Retrospective Assessment

Genotypic Analysis of SCN9A for Prediction of Postoperative Pain in Female Patients Undergoing Gynecological Laparoscopic Surgery

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Background: The SCN9A gene product is a critical component in human pain perception. Recent studies found that single-nucleotide polymorphisms (SNPs) in this gene contributed to the risk and severity of common pain phenotypes.

Objectives: In this study, we aimed to assess the use of SCN9A SNP screening for predicting postoperative pain.

Study Design: A retrospective assessment of patients who underwent gynecological laparoscopic surgery.

Setting: Department of anesthesiology, a teaching hospital, in a medical college, major metropolitan city, China.

Methods: Twenty-nine candidate and tag SCN9A SNPs were analyzed in this study. Four hundred twenty-one patients who underwent gynecological laparoscopic surgery and refused postoperative patient controlled analgesia (PCA) were recruited and completed the study protocol. An additional 578 patients who voluntarily received PCA treatment were included for verification. Postoperative pain intensity was evaluated in all patients using numerical rating scale (NRS), and for patients receiving PCA analgesic requirements were also recorded.

Outcomes Assessment: The outcome was assessment of postoperative pain NRS and PCA analgesic requirements.

Results: Ten different SCN9A SNPs exhibited significant associations with postoperative pain intensity, the incidence of severe postoperative pain, and postoperative PCA requirement. Of the candidate SCN9A SNPs, there was a statistically significant correlation between SNP rs6746030 and higher maximum NRS scores during the postoperative follow-up of non-PCA patients ($P < 0.05$). Furthermore, there was a significant association between the tag SNP rs4286289 and both increased postoperative maximum NRS scores ($P < 0.05$) and higher incidences of severe postoperative pain ($P < 0.05$) in non-PCA patients. Meanwhile, in PCA patients, rs4286289 exhibited the strongest association ($P = 0.001$) with increased requirements for postoperative analgesics, which indirectly strengthened the significant association between this SNP and higher postoperative pain.

Limitations: The limitations of this study include that it is an assessment of only Chinese women scheduled for gynecological laparoscopic surgery.

Conclusion: The current study provides evidence that postoperative pain was affected by SCN9A variability in gynecological patients. Notably, our results provide the first indication that SCN9A SNP rs4286289 can be used as a predictor for hypersensitivity to postoperative pain.

Key words: SCN9A, single-nucleotide polymorphisms, genotypic analysis, postoperative pain, female patients, gynecological laparoscopic surgery, genetic markers for pain, predictors of postoperative pain

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More than 230 million surgical procedures are performed each year (1), and surgery is the most common and predictable source of pain (2). However, inadequate postoperative pain control is still a clinical issue that urgently needs to be addressed (3,4). Indeed, implementation of appropriate preoperative screening methods for determining inter-individual differences in postoperative pain sensitivity might enable personalized postoperative pain therapies and improve postoperative pain control (5-7). Heritability studies demonstrated that genetic variation could explain a significant portion of the variability in human pain sensitivity (8-10). Therefore, genetic analyses might be an effective and promising method for predicting postoperative pain sensitivity (11).

The *SCN9A* gene, which encodes the voltage-gated sodium-channel type IV-α subunit (Nav1.7), is critical for human pain sensitivity (12,13). Whereas inactivating mutations in *SCN9A* resulted in congenital insensitivity to pain (14,15), gain-of-function mutations in this gene resulted in distinct pain syndromes such as inherited erythromelalgia and paroxysmal extreme pain disorder (16,17). Furthermore, recent studies identified genetic polymorphisms in *SCN9A* (e.g., rs6746030) that contributed to the risk or severity of more common pain phenotypes (18-20). Based on these findings, we speculated that Nav1.7 may be involved in the modulation of pain sensitivity within the general population, and that *SCN9A* gene screening could therefore serve as an optimal method for predicting postoperative pain.

Several studies have explored the effects of genetic variations, including variations in the genes encoding the mu-opioid receptor (21,22) and catechol-o-methyltransferase (23), on postoperative analgesia, and they proposed a way to predict which patients have greater analgesic requirements. However, it is quite difficult to accurately quantify the postoperative pain of the patients in these studies due to the application of persistent postoperative analgesia, which could alter these patients' pain perception. At the time of this study, there were multiple patients undergoing gynecological laparoscopic surgery in our hospital, who refused postoperative patient controlled analgesia (PCA) treatment. We therefore chose to recruit such patients, in addition to patients receiving PCA therapy, to investigate the association between *SCN9A* single-nucleotide polymorphisms (SNPs) and postoperative pain.

While recent studies investigated associations between SNPs in *SCN9A* and common pain phenotypes (18,20,24,25), these studies focused on only one SNP. Furthermore, no previous studies have comprehensively investigated the associations between *SCN9A* SNPs and postoperative pain. Therefore, in this study, we utilize a combined candidate and tag approach to select *SCN9A* SNPs. Using this approach, this study aimed to assess the potential of *SCN9A* genotypic analyses for predicting postoperative pain.

Methods

Patients

From August 2013 to August 2014, a total of 1,025 patients, aged 18 – 65, who were scheduled for gynecological laparoscopic surgery under general anesthesia were recruited into the present study. The study protocol was approved by the Institutional Ethics Committee, and was registered in ClinicalTrial.gov (ID: NCT01950078). Prior to the study, written informed consent was obtained from all participants.

