Opposite associations between the rs3845446 single-nucleotide polymorphism of the
CACNA1E gene and postoperative pain-related phenotypes in gastrointestinal surgery
versus previously reported orthognathic surgery

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ABSTRACT

Ca\textsubscript{v}2.3 (R-type) voltage-activated Ca\textsuperscript{2+} channels (VACCs), encoded by the \textit{CACNA1E} gene, are responsible for transmission of somatic inflammatory pain, and activation of antinociception elicited by visceral inflammatory pain stimuli. Carriers of the minor G allele of the rs3845446 single-nucleotide polymorphism (SNP) of the \textit{CACNA1E} gene reportedly exhibit a decrease in opioid requirements to control typical somatic inflammatory pain after orthognathic surgery (i.e., a painful cosmetic surgery), suggesting the downregulation of \textit{Ca}v\textsubscript{2.3} VACC function that is responsible for transmission of somatic inflammatory pain in these carriers. Gastrointestinal surgery involves both somatic and visceral inflammatory pain, in which visceral inflammatory pain stimuli activate \textit{Ca}v\textsubscript{2.3} VACC-mediated antinociception. Unknown is whether pain-related phenotypes after gastrointestinal surgery are affected in these carriers. The present study used a correlational design to examine the impact of the rs3845446 SNP on postoperative pain-related phenotypes in two groups of patients who underwent gastrointestinal surgery. Carriers of the minor G allele had higher opioid requirements after laparoscopic colectomy when intravenous patient-controlled analgesia was used, while reporting higher pain scores after open gastrointestinal surgery when postoperative analgesia was managed with continuous epidural analgesia and rescue analgesics. These results suggest that pain-related phenotypes after gastrointestinal surgery

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are enhanced in carriers of the minor G allele of the rs3845446 SNP, possibly through impairment of Ca\textsubscript{v}2.3 VACC function that is responsible for the activation of visceral inflammatory pain stimulus-elicited antinociception.

**PERSPECTIVE**

Carriers of the minor allele of the rs3845446 single-nucleotide polymorphism of the \textit{CACNA1E} gene required more opioid or reported higher pain scores after gastrointestinal surgery, while requiring less opioid after orthognathic surgery. The difference may result from the presence of visceral inflammatory pain stimulus that activates Ca\textsubscript{v}2.3 voltage-activated Ca\textsuperscript{2+} channels-mediated antinociception.

**KEYWORDS**

\textit{CACNA1E}; Ca\textsubscript{v}2.3; single-nucleotide polymorphism; postoperative pain; gastrointestinal surgery
Introduction

In internal organ surgery, postoperative pain consists of somatic, visceral, and referred pain components [5,19,39]. Facilitatory and inhibitory interactions between somatic and visceral afferents have been reported [3,36]. Referred pain is thought to result from the neurophysiological convergence of somatic and visceral afferents [36]. Certain noxious cutaneous and visceral stimuli inhibit the activity of visceral and cutaneous nociceptive neurons, respectively [31,32]. Preexposure to visceral inflammatory pain stimuli induces a long-lasting decrease in the response to somatic inflammatory pain stimuli [23]. In humans, gastric and rectal distention inhibits somatic nociceptive flexion [7,8]. Nevertheless, the mechanisms that underlie such inhibitory interactions between somatic and visceral afferents have not been clarified.

The rs3845446 single-nucleotide polymorphism (SNP) of the CACNA1E (calcium channel, voltage-dependent, R type, α1E subunit) gene that encodes Ca,2.3 (R-type) voltage-activated Ca^{2+} channels (VACCs) is an intronic Tag SNP in the linkage disequilibrium block from intron 46 to exon 47, a region that contains a stop codon [16]. The rs3845446 SNP is common in East Asian population (minor allele frequency > 25%) whereas it is relatively rare in other population (minor allele frequency < 13%) [29]. The rs3845446 SNP has been reported to be associated with opioid consumption to control pain after
orthognathic surgery (i.e., a painful cosmetic surgery), which involves somatic inflammatory pain [16]. Carriers of the minor G allele of this SNP required less postoperative fentanyl [16]. The CACNA1E gene is highly expressed in cortical neurons [27], and Ca\textsubscript{v}2.3 VACC function affects responses to inflammatory pain stimuli in mice [35]. Ca\textsubscript{v}2.3 VACCs are responsible for the transmission of somatic inflammatory pain [35]. Ca\textsubscript{v}2.3 VACC knockout mice exhibit a gene dose-dependent decrease in the response to somatic inflammatory pain stimuli [35]. Therefore, carriers of the minor G allele of the rs3845446 SNP required less postoperative fentanyl after orthognathic surgery, possibly through the impairment of Ca\textsubscript{v}2.3 VACC function that is responsible for the transmission of somatic inflammatory pain in these carriers [16]. This previous study discussed the possibility that genetic polymorphisms in the vicinity of the rs3845446 SNP affect the splicing mechanism of the CACNA1E gene, based on the fact that Ca\textsubscript{v}2.3 VACCs consist of several splicing variants, and splicing differences between the major and other isoforms occur between the LD block that contains the rs3845446 SNP and the preceding block [16].

