene polymorphism**

We used ELISA to detect the levels of CCL2, CALCA, and CX3CL1 in serum of all subjects. The results showed that the serum levels of CCL2, CALCA, and CX3CL1 in women with chronic pain after cesarean section were significantly higher than those in healthy controls, the difference were statistically significant ( $P<.001$ ) (Fig. 4A, C, E). The levels of CCL2, CALCA, and CX3CL1 in serum of different genotypes were analyzed. The results showed that the CCL2 rs4586 locus CC



**Figure 2.** Hierarchical interaction graphs and interaction dendrogram. (A) Hierarchical interaction graphs showed that the percentage at the bottom of the each polymorphism represented entropy of it, and the percentage on each line represented the interaction percentage of entropy between the two SNPs. The red line represented synergy redundancy interaction and the orange line represented redundancy interaction. (B) Interaction dendrogram showed that the red line represented synergy interaction and the orange line represented synergy interaction more weakly. From left to right the interaction was more intensive.

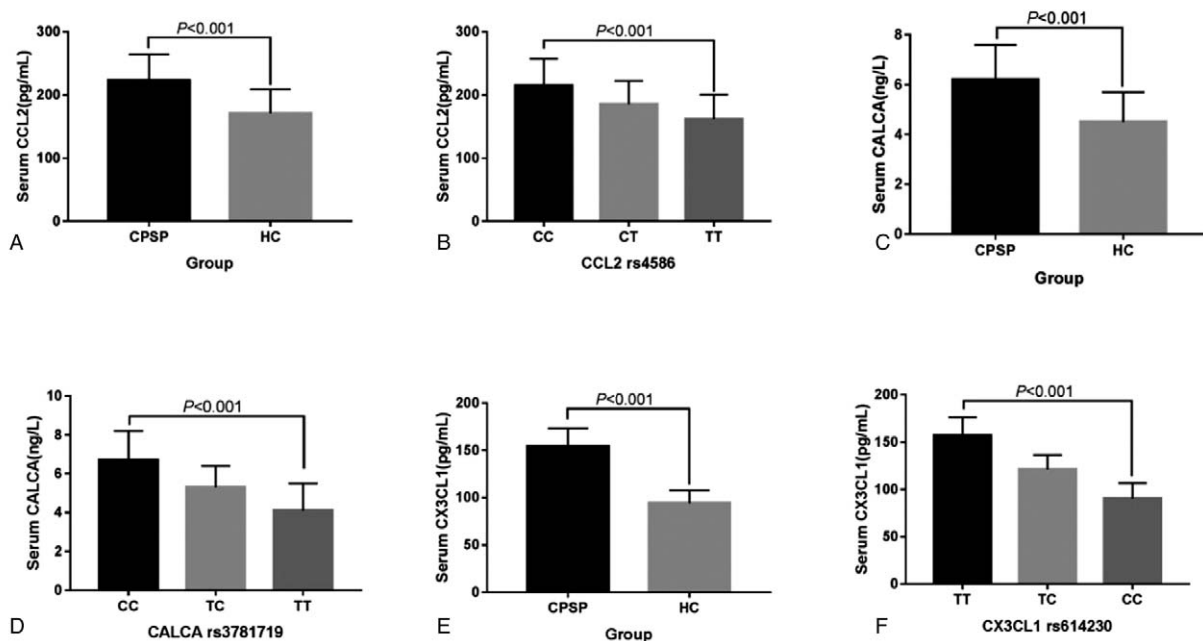


**Figure 3.** Distribution of high-risk and low-risk of hypertension in the best model. Dark gray and light gray boxes presented the high- and low-risk factor combinations, respectively. Left bars within each box represented chronic pain case while the right bars represented healthy control. The heights of the bars are proportional to the sum of samples in each group. Note that the patterns of high-risk and low-risk cells differ across each of the different multilocus dimensions. This is evidence of gene–gene interaction. The red box indicates the best combination for predicting CPSP risk.

genotype had the highest serum CCL2 protein level, the CT genotype was the second, followed by CT and TT ( $P < .001$ ) (Fig. 4B). The CALCA gene rs3781719 locus TT genotype had the highest serum CALCA level, followed by CT and TT (Fig. 4D). Subjects carrying the CX3CL1 gene rs614230 locus TT genotype had the highest serum CX3CL1 levels, followed by TC and CC (Fig. 4F).