Participants were included or rejected from the study based on set inclusion and exclusion criteria. The inclusion criteria were as follows: Han Chinese; and grouped based on the American Society of Anesthesiologists physical status I–II. The exclusion criteria were as follows: incapable of communicating; smoking, alcohol, or drug abuse; use of any analgesic medication over the previous 4 weeks.

Preoperative Management

On the day prior to the operation, research assistants screened patients on the gynecological wards. As shown in Fig. 1, of the enrolled patients, 424 (referred to as the non-PCA group) refused PCA. The other 581 patients (PCA group) voluntarily received PCA treatment and were included as a replication sample for verification in the final analysis (Fig. 1). Demographic data, including age, height, weight, history of dysmenorrhea (yes or no), and whether the patient underwent prior tumor excision surgery (yes or no), were collected. All patients were instructed on the parameters for assessing postoperative pain intensity (numerical rating scale [NRS] where 0 = no pain and 10 = unbearable pain) during the postoperative follow-up, and patients who voluntarily received PCA treatment were trained to use the analgesic pump prior to the operation.

Anesthetic Technique

Patients' blood pressure, electrocardiography, and pulse oxygen saturation were monitored in the operat-

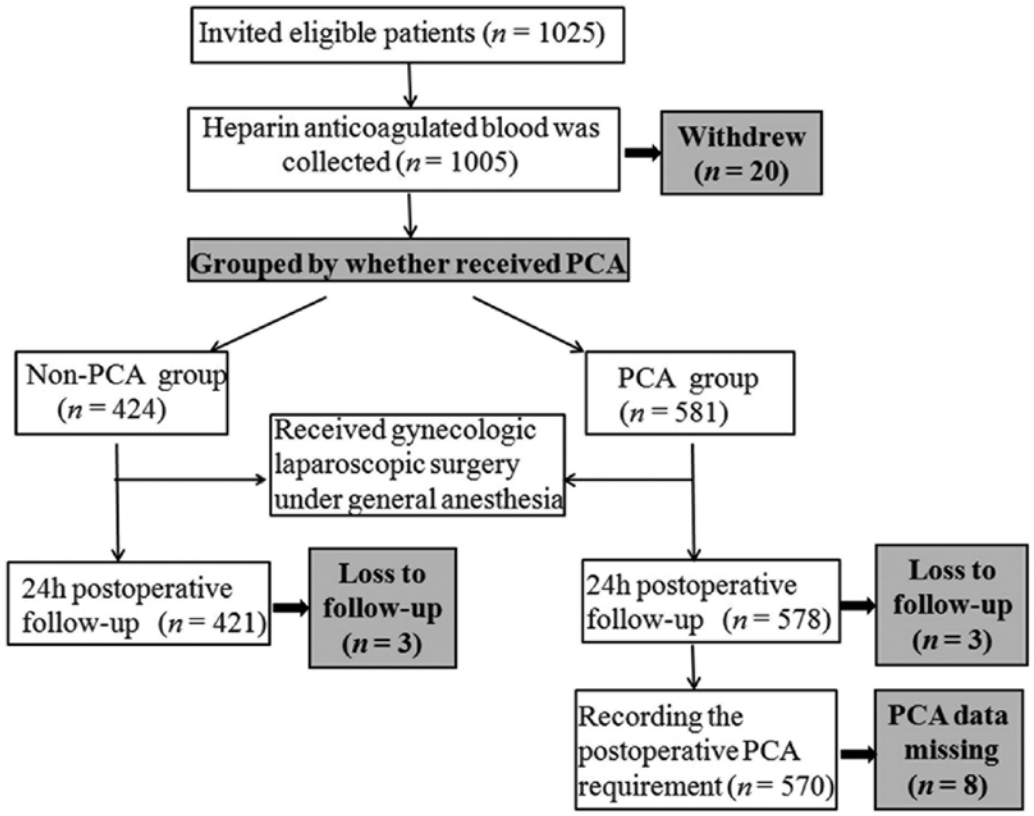

Fig. 1. *Flow chart depicting the set-up of the study. PCA = patient controlled analgesia.*

ing room, and a blood specimen was collected for DNA isolation prior to the operation. Standardized general anesthesia was utilized for all patients, and included 0.05 mg/kg midazolam, 2 mg/kg propofol, 0.5 μg/kg sufentanil, and 0.6 mg/kg rocuronium. Anesthesia was maintained using a combined intravenous and inhalation approach: inhalation of 1 – 2% sevoflurane, infusion of remifentanil (0.2 – 0.4 μg·kg-1·min-1) and propofol (6 – 10 mg·kg-1·h-1), and intravenous boluses of rocuronium (0.2 mg/kg). The Narcotrend system (MonitorTechnik, Bad Bramstedt, Germany) was used to maintain the depth of anesthesia.