In addition to the transmission of somatic inflammatory pain, Ca\textsubscript{v}2.3 VACCs are involved in activating the serotonergic descending antinociceptive pathway, which is activated primarily by visceral inflammatory pain stimuli [35]. Ca\textsubscript{v}2.3 VACC knockout mice that are presensitized with visceral inflammatory pain stimuli exhibit an increase in the
response to somatic inflammatory pain stimuli compared with non-sensitized knockout mice, whereas wildtype mice respond in an opposite manner, depending on impaired or intact functions of Ca\(_{\text{v}}\)2.3 VACCs responsible for antinociception elicited by visceral inflammatory pain stimuli [35].

Unknown is whether pain-related phenotypes after internal organ surgery involving both somatic and visceral inflammatory pain, in which visceral inflammatory pain stimulus should activate Ca\(_{\text{v}}\)2.3 VACC-mediated antinociception [35], are affected in carriers of the minor G allele of the rs3845446 SNP. The present study examined associations between the rs3845446 SNP and postoperative pain-related phenotypes in patients who underwent gastrointestinal surgery (i.e., a typical internal organ surgery). Considering the possible impairment of Ca\(_{\text{v}}\)2.3 VACC function that is responsible for the activation of visceral inflammatory pain stimulus-elicited antinociception in carriers of the minor G allele, we hypothesized that the associations between the rs3845446 SNP and postoperative pain-related phenotypes after gastrointestinal surgery would be opposite to such associations after orthognathic surgery.

**Methods**

*Design*
The present study used a correlational design to examine the impact of the rs3845446 SNP on postoperative analgesic consumption and pain scores in two groups of patients who underwent gastrointestinal surgery.

_Ethics statement_

The study protocol was approved by the Institutional Review Boards of Saitama Medical University (Iruma-gun, Japan), The University of Tokyo (Tokyo, Japan), Toho University Sakura Medical Center (Sakura City, Japan), and Tokyo Metropolitan Institute of Medical Science (Tokyo, Japan). Written informed consent was obtained from all of the subjects for the genetics studies.

_Patients_

Two groups of patients were enrolled in the study. Patients with chronic pain, patients who took any analgesics, psychotherapeutic drugs, anti-anxiety drugs, or anticonvulsants, and patients with an American Society of Anesthesiologists Physical Status of III or higher were excluded.

Group 1 (laparoscopic colectomy)
Group 1 included 351 unrelated patients who were scheduled to undergo laparoscopic colectomy for colon cancer at Saitama Medical University International Medical Center, during the period from January 2011 to March 2013. Postoperative pain was primarily managed by intravenous fentanyl patient-controlled analgesia (PCA). Peripheral blood was collected for gene analysis.

Group 2 (open gastrointestinal surgery)

Group 2 included 112 unrelated patients who were scheduled to undergo open gastrectomy for gastric cancer or open colectomy for colon cancer at the Research Hospital, Institute of Medical Science, The University of Tokyo, or Toho University Sakura Medical Center, during the period from January 2002 to December 2004. Postoperative pain was managed by a combination of epidural analgesia infused at a constant rate and rescue analgesics. Peripheral blood or oral mucosa samples were collected for gene analysis.

Anesthesia and surgery

Group 1 (laparoscopic colectomy)

In Group 1, the surgical and anesthetic protocols were fundamentally the same as in a previous study [26]. General anesthesia was induced with oxygen, 1-2 mg/kg propofol and
100 µg fentanyl. Rocuronium was administered appropriately to facilitate intubation and surgery. General anesthesia was maintained with oxygen-enriched air, sevoflurane (1.2% in end-tidal concentration, corresponding to 0.67 minimal alveolar concentration [MAC]) and 0.2-0.5 µg/kg/min remifentanil. The average dose of remifentanil was recorded. The Bilateral Index (BIS) was recorded and controlled to be in the range of 40 to 60.