#### 4. Discussion

In this study, we analyzed the relationship between chronic pain after cesarean section in Chinese Han women and CCL2 gene rs4586, CALCA gene rs3781719 locus, and CX3CL1 gene rs614230 locus single nucleotide polymorphism, and found CCL2 gene rs4586, CALCA rs3781719, CX3CL1 rs614230



**Figure 4.** The comparison of CCL2, CALCA, and CX3CL1 protein levels. CPSP represents chronic postsurgical pain. HC=healthy control.

gene polymorphism were associated with the risk of CPSP in Chinese Han women.

Pain, tension, and anxiety are common problems after cesarean section, which can affect maternal eating and rest, and thus affect rehabilitation and lactation. Clinical studies have found that the incidence of chronic pain after cesarean section is 15% at 3 months to 11% at 12 months or longer.<sup>[17]</sup>

The causes of CPSP and its relationship with acute pain are still not clear. The possible mechanisms include: pain amplification mechanism caused by peripheral and central sensitization, inhibitory regulation of central nervous system and sympathetic nervous system regulation mechanism.<sup>[17]</sup> Inflammatory reactions, especially peripheral and central nervous system inflammation, play an important role in the formation of CPSP.<sup>[18]</sup> The repair process of peripheral tissue injury after operation includes three stages: inflammatory reaction, inflammatory cell infiltration, and tissue remodeling. The pain caused by acute inflammatory response is mainly caused by the release of inflammatory neuropeptides, including substance P, calcitonin gene-related peptide (CGRP), and C-nerve fiber neurokinin A (NKA); all of them are important priming factors in the process of neural system remodeling.<sup>[19]</sup> Acute surgical trauma can lead to severe pain and pain hypersensitivity, but it usually relieves as the wound heals. The duration of acute inflammatory reaction is related to wound healing speed and the patient's basic physical condition, which lasts for 24h to 2 weeks. When the wound repair is not good, the chronic inflammatory reaction in the injured site of hand operation can persist, leading to the occurrence of CPSP.<sup>[20]</sup>

The *CCL2* gene is located in 17q12 and consists of three exons and two introns. There is a proximal and a distal regulatory regions in the *CCL2* gene, both located upstream of the transcription start point, and the proximal regulatory region can bind to transcription factors, affecting the level of transcription of the gene.<sup>[21]</sup> The distal regulatory region contains a NF- $\kappa$ B binding site necessary for cytokine to have *CCL2* expression.<sup>[22]</sup> *CCL2* gene rs4586 is located in the second exon, and this mutation is associated with the occurrence of various diseases. For example, Sunakawa et al<sup>[23]</sup> showed that *CCL2* gene rs4586 is associated with gastric cancer progression. We believe that this mutation may affect the expression of *CCL2* protein, because from the results of this study, the level of *CCL2* protein in the serum of mutant individuals is obviously higher, and the corresponding clinical symptoms show that the risk of CPSP in mutant individuals is lower. However, it is not yet fully confirmed that *CCL2* gene rs4586 locus SNP affects the expression of *CCL2* protein and leads to the occurrence and development of CPSP, because we lack cell model validation. The level of *CCL2* protein in serum does not fully reflect the expression level of this protein, as other factors may influence the level of *CCL2* protein in serum.<sup>[24]</sup>

The *CALCA* gene encodes calcitonin gene-related peptide (CGRP), which is an important priming factor in the process of neural system remodeling. Lassen et al<sup>[25]</sup> found that the increase in CGRP observed during the onset of spontaneous migraine suggests that CGRP may play a role in the pathogenesis of spontaneous migraine. Our study also showed that CGRP levels were higher in the CPSP group, which is consistent with the results of Lassen's study. The *CALCA* level of *CALCA* gene rs3781719 site mutation (CC) carriers was significantly higher than that of other genotype carriers. This phenomenon suggests that *CALCA* gene rs3781719 site mutation may be the intrinsic

cause of *CALCA* expression level change, and further research is needed.