Postoperative Pain Treatment and Assessment

Standard analgesia, according to the protocol of acute pain service (APS), including preoperative analgesia and postoperative intravenous PCA, was provided for the patients in the PCA group. At 15 minutes prior to incision, 40 mg of parecoxib sodium and 2 mg of tropisetron hydrochloride were administered intravenously for postoperative pain treatment and prevention of postoperative nausea and vomiting, respectively. The PCA pump was started immediately following surgery, and patients were provided sufentanil (0.5 μg/mL) and tramadol (5 mg/mL). The pump was programmed to use a loading dose of 2 mL, a background infusion rate of 1.5 – 2 mL/h, a PCA dose of 1 mL, and a lockout period of 10 minutes. Meanwhile, the patients in the non-PCA group were provided pain treatment on demand with loading doses of nonopioid analgesia, e.g., parecoxib sodium or diclofenac sodium suppositories.

The rest and movement NRS pain scores were recorded for all patients at 30 minutes after surgery in the post-anesthesia care unit (PACU), and again at 9 to 12 hours, and at 21 to 24 hours post-surgery during patient follow-up. Pain was assessed during motion by asking the patient to cough. As in some previous studies (26,27), patients presenting NRS scores ≥ 6 while at rest at any of these time points were grouped as indi-

viduals that experience severe postoperative pain (SPP) while at rest. Meanwhile, patients who recorded NRS scores ≥ 6 during movement were grouped as individuals that experience SPP during motion. The postoperative outcomes of the present study were the maximum NRS values that patients presented during the postoperative follow-ups, and also the incidence of SPP at rest and during motion. Furthermore, for patients in the PCA group, the total PCA consumption from the PCA pump at 24 hours post-operation were recorded at the end of analgesic therapy. To exclude any impact due to differences in patients' weights, the data were analyzed as mL/kg of body weight.

SNP Selection and Genotyping Analysis

A combined candidate and tag SNP approach was used to select SNPs within *SCN9A*. The tag SNP selection was based on phase 3 data of the HapMap CHB reference population database and was performed using the Tagger program included in the Haploview 4.2 software (28). The aim was to capture all SNPs with a minor allele frequency of greater than 5% in the HapMap database by setting the limit for the pair-wise $r^2 \geq 0.8$. Twenty-five tag SNPs, including rs4369876 (18) and rs6746030 (19), were selected to capture 100% of the allelic variation in 119 SNPs across the entire gene, with a mean r^2 value of 0.963. Three additional SNPs (rs7595255, rs11898284, and rs12622743), which were selected based on their potential association with the human pain perception phenotype, were not available in the phase 3 data (19,29). In addition, 4 SNPs (rs74401238, rs58022607, rs12478318, and rs41268673) were selected based on their positions in the exonic areas and whether they induced amino acid substitutions.

Genomic DNA was extracted from blood samples using the guanidiniumisothiocyanate method. Genotyping of the 32 SNPs was performed by the Shanghai BioWing Applied Biotechnology Company (www.biowing.com.cn/) using ligase detection reactions (LDRs).

Statistical Analyses

All variables were summarized using standard descriptive statistics. Demographic analyses, Pearson's Chi-square tests, and analysis of variance (ANOVA) were conducted using the SPSS Statistics Version 17.0 statistical package (SPSS Statistics, Inc., Chicago, IL, USA). A 2-tailed probability value of $P < 0.05$ was used as the criterion for judging statistical significance. Genetic association analyses between *SCN9A* SNPs and the observed data were conducted using PLINK Version 1.07 (30). Summary statistics were calculated, and the samples from the PCA and non-PCA groups were tested to determine whether the null hypothesis of the Hardy-Weinberg equilibrium (HWE) model could be rejected by applying the chi-square method. SNPs exhibiting deviation from the HWE ($P < 0.01$) were excluded from the final analysis.

Logistic regression analysis was used to investigate associations between the *SCN9A* SNPs and the presentation of SPP. Associations between the *SCN9A* SNPs and the PCA requirements for patients in the PCA group were examined using linear regression analysis. Three models, including additive, dominant, and recessive models, were considered. Association analyses were performed by adjusting for potential confounding variables, including age, body mass index (BMI), history of dysmenorrhea (yes or no), and whether the patient underwent prior tumor excision surgery (yes or no). For logistic regression analyses, patients were classified by age (18 – 40 years and 41 – 65 years) and BMI (< 18.5, 18.5 – 24, and ≥ 24).

Results

General Results

As depicted in Fig. 1, 20 patients chose to withdraw from the study, and a total of 6 patients were lost to follow-up during the study procedure. Therefore, the information from 421 patients in the non-PCA group and 578 patients in the PCA group were included for the analyses of postoperative pain, respectively. In addition, due to missing PCA data, 570 of the patients in the PCA group were included for the analyses of PCA requirements (Fig. 1). The demographic and perioperative data from these patients are shown in Table 1. There were statistical differences between the PCA and non-PCA groups in age ($P < 0.001$), weight ($P < 0.001$), BMI ($P < 0.001$), surgery during ($P < 0.001$) and surgery status ($P < 0.001$).

Of the 32 SNPs detected in this study, 3 (rs74401238, rs41268673, and rs58022607) exhibited no polymorphisms in our samples and were excluded. The HWE values, minor allele frequencies, and genotype counts of the other 29 SNPs for patients in the non-PCA and PCA groups are presented in Appendix 1. The total success rates for non-PCA and PCA patients were 0.981 and 0.977, respectively. No SNP in the samples from the PCA and non-PCA groups were rejected by HWE tests (P for each > 0.01, Fig. 2).