At the end of surgery, 100 µg fentanyl was intravenously administered. After emergence from anesthesia and tracheal extubation, pain was assessed using a four-point verbal rating scale (0, no pain; 1, mild pain; 2, moderate pain; 3, severe pain). If the pain score was ≥ 2, then 50 µg fentanyl was titrated at 3 min intervals until the pain score was < 2. After sufficient analgesia was attained, intravenous fentanyl PCA (CADD-Legacy PCA pump, Model 6300, Smiths Medical Japan, Tokyo, Japan) began. Fentanyl (1 mg) was diluted in normal saline in a total volume of 100 ml. A bolus dose of 20 µg fentanyl on demand and a lockout time of 5 min were set. Continuous background infusion was not employed. Patient-controlled analgesia was continued for at least 24 h postoperatively. In cases of treatment-refractory adverse effects, PCA was discontinued. Intravenous flurbiprofen (50 mg) or pentazocine (30 mg) was prescribed as a rescue analgesic for significant postoperative pain despite the maximum usage of fentanyl PCA. Fentanyl doses that were administered before fentanyl PCA began and fentanyl consumption via PCA at 2, 4, 6, 12, 18, and 24 h.
postoperatively were recorded. The cumulative fentanyl dose at each time point was normalized to body weight. The intensity of pain was evaluated using an 11-point numerical rating scale (NRS; 0, no pain; 10, most severe pain imaginable) at each postoperative time point.

Group 2 (open gastrointestinal surgery)

In Group 2, open gastrectomy or colectomy was performed under a combination of general and epidural anesthesia [15]. A lower thoracic or upper lumbar epidural catheter was placed before the induction of general anesthesia. General anesthesia was induced with oxygen, 1-2 mg/kg propofol, and 100 µg fentanyl. Vecuronium was administered as appropriate to facilitate intubation and surgery. General anesthesia was maintained with oxygen-enriched air and sevoflurane. A single dose of 6 ml of 0.5% bupivacaine with fentanyl or morphine was administered through the epidural catheter before surgery. Additional dosages of epidural local anesthetic were administered intermittently according to the preceding state of surgery.

Postoperative pain was managed primarily with continuous epidural analgesia, which was started before emergence from anesthesia. Fentanyl or morphine was diluted with 0.25% bupivacaine in a total volume of 100 ml and infused at a constant rate of 2 ml/h.
Whenever the patient complained of significant postoperative pain despite continuous epidural analgesia, appropriate doses of opioids and/or nonsteroidal antiinflammatory drugs (NSAIDs) were administered as rescue analgesics.

The frequency and doses of rescue analgesics, highest pain scores using a 5-point NRS (0, no pain; 4, most severe pain imaginable), and doses of opioids that were infused epidurally during the first 24-h postoperative period were recorded. The doses of rescue analgesics that were administered during the first 24-h postoperative period were converted to equivalent doses of systemic fentanyl [15]. Perioperative analgesic doses were calculated as the sum of doses of epidural opioids and doses of rescue analgesics, converted to equivalent doses of systemic fentanyl [15].

Genotyping

Group 1 (laparoscopic colectomy)

In Group 1, total genomic DNA was extracted from peripheral blood using standard procedures. Genotype data of the rs3845446 SNP were obtained from the results of whole-genome genotyping, which was performed using HumanOmniExpressExome-8 BeadChips and Infinium assay II with an iScan system (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions, as described in detail in a previous study [26].
The genotyping results for the rs3845446 SNP were qualified by a data-cleaning process using GenomeStudio with the Genotyping v3.3.7 module (Illumina). In the data-cleaning process, markers with a genotype call frequency of less than 0.95, “Cluster sep” (i.e., an index of genotype cluster separation) of less than 0.1, or $P$ values ($df = 1$) less than 0.001 in the Hardy-Weinberg equilibrium tests were excluded from the subsequent association study.

Group 2 (open gastrointestinal surgery)

In Group 2, total genomic DNA was extracted from peripheral blood or oral mucosa samples using standard procedures. The TaqMan allelic discrimination assay (Life Technologies, Tokyo, Japan) was used to genotype the rs3845446 SNP. To perform the TaqMan allelic discrimination assay with a LightCycler 480 (Roche Diagnostics K.K., Tokyo, Japan), TaqMan SNP Genotyping Assays (Life Technologies, Tokyo, Japan) that contained sequence-specific forward and reverse primers to amplify the polymorphic sequence and two probes that were labeled with VIG and FAMTM dye to detect both alleles of the rs3845446 SNP (Assay ID: C_7539287_30) were used. Real-time polymerase chain reaction was performed in a final volume of 10 µl that contained 2×LightCycler 480 Probes Master (Roche Diagnostics K.K., Tokyo, Japan), 20×TaqMan Gene Expression Assays, 5 ng genomic DNA as the template, and up to 10 µl of H$_2$O that was included with 2×LightCycler 480 probe
Master. The thermal conditions were the following: 95°C for 10 min, followed by 45 cycles of 95°C for 10 s and 60°C for 60 s, with final cooling at 50°C for 30 s. Afterward, endpoint fluorescence was measured for each sample well, and the A/A, A/G, and G/G genotypes were determined based on the presence or absence of each type of fluorescence.