Chemokine (C-X3-C motif) ligand 1 gene (*CX3CL1*, also known as fractalkine), located at 16q13, is a member of CX3C chemokines and plays an important role in various diseases.<sup>[15,26–28]</sup> *CX3CL1* rs614230 gene SNP is a disease-related site, and Jin et al<sup>[29]</sup> showed that the change of point SNP was associated with coronary artery disease (CAD). The results of this study showed that this mutant was a protective factor for chronic pain. The serum *CX3CL1* level in mutants was also significantly lower than that in wild-type. We speculate that this correlation may be the cause of CPSP. The wild-type individual has a higher level of *CX3CL1*, which is a pro-inflammatory cytokines can act directly or indirectly on noxious sensory receptors, activation of many complex signal transduction pathways, such as protein kinase A, protein excitase C, p38 mitogen-activated protein kinase (MAPK), in order to reduce the excitatory threshold of peripheral neurons and produce short-term peripheral sensitization.<sup>[8]</sup>

In addition, we also studied whether *CCL2*, *CALCA*, and *CX3CL1* genes have synergistic effects on CPSP, and the results show that there is a potential gene–gene interaction between the *CALCA* gene rs3781719 locus and the *CX3CL1* gene rs614230 locus. Gene–gene interactions and gene–environment interactions may be major factors influencing the occurrence of CPSP. The role of a single gene may be very limited, so screening for more genes and related environmental factors, and analyzing the effects of gene–gene and gene–environmental factors may be very meaningful.

The results of our study suggest an association between *CCL2*, *CALCA*, and *CX3CL1* gene polymorphisms and CPSP. Nevertheless, this hypothesis requires further investigation, and it may be necessary to expand the sample size and set up in vitro cell experiments.

## 5. Conclusion

*CCL2* gene rs4586, *CALCA* rs3781719, *CX3CL1* rs614230 gene polymorphism are associated with the occurrence of chronic pain after cesarean section in Chinese Han women.

## Author contributions

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## References

- [1] Haroutiunian S, Nikolajsen L, Finnerup NB, et al. The neuropathic component in persistent postsurgical pain: a systematic literature review. *Pain* 2013;154:95–102.
- [2] Shanthanna H, Aboutouk D, Poon E, et al. A retrospective study of open thoracotomies versus thoracoscopic surgeries for persistent postthoracotomy pain. *J Clin Anesth* 2016;35:215–20.
- [3] Wang HT, Liu W, Luo AL, et al. Prevalence and risk factors of chronic post-thoracotomy pain in Chinese patients from Peking Union Medical College Hospital. *Chin Med J (Engl)* 2012;125:3033–8.