Table 1. *Demographic and perioperative data.*

	Non-PCA group (n = 421)	PCA group (n = 578)	*P* value
Age (year)	33.8 ± 9.8	41.9 ± 10.4	< 0.001
Height (cm)	160.6 ± 4.6	160.1 ± 5.0	0.078
Weight (kg)	55.9 ± 8.5	58.3 ± 9.4	< 0.001
BMI (kg/m2)	21.6 ± 3.1	22.7 ± 3.5	< 0.001
Tumor excision surgery (yes/no)	123 (29.2%)/298 (70.8%)	349 (60.4%)/229 (39.6%)	< 0.001
History of dysmenorrhea (yes/no)	32 (7.6%)/389 (92.4%)	55 (9.5%)/523 (90.5%)	0.289
Surgery duration (min)	123.9 ± 59.3	168.5 ± 70.7	< 0.001
Maximum rest NRS	2.8 ± 2.3	3.0 ± 2.6	0.149
Maximum movement NRS	3.5 ± 2.5	3.8 ± 2.7	0.070
SPP at rest (yes/no)	57 (13.5%)/364 (86.5%)	109 (18.9%)/469 (81.1%)	0.026
SPP during motion (yes/no)	86 (20.4%)/335 (79.6%)	146 (25.3%)/432 (74.7%)	0.074

Data were presented as mean ± S.D. or as numbers (percentage).
BMI = body mass index; NRS = numerical rating scale; PCA = patient controlled analgesia; SPP = severe postoperative pain

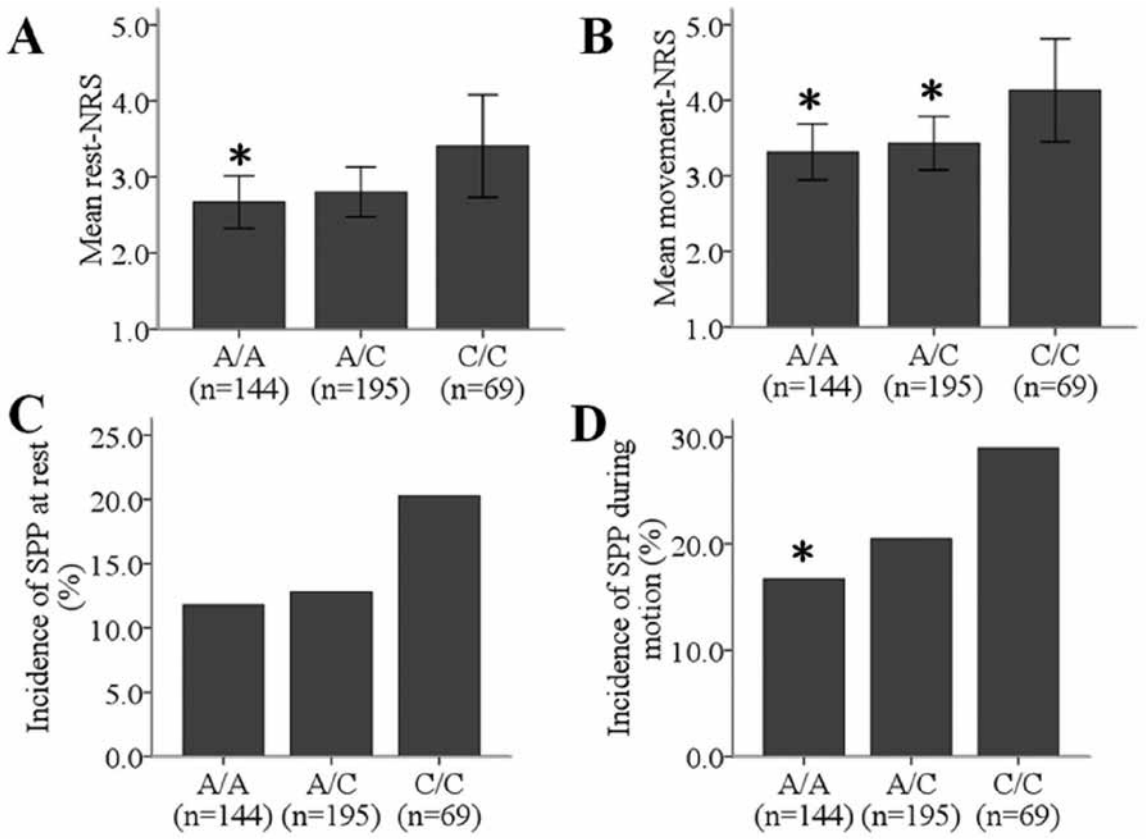

Fig. 2. *The mean maximum numerical rating scale (NRS) values at rest (A) and during movement (B), and the incidences of severe postoperative pain (SPP) at rest (C) and during motion (D) associated with the different rs4286289 genotypes during the postoperative follow-ups.*
* $P < 0.05$, compared to C/C.