*Statistical analysis*

The statistical analysis was performed using SPSS 20.0.0 software (IBM, Tokyo, Japan). In the present study, none of the clinically measured endpoints that were related to analgesic requirements (cumulative fentanyl dose in Group 1, and frequency of rescue analgesics, fentanyl-converted dose of rescue analgesics, and fentanyl-converted total dose of analgesics in Group 2) or NRS pain scores were normally distributed. Therefore, nonparametric analyses, including the Mann-Whitney U-test, Kruskal-Wallis test, and Spearman’s rank correlation test, were used to detect possible associations between the rs3845446 SNP and clinical endpoints that were related to analgesic requirements or NRS pain scores.

In the analysis of patients who underwent laparoscopic colectomy (Group 1), the cumulative fentanyl dose (in µg/kg) and NRS pain scores during the 2-, 4-, 6-, 12-, 18-, and 24-h postoperative periods were used as pain-related phenotypes. In the analysis of patients
who underwent open gastrointestinal surgery (Group 2), the total dose of rescue analgesics
(converted to fentanyl dose [in µg/kg]), sum of epidurally infused opioids and rescue
analgesics (converted to fentanyl dose [in µg/kg]), and highest NRS pain scores during the
first 24-h postoperative period were used as pain-related phenotypes.

To explore the association between the rs3845446 SNP and pain-related phenotypes,
the Mann-Whitney U-test was performed for both groups. For this analysis, pain-related
phenotypes and genotype data of the rs3845446 SNP were incorporated as dependent and
independent variables, respectively.

Factors other than genotype (e.g., sex, age, and surgical sites) may also influence
pain-related phenotypes. In a previous study, the difference in cumulative fentanyl dose
among genotypes of the rs3845446 SNP was not influenced by age or sex [16]. The same
study also found that the cumulative fentanyl dose was significantly less in males at certain
time points and in the elderly at all time points [16]. In the present study, the effects of sex,
age, and surgical site on pain-related phenotypes were evaluated using nonparametric
analyses.

Values of $P < 0.05$ were considered statistically significant. In Group 1, outcomes
were measured at six time-points. To examine the impact of the rs3845446 SNP on
pain-related phenotypes and fully utilize the obtained data, we performed statistical analyses
at each time-point. Bonferroni correction was applied for multiple comparisons. Values of $P < 0.008 (= 0.05 / 6)$ were considered significant for Group 1. The power and effect size ($r$) of significant SNP analyses were calculated for pain-related phenotypes.

**Results**

The minor allele frequency of the rs3845446 SNP was 28% (Group 1) and 32% (Group 2) in Japanese population of the present study. Homozygotes of the minor G allele were rare (8% in Group 1 and 9% in Group 2); therefore, a dominant model (AA vs. AG + GG) was selected to divide the patients.

Consistent with a previous study [16], Pearson’s $\chi^2$ test revealed no significant difference in sex among the subjects who carried different genotypes of the rs3845446 SNP in group 1 ($P = 0.974$) and group 2 ($P = 0.745$), indicating that the clinical outcomes among genotypes were not influenced by sex. The Mann-Whitney test revealed that sex was not significantly associated with any of the pain-related phenotypes in groups 1 and 2 ($P > 0.05$).

**Group 1 (laparoscopic colectomy)**

Four patients were excluded from the analysis for the following reasons: surgery was converted to open colectomy in two patients, reoperation was performed before emergence
from anesthesia in one patient, and the record of pain-related phenotypes was incomplete in one patient. Thus, a total of 347 patients were included in the analysis.

Effects of age on pain-related phenotypes

Spearman’s rank correlation test revealed that age was not significantly associated with fentanyl use (Spearman’s rank correlation coefficient = -0.026, -0.091, -0.092, -0.088, -0.050, and -0.039 at 2, 4, 6, 12, 18, and 24 h, respectively; $P = 0.63, 0.092, 0.087, 0.10, 0.35, \text{and } 0.47, \text{respectively}$) or NRS pain scores (Spearman’s rank correlation coefficient = -0.073, -0.020, -0.079, -0.069, -0.12, and -0.085 at 2, 4, 6, 12, 18, and 24 h, respectively; $P = 0.18, 0.71, 0.16, 0.23, 0.040, \text{and } 0.15, \text{respectively}$).