- [4] Deumens R, Steyaert A, Forget P, et al. Prevention of chronic postoperative pain: cellular, molecular, and clinical insights for mechanism-based treatment approaches. *Prog Neurobiol* 2013;104:1–37.
- [5] Peng J, Gu N, Zhou L, et al. Microglia and monocytes synergistically promote the transition from acute to chronic pain after nerve injury. *Nat Commun* 2016;7:12029.
- [6] Richebe P, Julien M, Brulotte V. Potential strategies for preventing chronic postoperative pain: a practical approach: Continuing Professional Development. *Can J Anaesth* 2015;62:1329–41.
- [7] Chapman CR, Vierck CJ. The transition of acute postoperative pain to chronic pain: an integrative overview of research on mechanisms. *J Pain* 2017;18:359e1–38.
- [8] Ellis A, Bennett DL. Neuroinflammation and the generation of neuropathic pain. *Br J Anaesth* 2013;111:26–37.
- [9] Kiguchi N, Kobayashi Y, Kishioka S. Chemokines and cytokines in neuroinflammation leading to neuropathic pain. *Curr Opin Pharmacol* 2012;12:55–61.
- [10] Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain* 2011;152:S2–15.
- [11] Zhang ZJ, Dong YL, Lu Y, et al. Chemokine CCL2 and its receptor CCR2 in the medullary dorsal horn are involved in trigeminal neuropathic pain. *J Neuroinflammation* 2012;9:136.
- [12] Teler J, Tarnowski M, Safranow K, et al. CCL2, CCL5, IL4 and IL15 gene polymorphisms in women with gestational diabetes mellitus. *Horm Metab Res* 2017;49:10–5.
- [13] Sutherland HG, Buteri J, Menon S, et al. Association study of the calcitonin gene-related polypeptide-alpha (CALCA) and the receptor activity modifying 1 (RAMP1) genes with migraine. *Gene* 2013;515:187–92.
- [14] Poniatowski LA, Wojdasiewicz P, Krawczyk M, et al. Analysis of the role of CX3CL1 (Fractalkine) and its receptor CX3CR1 in traumatic brain and spinal cord injury: insight into recent advances in actions of neurochemokine agents. *Mol Neurobiol* 2017;54:2167–88.
- [15] Kehlen A, Greither T, Wach S, et al. High coexpression of CCL2 and CX3CL1 is gender-specifically associated with good prognosis in soft tissue sarcoma patients. *Int J Cancer* 2014;135:2096–106.
- [16] Lou XY, Chen GB, Yan L, et al. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. *Am J Hum Genet* 2007;80:1125–37.
- [17] Weibel S, Neubert K, Jelting Y, et al. Incidence and severity of chronic pain after caesarean section: a systematic review with meta-analysis. *Eur J Anaesthesiol* 2016;33:853–65.
- [18] Omoigui S. The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3—inflammatory profile of pain syndromes. *Med Hypotheses* 2007;69:1169–78.
- [19] Talbot RM, McCarthy KF, McCrory C. Central and systemic inflammatory responses to thoracotomy—potential implications for acute and chronic postsurgical pain. *J Neuroimmunol* 2015;285:147–9.
- [20] Rashid S, Dick BD. Post-surgical pain syndromes: a review for the non-pain specialist. *Can J Anaesth* 2014;61:123–30.
- [21] Sato S, Sakurai T, Ogasawara J, et al. A circadian clock gene, *Rev-erbalpha*, modulates the inflammatory function of macrophages through the negative regulation of *Ccl2* expression. *J Immunol* 2014;192:407–17.
- [22] Messeha SS, Zarmouh NO, Mendonca P, et al. The inhibitory effects of plumbagin on the NF- $\kappa$ B pathway and CCL2 release in racially different triple-negative breast cancer cells. *PLoS One* 2018;13:e0201116.
- [23] Sunakawa Y, Stremtitz S, Cao S, et al. Association of variants in genes encoding for macrophage-related functions with clinical outcome in patients with locoregional gastric cancer. *Ann Oncol* 2015;26:332–9.
- [24] McDermott DH, Yang Q, Kathiresan S, et al. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. *Circulation* 2005;112:1113–20.
- [25] Lassen LH, Haderslev PA, Jacobsen VB, et al. CGRP may play a causative role in migraine. *Cephalalgia* 2002;22:54–61.
- [26] Ferretti E, Pistoia V, Corcione A. Role of fractalkine/CX3CL1 and its receptor in the pathogenesis of inflammatory and malignant diseases with emphasis on B cell malignancies. *Mediators Inflamm* 2014;2014:480941.
- [27] Guo Y, Apostalakis S, Blann AD, et al. Plasma CX3CL1 levels and long term outcomes of patients with atrial fibrillation: the West Birmingham Atrial Fibrillation Project. *Cerebrovasc Dis* 2014;38:204–11.
- [28] Tsang JY, Ni YB, Chan SK, et al. CX3CL1 expression is associated with poor outcome in breast cancer patients. *Breast Cancer Res Treat* 2013;140:495–504.
- [29] Jin SG, Chen GL, Yang SL, et al. Gene-gene interactions among CX3CL1, LEPR and IL-6 related to coronary artery disease in Chinese Han population. *Int J Clin Exp Pathol* 2015;8:5968–73.