The Effects of *SCN9A* SNPs on Postoperative Pain in the Non-PCA Group

First, we compare the maximum NRS values presented by non-PCA patients with different *SCN9A* SNPs during the postoperative follow-ups. Three SNPs, i.e., candidate SNP rs6746030 and the tag SNPs rs9646772 and rs4286289, exerted significant effects on the maximum NRS scores (Table 2). Three patients carried minor homozygotes of SNP rs6746030. As a result, these 3 patients were not included in the comparison. As shown in Table 2, the mean maximum NRS value for heterozygote carriers of rs6746030 was higher than that of the major homozygote carriers, both at rest ($P = 0.010$) and during movement ($P = 0.039$). As shown in Fig. 2, the mean rest maximum NRS value for minor homozygote carriers of rs4286289 was higher than that of the major homozygote carriers ($P = 0.031$), and approximately significantly higher than heterozygote carriers ($P = 0.065$). And the patients who carried minor homozygote of rs4286289 showed higher movement NRS than those who carried heterozygote ($P = 0.044$) and major homozygote ($P = 0.025$). Furthermore, no significant differences ($P > 0.05$) were found in age, BMI, surgery status, or history of dysmenorrhea among non-PCA patients with different genotypes of these 3 SNPs.

Next, we utilized logistic regression analysis to explore the ability of *SCN9A* SNPs to predict SPP (NRS ≥ 6). In the non-PCA group, 5 SNPs, depending on the model used, were associated ($P < 0.05$) with SPP (Table 3). Of these, rs4286289 was associated with SPP both at rest ($P = 0.034$) and during motion ($P = 0.033$) in the recessive model, and with SPP at rest in the additive model ($P = 0.031$). The respective odds rates (OR) for rs4286289 as a predictor of SPP at rest and during motion were 2.13 (95% CI: 1.06 to 4.30) and 1.92 (95% CI: 1.05 to 3.51) in the recessive model, respectively, indicating that a minor effect of rs4286289 was to increase the risk of presenting SPP. The actual incidences of SPP in non-PCA patients of the different rs4286289 genotypes (A/A vs. A/C vs. C/C; 11.8% vs. 12.8% vs. 20.3% for SPP at rest; and 16.7% vs. 20.5% vs. 29.0% for SPP during motion, respectively) are shown in Fig. 2.

The Effects of *SCN9A* SNPs on Postoperative Pain in the PCA group

In contrast to the non-PCA group, there was no SNP that exhibited a significant association with the maximum NRS values in the PCA group during the postop-

Table 2. *Maximum NRS values associated with different SNPs during the postoperative follow-up of patients in the Non-PCA group.*

SNP	Pain perception phenotypes	Major homozygote	Heterozygote	Minor homozygote	*P* value
rs6746030	Rest NRS	2.8 ± 2.2	3.9 ± 2.9*	Null	0.010
	Movement NRS	3.4 ± 2.4	4.4 ± 2.9*	Null	0.039
rs9646772	Rest NRS	3.1 ± 2.4	2.6 ± 2.2*	3.0 ± 2.3	0.076
	Movement NRS	3.9 ± 2.6	3.1 ± 2.3*	3.6 ± 2.4	0.020
rs4286289	Rest NRS	2.6 ± 2.1	2.8 ± 2.3	3.4 ± 2.8*	0.088
	Movement NRS	3.3 ± 2.2	3.4 ± 2.5	4.1 ± 2.8*†	0.068

Data were presented as mean ± S.D.
SNP = single nucleotide polymorphism; NRS = numerical rating scale;
*Significant difference ($P < 0.05$) between heterozygote, minor homozygote, and major homozygote; †significant difference ($P < 0.05$) between minor homozygote and heterozygote

Table 3. *The use of SCN9A SNPs for prediction of severe postoperative pain in the non-PCA group.*

Pain phenotype	Model	SNP	OR (95%CI)	Observed *P* Value
SPP at rest	Recessive model	rs12619987	2.80 (1.24 to 6.32)	0.013
		rs4286289	2.13 (1.06 to 4.30)	0.034
SPP during motion	Additive model	rs4286289	1.47 (1.05 to 2.09)	0.031
	Dominant model	rs9646772	0.52 (0.32 to 0.84)	0.008
		rs6724623	0.85 (0.34 to 0.92)	0.023
		rs6739404	0.58 (0.35 to 0.96)	0.033
	Recessive model	rs4286289	1.92 (1.05 to 3.51)	0.033

OR = odds rate; PCA = patient-controlled analgesia; SNP = single nucleotide polymorphism; SPP = severe postoperative pain

erative follow-ups. The results of the logistic regression analysis for predicting severe postoperative pain (NRS ≥ 6) demonstrated that rs4303728 and rs13017637 were associated with SPP at rest in patients in the PCA group. Specifically, rs4303728 was associated with SPP in the recessive model (OR = 3.02 [95% CI: 1.14 to 7.97], *P* = 0.026), while rs13017637 was associated with SPP (OR = 0.56 [95% CI: 0.33 to 0.95], *P* = 0.033) in the dominant model; however, no SNP exhibited a significant correlation with SPP during motion.