Effects of surgical sites on pain-related phenotypes

The Mann-Whitney test revealed that the type of surgery (colectomy, $n = 188$; proctectomy, $n = 159$) was not significantly associated with fentanyl use ($P = 0.68, 0.93, 0.97, 0.47, 0.68, \text{and } 0.88 \text{ at } 2, 4, 6, 12, 18, \text{and } 24 \text{ h, respectively}$) or NRS pain scores ($P = 0.026, 0.081, 0.13, 0.53, 0.97, \text{and } 0.29 \text{ at } 2, 4, 6, 12, 18, \text{and } 24 \text{ h, respectively}$). The Kruskal-Wallis test revealed that the extent of lymph node dissection (D1, D2, or D3) was not significantly associated with fentanyl use ($P = 0.87, 0.66, 0.71, 0.99, 0.75, \text{and } 0.70 \text{ at } 2, 4, 6, 12, 18, \text{and } 24 \text{ h, respectively}$).
24 h, respectively) or NRS pain scores ($P = 0.71, 0.35, 0.12, 0.14, 0.082, \text{ and } 0.11$ at 2, 4, 6, 12, 18, and 24 h, respectively).

Effects of rs3845446 SNP on pain-related phenotypes

The Mann-Whitney test revealed that carriers of the minor G allele of the rs3845446 SNP required significantly more fentanyl at 6-24 h postoperatively even after the Bonferroni correction ($P = 0.16, 0.025, 0.003, 0.001, 0.001, \text{ and } 0.002$; power $= 0.811, 0.892, 0.876, \text{ and } 0.851$; $r = 0.15, 0.17, 0.16, \text{ and } 0.16$ at 2, 4, 6, 12, 18, and 24 h, respectively; Fig. 1). The rs3845446 SNP was not significantly associated with postoperative NRS pain scores ($P = 0.24, 0.81, 0.58, 0.17, 0.57, \text{ and } 0.57$ at 2, 4, 6, 12, 18, and 24 h, respectively; Table 1).

*Group 2 (open gastrointestinal surgery)*

A total of 112 patients were included in the analysis.

Effects of age on pain-related phenotypes

Spearman’s rank test revealed that age was not associated with NRS scores

(Spearman’s rank correlation coefficient $= -0.136, P = 0.202$) or total analgesic doses

(converted to fentanyl doses; Spearman’s rank correlation coefficient $= -0.175, P = 0.065$).
Older age was significantly associated with a lower frequency of rescue analgesics (Spearman’s rank correlation coefficient = -0.236, \( P = 0.012 \)) and lower doses of rescue analgesics (converted to fentanyl doses; Spearman’s rank correlation coefficient = -0.210, \( P = 0.026 \)).

Effects of rs3845446 SNP on pain-related phenotypes

The Mann-Whitney test revealed that carriers of the minor G allele of the rs3845446 SNP had higher NRS pain scores (\( P = 0.013 \), Power = 0.765, \( r = 0.28 \); Fig. 2). The rs3845446 SNP was not significantly associated with the frequency of rescue analgesics (\( P = 0.480 \)), rescue analgesic doses (converted to fentanyl doses; \( P = 0.399 \)), or perioperative analgesic doses (converted to fentanyl doses; \( P = 0.988 \); Table 2).

Multiple regression analyses were performed, with rescue analgesic requirements as the dependent variable and age and genotype data of the rs3845446 SNP as the independent variables. Multiple regression revealed that the rs3845446 SNP was not associated with rescue analgesic requirements after multivariate analysis corrections for age: frequency of rescue analgesics as the dependent variable (multiple coefficient of determination = 0.083; \( P \)-value of the regression model = 0.009; unstandardized coefficient and \( P \)-value of age = -0.028 and 0.002; unstandardized coefficient and \( P \)-value of the rs3845446 SNP = 0.083 and
and rescue analgesic doses as the dependent variable (multiple coefficient of
determination = 0.058; \( P \)-value of the regression model = 0.039; unstandardized coefficient
and \( P \)-value of age = -0.027 and 0.012; unstandardized coefficient and \( P \)-value of the
rs3845446 SNP = 0.093 and 0.665).