Associations between the *SCN9A* SNPs and PCA Requirements in the PCA Group

Four SNPs exhibited significant associations with PCA requirements in patients from the PCA group (Table 4). Of these, rs4286289 exhibited the strongest link (*P* = 0.001) to increased PCA requirements. The regression coefficients for rs4286289 were 0.05, 0.06, and 0.08 in the additive, dominant, and recessive models, respectively. As shown in Fig. 3, ANOVA analysis detected significant differences among the patients who carried different SNPs at rs4286289 (C/C vs. A/C vs. A/A; 0.76 ± 0.25 vs. 0.69 ± 0.26 vs. 0.66 ± 0.24, respectively; *P* = 0.006), rs4387806 (C/C vs. C/T vs. T/T; 0.71 ± 0.26 vs. 0.66 ± 0.23 vs. 0.59 ± 0.24, respectively; *P* = 0.028), rs16851799 (T/T vs. C/T vs. C/C; 0.78 ± 0.24 vs. 0.71 ± 0.27 vs. 0.67 ± 0.25, respectively; *P* = 0.039), and rs12994338 (C/C vs. C/T vs. T/T; 0.72 ± 0.27 vs. 0.66 ± 0.22 vs. 0.67 ± 0.29, respectively; *P* = 0.019).

Discussion

In this study, we explored the associations between 29 *SCN9A* SNPs with postoperative pain in gynecological patients. A novel association between rs4286289 and postoperative pain was identified in non-PCA patients, and these results were strengthened by the significant association detected between this SNP and PCA analgesic requirements in PCA patients.

Previous studies have established that gender is a factor that affects pain sensitivity (31,32). Therefore, to avoid this issue, only women were recruited for this study. Indeed, certain patients receiving gynecological laparoscopic surgery in our hospital refused postoperative PCA because of both subjective factors, such as fear of addiction, and objective factors, including economic issues. In addition, according to "anticipated pain level" (33), the lower abdominal laparoscopic gynecological surgery is classified as "minor" to "moderate" surgery, which might be another reason that patients refused PCA pretreatment. Regardless, this made it possible to recruit patients that were free of postoperative persistent analgesia, thereby reducing the effects of analgesics on postoperative pain.

Moreover, selecting such a population allowed us to eliminate potential differences based on surgical sites and surgical methods, which were previously shown to affect postoperative pain intensity and analgesic requirements (7). Based on these reasons, we selected gynecological laparoscopic surgery patients as participants in this study to investigate the effects of *SCN9A* mutations on postoperative pain. Furthermore, our results showed that the percentage of patients who underwent tumor excision surgery in the PCA group (60.4%) was markedly greater than that in the non-PCA group (29.2%), and that there were significant differences between the 2 groups in certain characteristic data, including age, BMI, and surgery duration. These findings indicated that the 2 populations were not homogenous, and that the PCA population could therefore be considered independent to the non-PCA

Table 4. *Statistically significant associations between PCA requirements and SCN9A SNPs in the PCA group.*

PCA requirement (mL/kg)	SNP	β	Observed *P* Value
Additive model	rs4286289	0.05 (0.02 to 0.08)	0.001
	rs4387806	-0.05 (-0.09 to -0.01)	0.009
	rs16851799	0.04 (0.01 to 0.08)	0.016
	rs12994338	-0.04 (-0.08 to -0.01)	0.015
Dominant model	rs4286289	0.06 (0.01 to 0.10)	0.013
	rs4387806	-0.05 (-0.10 to -0.01)	0.022
	rs16851799	0.05 (0.00 to 0.09)	0.037
	rs12994338	-0.06 (-0.10 to -0.02)	0.005
Recessive model	rs4286289	0.08 (0.02 to 0.14)	0.006

β = regression coefficients; PCA = patient-controlled analgesia; SNP = single nucleotide polymorphism

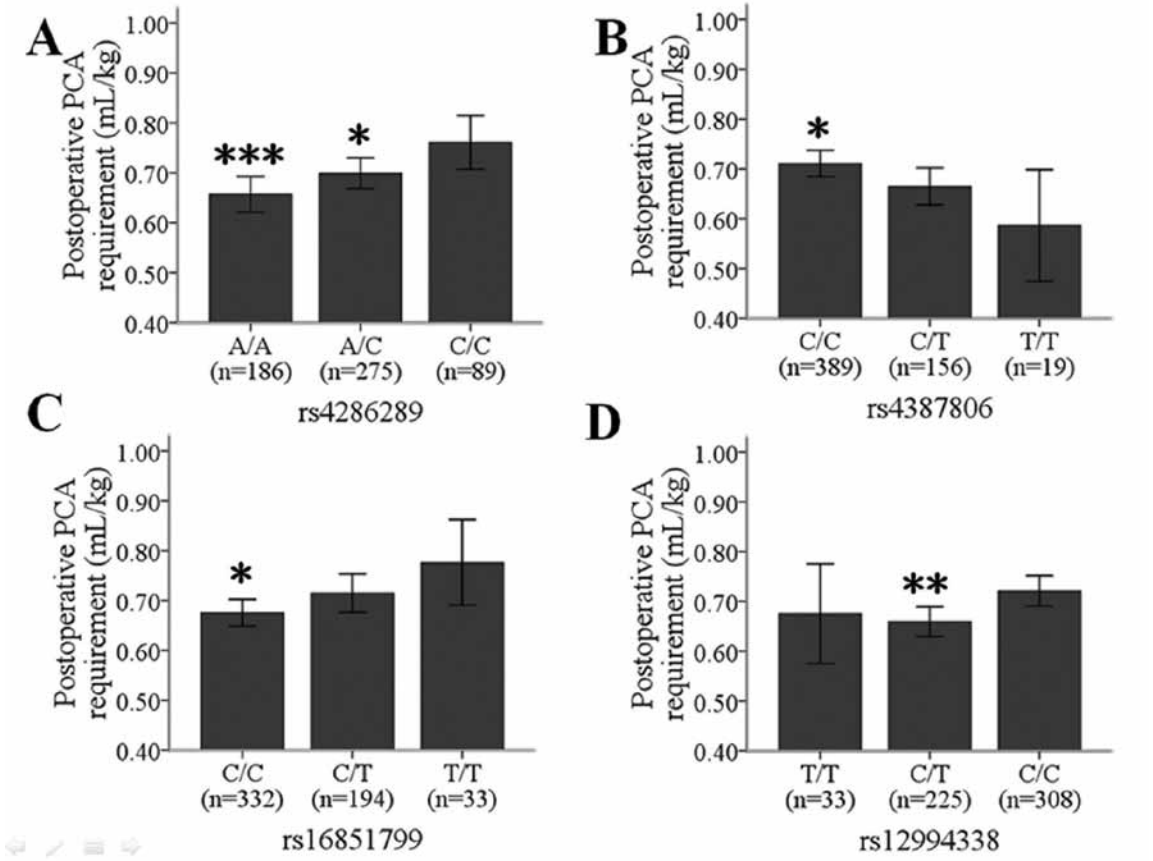

Fig. 3. *The mean postoperative patient controlled analgesia (PCA) requirements for each rs4286289 (A), rs4387806 (B), rs16851799 (C), and rs12994338 (D) genotype.*
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared to the third genotype in each graph.

population, making it possible to verify the association results in the non-PCA group.

Three *SCN9A* SNPs were associated with significant effects on postoperative pain intensity, and 5 SNPs exhibited significant associations with SPP in the non-PCA patients. Nevertheless, these significant links between *SCN9A* SNPs and either postoperative NRS values or the presentation of SPP were not replicated in the PCA group. Previous studies showed that Nav1.7, which is encoded by the *SCN9A* gene, accumulates at peripheral nociceptive neurons and amplifies small subthreshold depolarizations, suggesting that this protein acts as a major contributor to pain signaling in humans (34,35). Therefore, it is conceivable that *SCN9A* SNPs have a significant effect on postoperative pain in the non-PCA group. Conversely, in the PCA group, all patients received postoperative persistent analgesia, which had the potential to alter the patients' pain sensitivity (36,37), and thereby reduce or eliminate the actual associations between postoperative pain and the different *SCN9A* SNPs. Obviously, it was necessary to include patients who did not receive postoperative persistent analgesia when exploring potential associations between the *SCN9A* SNPs and postoperative pain.

Of the candidate *SCN9A* SNPs analyzed in the current study, only the minor effects of rs6746030 were found to be significantly linked to increases in the mean maximum NRS values during the postoperative follow-ups of non-PCA patients. As we know, rs6746030 was first reported to be associated with increased pain in patients with osteoarthritis, and the functional effects of this SNP were confirmed by patch-clamp experiments (19,38). Therefore, the effect direction of rs6746030 on pain sensitivity in the current study was the same as this

initial finding, partly indicating that the patients and methods included in our study were reliable for exploring the associations between *SCN9A* SNPs and postoperative pain.

In addition to these candidate SNPs, we detected a significant association between the tag SNP rs4286289 and the higher mean maximum NRS values. Furthermore, we observed that only rs4286289 was a significant predictor of both SPP at rest and during motion in the patients of the non-PCA group. These findings indicated that rs4286289 exhibited a steady association with the incidence of SPP. Meanwhile, the ORs of rs4286289 were 2.13 and 1.92 in the recessive model, suggesting that minor homozygote carriers (69/408, 16.9%) of this SNP were more hypersensitive to postoperative pain, which was consistent with the significant effect of this SNP on the mean maximum NRS value. Therefore, rs4286289 could potentially be used to identify patients who are more likely to present severe postoperative pain.

Of the SNPs examined in this study, rs4286289 exhibited the strongest association (P = 0.001) with PCA requirements in the PCA group. Indeed, even minor allele effects of rs4286289 increased the mean PCA requirement. Minor homozygote carriers (89/550, 16.1%) of this *SCN9A* SNP exhibited approximately a 12% increase in PCA compared to other patients, indicating that these minor carriers often require additional analgesic treatment. PCA requirement, which corresponds to the patient's actual demand for relief of postoperative pain, could serve as an indirect pain assessment method (39). Thus, this result indirectly reflected that minor carriers of rs4286289 exhibited higher intensities of postoperative pain than other carriers, which was consistent with the association between rs4286289 and postoperative pain in non-PCA patients. Based on these findings, we inferred that rs4286289 may be an optimal SNP for predicting postoperative pain.