**Discussion**

In the present study, carriers of the minor G allele of the rs3845446 SNP required
more intravenous PCA fentanyl after laparoscopic colectomy than non-carriers to achieve
comparable levels of pain control, and carriers of the minor G allele of the rs3845446 SNP
reported higher pain scores than non-carriers at comparable analgesic doses after open
gastrointestinal surgery. Correlations between postoperative analgesic consumption or pain
scores and sensitivity to experimental pain have been previously reported [9,10]. In these
studies, pain perception thresholds for experimental pain correlated with postoperative opioid
consumption when PCA was used [10], and these thresholds also correlated with
postoperative pain scores when analgesics were administered intermittently on demand [9].
These studies suggest that postoperative analgesic consumption or pain scores can be used as
reliable indicators of responses to postoperative pain. The results from both groups in the
present study indicated that carriers of the minor G allele of the rs3845446 SNP exhibited an
increase in responses to pain after gastrointestinal surgery, in contrast to a decrease in responses to pain after orthognathic surgery, in which carriers of the minor G allele of the rs3845446 SNP required less intravenous PCA fentanyl [16].

Opposite responses to pain were found in carriers of the minor G allele of the rs3845446 SNP after orthognathic surgery, which involves somatic inflammatory pain alone, and after gastrointestinal surgery, which involves both somatic and visceral inflammatory pain. These responses appear to resemble those of Ca\textsubscript{v}2.3 V ACC knockout mice to inflammatory pain stimuli (Table 3). Ca\textsubscript{v}2.3 V ACC knockout mice exhibit a gene dose-dependent decrease in responses to somatic inflammatory pain stimuli, which is most likely attributable to an impairment of Ca\textsubscript{v}2.3 V ACC-mediated transmission of somatic inflammatory pain [35]. Ca\textsubscript{v}2.3 V ACC knockout mice exhibit an increase in responses to somatic inflammatory pain stimuli after sensitization with visceral inflammatory pain stimuli, which is most likely attributable to an impairment of Ca\textsubscript{v}2.3 V ACC-mediated descending antinociceptive pathway activation [35] that could be elicited particularly by visceral inflammatory pain stimuli [23,35]. Thus, opposite responses to postoperative pain between different types of surgery observed in the previous [16] and present studies are highly likely to result from the presence or absence of visceral inflammatory pain stimuli. The $\text{CACNA1E}$ gene has been suggested to be downregulated in carriers of the minor G allele of the
rs3845446 SNP [16]. Therefore, responses to pain after orthognathic surgery decreased in carriers of the minor G allele [16], which was possibly attributable to impairment of the Ca₂⁺,3 VACC-mediated transmission of somatic inflammatory pain. In patients who undergo internal organ surgery, perioperative visceral inflammatory pain might elicit Ca₂⁺,3 VACC-mediated antinociception, such as in mice that are preexposed to visceral inflammatory pain stimuli [35]. Activation of this Ca₂⁺,3 VACC-mediated antinociceptive pathway might be impaired in carriers of the minor G allele, thereby resulting in an increase in responses to pain after gastrointestinal surgery.

Visceral afferents project to the central nervous system through autonomic nerves [12]. Among visceral afferents, vagal and pelvic nerves contribute to parasympathetic innervation [14]. Several studies have reported a relationship between visceral pain transmission and vagal afferents. Vagal nerve activation affects visceral pain transmission [33] and *vice versa* [40]. The vagal nerve reportedly plays a direct role in transmitting nociceptive information related to experimental visceral inflammatory pain [40]. Vagal afferent stimulation reportedly has analgesic effects on experimental pain [18,33,38], including somatic inflammatory pain [6] and visceral inflammatory pain [40]. In humans, vagal nerve stimulation has been reported to elicit analgesia in experimental pain [11,21,22,24], chronic pelvic pain [28], and headache [2,30,37]. Interestingly, antinociception
that is elicited by vagal nerve stimulation [38] and experimental visceral inflammatory pain stimuli [23] is similar in that they are both mediated by serotonergic pathways [23,35,38]. The presence of visceral inflammatory pain necessarily involves vagal nerve activation [40]. Therefore, there may be a common pathway between antinociception elicited by vagal nerve stimulation and that elicited by visceral inflammatory pain stimuli. Visceral inflammatory pain stimuli increase vagal activity [40], thus possibly eliciting vagal nerve-mediated antinociception. In addition to the aforementioned cortical neurons [27], Ca,2.3 VACCs are also expressed in smooth muscle cells in various tissues [13,17] and myenteric neurons [4], which are known to be controlled by the autonomic nervous system and where visceral nociceptors are distributed. Ca,2.3 VACC function is likely inhibited in subjects with the minor G allele of the rs3845446 SNP [16]. Therefore, impairment in Ca,2.3 VACC function in knockout mice or humans who carry the minor G allele of the rs3845446 SNP may inhibit antinociception that is elicited by visceral inflammatory pain stimuli or vagal nerve activation, thereby increasing pain-related phenotypes after sensitization to visceral inflammatory pain.

There are some limitation of the present study. First, the two patient groups in the present study and the patient group in the previous study [16] presented some differences in sites of surgery, modes of anesthesia and postoperative pain control, which may hinder direct comparisons among groups. Group 1 included patients who underwent laparoscopic
colectomy and received intraoperative analgesia with remifentanil and postoperative analgesia with IV-PCA fentanyl. Group 2 included patients who underwent open gastrointestinal surgery and received intraoperative and postoperative analgesia with epidural analgesia using a local anesthetic and an opioid. The group of patients in the previous study [16] included patients who underwent orthognathic surgery and received intraoperative analgesia with fentanyl and postoperative analgesia with IV-PCA fentanyl (Group 3). The different surgical sites also imply different pain transmission pathways, which may especially hinder the direct comparison between the present study (gastrointestinal surgery) and the previous study (orthognathic surgery) [16]. The differences in analgesic regimens may also result in differences in postoperative pain-related phenotypes, although it has been reported that different regimens of opioid (fentanyl or remifentanil) administration used for induction and maintenance of general anesthesia does not affect the total IV-PCA fentanyl doses required after orthognathic surgery [1]. Second, acute opioid tolerance or opioid-induced hyperalgesia after intraoperative fentanyl or remifentanil administration may influence postoperative pain-related phenotypes, although they are still matters of controversy [20,25,34]. Clearly, standardized regimens of analgesic administration are required to make more precise comparisons between postoperative pain-related phenotypes after different types of surgery.
Conclusion

Carriers of the minor G allele of the rs3845446 SNP exhibited enhanced pain-related phenotypes after gastrointestinal surgery. The pain-related phenotypes of carriers of the minor allele of this SNP after gastrointestinal surgery were opposite to such phenotypes after orthognathic surgery. This difference appeared to result from the presence or absence of visceral inflammatory pain stimuli that activate the descending Ca\(_{\text{v}}\)2.3 VACC-mediated antinociceptive pathway. Postoperative pain-related responses in carriers of the minor G allele resembled responses to inflammatory pain in Ca\(_{\text{v}}\)2.3 VACC knockout mice, strongly suggesting the lower expression of or dysfunctional changes in the CACNA1E gene in carriers of the minor G allele. Associations between the rs3845446 SNP and pain-related phenotypes in ethnic groups other than the Japanese population have not been investigated, and further studies are required. Nonetheless, our data suggest that postoperative pain-related phenotypes are significantly affected in subjects with the minor allele of the rs3845446 SNP, depending on the presence or absence of visceral inflammatory pain stimuli. These findings provide valuable information that may improve pain management in the near future.

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**Figure Legends**

Fig. 1. Time course of associations between genotypes of the rs3845446 SNP and cumulative fentanyl doses after laparoscopic colectomy ($n = 347$). The data are expressed by box and whisker plots. The upper and lower ends of the boxes represent the 75th and 25th percentiles, respectively. Whiskers represent the highest and lowest values. The median is depicted by a horizontal solid line in the box. Outliers and extreme values are shown as circles and triangles, respectively. **$P < 0.008$.**

Fig. 2. Associations between genotypes of the rs3845446 SNP and the highest numerical rating scale pain scores during the 24-h postoperative period for open gastrointestinal surgery ($n = 112$). The data are expressed by box and whisker plots. The upper and lower ends of the boxes represent the 75th and 25th percentiles, respectively. Whiskers represent the highest and lowest values. The median is depicted by a horizontal solid line in the box. There were no outliers or extreme values. *$P < 0.05$.**
**Highlight points**

1. Carriers of the minor G allele of the rs3845446 SNP require less opioid analgesics to control pain after orthognathic surgery (which involves somatic inflammatory pain alone) compared with non-carriers.

2. Numerical rating scale pain scores and opioid requirements to control pain increased after gastrointestinal surgery (which involves both somatic and visceral inflammatory pain) in carriers of the minor G allele of the rs3845446 SNP compared with non-carriers.

3. Pain-related phenotypes of carriers of the minor G allele of the rs3845446 SNP resembled the phenotypes of Ca_{2.3} (R-type) voltage-activated Ca^{2+} channel knockout mice.