To the best of our knowledge, the association between rs4286289 and postoperative pain detected in this study has not been described before. The rs4286289 SNP is located on an intronic region, and its functional effect on pain phenotypes is not immediately clear. Although the tag SNP rs4286289, together with the SNPs exhibiting LDs $\geq$ 0.8 in the phase 3 data of the HapMap CHB reference population database, is located on an intronic region, this region is involved in RNA binding protein-mediated post-transcriptional regulation, and may alter the expression of a nearby IFIH1 gene, according to rSNPBase (40). Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes, including inflammatory diseases that affect the brain and skin via upregulation of type I interferon signaling (41). However, rSNPBase searches did not provide evidence that this SNP may affect *SCN9A* expression levels. Therefore, the effects of rs4286289 on *SCN9A* activity require further studies.

There were several limitations considered when interpreting our study results. First, the tag SNPs examined in the current study were selected on the basis of the HapMap CHB reference population. As a result, our study cannot address the possibility that these tag SNPs may not have captured all variations in *SCN9A* in non-Chinese populations. Whether these findings would be similar in other race groups also remains to be tested. Second, to obtain a more accurate analysis of the association between a genetic factor and human pain perception, only women scheduled for gynecological laparoscopic surgery were included in the current study. Thus, our results are based on this special cohort, and further validation using another independent cohort is needed to strengthen these findings. Third, although it is commonly accepted that when exploring earlier findings the criteria needed for the association analysis can be less stringent, the fact that the analyses did not correct for the number of statistical tests that were performed is a potential limitation of the current study.

Conclusion

In conclusion, this study provides evidence that postoperative pain in gynecological patients is affected by variants in the *SCN9A* gene, including rs6746030. Moreover, the tag SNP rs4286289 identified in the current study has the potential to be used as a predictor for hypersensitivity to postoperative pain. This finding could therefore provide important insights for the use of *SCN9A* genotyping for future guidance of postoperative pain control.

Appendix 1. List of genotyped *SCN9A* SNPs detected in participating patients in the non-PCA and PCA groups.

SNP	Position (dbSNP Build 130)	Minor Alleles	Genotype Counts-1	MAF-1	HWE P Value-1	Genotype Counts-2	MAF-2	HWE P Value-2
rs3750904	Chr2:166763639	C	17/110/284	0.175	0.127	15/121/426	0.134	0.099
rs4303728	Chr2:166764674	A	18/138/264	0.207	1.000	19/138/410	0.155	0.107
rs16851778	Chr2:166769555	G	43/185/177	0.334	0.656	69/256/224	0.359	0.782
rs6432885	Chr2:166780936	T	66/200/142	0.407	0.837	97/284/176	0.429	0.387
rs7595255*	Chr2:166791224	T	3/32/381	0.046	0.046	3/55/517	0.053	0.208
rs16851799	Chr2:166794660	T	23/138/250	0.224	0.479	34/191/341	0.229	0.286
rs6746030*	Chr2:166807404	A	3/31/381	0.045	0.040	3/55/512	0.054	0.212
rs7563540	Chr2:166811219	C	28/142/250	0.236	0.222	29/166/372	0.198	0.084
rs10930214	Chr2:166814099	G	74/207/135	0.427	0.764	108/296/169	0.447	0.311
rs4426541	Chr2:166829202	A	3/56/361	0.074	0.484	3/68/496	0.065	0.724
rs4443014	Chr2:166832467	A	29/155/227	0.259	0.701	28/173/361	0.203	0.241
rs4369876*	Chr2:166837502	A	3/57/348	0.077	0.722	3/65/489	0.064	0.482
rs12478318*	Chr2:166841786	G	3/57/360	0.075	0.719	3/63/501	0.061	0.453
rs6739404	Chr2:166844276	A	30/161/217	0.271	1.000	41/226/290	0.276	0.832
rs6724623	Chr2:166844511	T	49/182/174	0.346	0.913	73/236/240	0.348	0.222
rs6722503	Chr2:166850375	T	37/174/204	0.299	1.000	47/232/295	0.284	0.918
rs4387806	Chr2:166859060	T	13/119/288	0.173	0.864	19/157/396	0.170	0.461
rs11688164	Chr2:166862035	C	68/215/133	0.422	0.269	102/275/193	0.420	0.797
rs12619987	Chr2:166866219	A	40/183/188	0.320	0.734	72/241/249	0.342	0.261
rs12620053	Chr2:166866532	C	64/203/150	0.397	0.759	91/281/202	0.403	0.729
rs13017637	Chr2:166868192	T	7/112/297	0.151	0.445	15/141/417	0.149	0.509
rs12994338	Chr2:166868275	T	22/147/248	0.229	1.000	33/228/313	0.256	0.380
rs6432896	Chr2:166869666	G	44/173/191	0.320	0.649	57/241/259	0.319	0.923
rs4605385	Chr2:166869844	G	8/113/295	0.155	0.575	12/143/418	0.146	1.000
rs4286289	Chr2:166869957	C	69/195/144	0.408	0.838	92/275/190	0.412	0.726
rs9646772	Chr2:166871445	T	73/195/149	0.409	0.543	76/297/201	0.391	0.044
rs4131159	Chr2:166877061	T	71/197/143	0.412	0.839	89/290/183	0.416	0.165
rs11898284*	Chr2:166889773	G	12/137/262	0.196	0.276	17/176/369	0.187	0.578
rs12622743*	Chr2:166902637	G	11/132/262	0.190	0.331	15/164/370	0.177	0.659

HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; SNP = single nucleotide polymorphism; -1 = Non-PCA group; -2 = PCA group.
*Candidate SNP

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