4. Activation of the serotonergic descending antinociceptive pathway, which is activated primarily by visceral inflammatory pain stimuli, was likely to be decreased in carriers of the minor G allele of the rs3845446 SNP compared with non-carriers.
5. Pain-related phenotypes (analgesic requirements and/or pain scores) for pain with or without different modalities are differentially influenced by physiological and genetic factors.
Table 1. Clinical data of patients who underwent laparoscopic colectomy (Group 1).

<table>
<thead>
<tr>
<th>rs3845446 SNP genotype</th>
<th>A/A</th>
<th>A/G + G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (male/female)</td>
<td>181 (112/69)</td>
<td>166 (103/63)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.2 ± 10.3</td>
<td>63.1 ± 10.6</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>160.5 ± 9.7</td>
<td>161.2 ± 8.7</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>59.9 ± 11.2</td>
<td>60.3 ± 10.7</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>23.1 ± 3.1</td>
<td>23.1 ± 3.2</td>
</tr>
<tr>
<td>Flurbiprofen used/not used</td>
<td>72/109</td>
<td>80/86</td>
</tr>
<tr>
<td>Surgery time (min)</td>
<td>206 ± 73</td>
<td>212 ± 73</td>
</tr>
<tr>
<td>Anesthetic time (min)</td>
<td>271 ± 76</td>
<td>276 ± 76</td>
</tr>
<tr>
<td>Average remifentanil dose (µg kg⁻¹ min⁻¹)</td>
<td>0.21 [0.18, 0.26]</td>
<td>0.22 [0.18, 0.26]</td>
</tr>
<tr>
<td>NRS pain score at rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>3.5 [2.5, 5.0]</td>
<td>4.0 [2.5, 5.0]</td>
</tr>
<tr>
<td>4 h</td>
<td>3.0 [1.5, 4.0]</td>
<td>3.0 [1.5, 5.0]</td>
</tr>
<tr>
<td>6 h</td>
<td>3.0 [1.0, 4.0]</td>
<td>3.0 [1.0, 4.0]</td>
</tr>
<tr>
<td>12 h</td>
<td>2.0 [0, 3.0]</td>
<td>2.0 [0, 3.5]</td>
</tr>
<tr>
<td>18 h</td>
<td>2.0 [1.0, 3.5]</td>
<td>2.5 [1.5, 3.5]</td>
</tr>
<tr>
<td>24 h</td>
<td>2.0 [1.0, 3.0]</td>
<td>2.0 [1.0, 3.0]</td>
</tr>
</tbody>
</table>

The data are expressed as numbers, mean ± SD (range), or median [interquartile range].
Table 2. Clinical data of patients who underwent open gastrointestinal surgery (Group 2).

<table>
<thead>
<tr>
<th>rs3845446 SNP genotype</th>
<th>A/A</th>
<th>A/G + G/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (male/female)</td>
<td>52 (27/25)</td>
<td>60 (33/27)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.7 ± 10.7</td>
<td>63.6 ± 9.3</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>158.6 ± 7.9</td>
<td>157.3 ± 8.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>56.0 ± 10.4</td>
<td>55.7 ± 9.5</td>
</tr>
<tr>
<td>Body mass index (kg m(^{-2}))</td>
<td>22.2 ± 3.5</td>
<td>22.5 ± 3.6</td>
</tr>
<tr>
<td>Frequency of rescue analgesics in 24 h</td>
<td>0 [0, 1]</td>
<td>1 [0, 1]</td>
</tr>
<tr>
<td>Rescue analgesic dose converted to fentanyl (µg kg(^{-1}))</td>
<td>0 [0, 1.4]</td>
<td>0.5 [0, 1.2]</td>
</tr>
<tr>
<td>Perioperative analgesic dose converted to fentanyl (µg kg(^{-1}))</td>
<td>8.3 [6.7, 10.5]</td>
<td>8.5 [6.6, 10.6]</td>
</tr>
</tbody>
</table>

The data are expressed as numbers, mean ± SD (range), or median [interquartile range].
Table 3. Comparison of pain-related responses of subjects with the G allele of the rs3845446 SNP and Ca,2.3 VACC knockout mice.

<table>
<thead>
<tr>
<th>Subjects with G allele of rs3845446 SNP</th>
<th>Ca,2.3 VACC knockout mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surgery</strong></td>
<td><strong>Pain-related responses</strong></td>
</tr>
<tr>
<td>Mandibular osteotomy</td>
<td>Decreased [16]</td>
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<td></td>
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<tr>
<td>Gastrointestinal surgery</td>
<td>Increased</td>
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