




Effects of rs958804 and rs7858836 single-nucleotide polymorphisms of the *ASTN2* gene on pain-related phenotypes in patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy

Rie Inoue^{1,2} | Daisuke Nishizawa² | Junko Hasegawa² | Kyoko Nakayama² | Ken-ichi Fukuda³ | Tatsuya Ichinohe⁴ | Tsutomu Mieda⁵ | Miki Tsujita⁶ | Hideyuki Nakagawa⁶ | Akira Kitamura⁶ | Hiroyuki Sumikura¹ | Kazutaka Ikeda²  | Masakazu Hayashida^{1,2,6}

¹Department of Anesthesiology and Pain Medicine, Graduate School of Medicine, Juntendo University, Tokyo, Japan

²Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

³Department of Oral Health and Clinical Science, Tokyo Dental College, Tokyo, Japan

⁴Department of Dental Anesthesiology, Tokyo Dental College, Tokyo, Japan

⁵Department of Anesthesiology, Saitama Medical University Hospital, Saitama, Japan

⁶Department of Anesthesiology, Saitama Medical University International Medical Center, Saitama, Japan

Correspondence

Kazutaka Ikeda, Department of Psychiatry and Behavioral Sciences, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan.
Email: ikeda-kz@igakuken.or.jp; <http://www.igakuken.or.jp/english/>

Funding information

Japan Society for the Promotion of Science, Grant/Award Number: 17H04324, 17K08970, 17K09052, 18K08829, 20K07774, 20K09259, 22790518, 23390377, 24790544, 26293347 and JP16H06276 (AdAMS); Smoking Research Foundation; Japan Research Foundation for Clinical Pharmacology; Ministry of Health, Labour and Welfare

Abstract

Background: Opioids are widely used as effective analgesics, but opioid sensitivity varies widely among individuals. The underlying genetic and nongenetic factors are not fully understood. Based on the results of our previous genome-wide association study, we investigated the effects of single nucleotide polymorphisms (SNPs) of the astrotactin 2 (*ASTN2*) gene on pain-related phenotypes in surgical patients.

Methods: We investigated the effects of two SNPs, rs958804 T/C and rs7858836 C/T, of the *ASTN2* gene on eight and seven pain-related phenotypes in 350 patients who underwent laparoscopic colectomy (LAC) and 358 patients who underwent mandibular sagittal split ramus osteotomy (SSRO), respectively. In both surgical groups, intravenous fentanyl patient-controlled analgesia (PCA) was used for postoperative analgesia, and 24-hour postoperative PCA fentanyl use was the primary endpoint.

Results: The association analyses among the two SNPs and pain-related traits showed that 24-hour fentanyl use was significantly associated with the two SNP genotypes in both surgical groups. The Mann-Whitney test showed that 24-hour fentanyl use was lower in patients with the C allele than in patients with the TT genotype of the rs958804 T/C SNP ($P = .0019$ and $.0200$ in LAC and SSRO patients, respectively), and it was lower in patients with the T allele than in patients with the CC genotype of the rs7858836 C/T SNP ($P = .0017$ and $.0098$ in LAC and SSRO patients, respectively).

Conclusion: The two SNPs of the *ASTN2* gene were consistently associated with fentanyl requirements after two different types of surgery. These findings may contribute to personalized pain control.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. *Neuropsychopharmacology Reports* published by John Wiley & Sons Australia, Ltd on behalf of the Japanese Society of Neuropsychopharmacology.

**KEYWORDS**

ASTN2, laparoscopic colectomy, mandibular sagittal split ramus osteotomy, opioids, pain-related phenotypes, single nucleotide polymorphisms

1 | INTRODUCTION

Opioid analgesics, such as fentanyl and morphine, are widely used for the treatment of moderate to severe pain. However, the analgesic efficacy of opioids varies widely among individuals.^{1,2} Individual differences may be related to various genetic and non-genetic factors.^{1,2} Previous twin studies showed that heritable genetic factors can account for a few tens to several tens of percent of individual differences in the sensitivity to pain and/or opioids.³⁻⁵ However, distinctive genetic factors remain to be identified that can affect individual differences in opioid sensitivity. To date, several candidate gene polymorphisms have been reported to affect opioid sensitivity in humans.^{1,2,6-22}

In a previous genome-wide association study that sought to comprehensively explore single nucleotide polymorphisms (SNPs) that affect fentanyl requirements for pain control after laparoscopic colectomy, we found that a nonsynonymous SNP, rs207622, of the *LAMB3* gene (which encodes laminin β 3) was most strongly associated with postoperative fentanyl requirements, and we further examined the effects of this SNP on various pain-related phenotypes.¹⁸ In this previous study, we also found that two SNPs, rs958804 and rs7858836, of the *ASTN2* gene (which encodes astrotactin 2) were among the top candidate SNPs that affected fentanyl requirements after laparoscopic colectomy. In this previous study, however, we did not examine the effects of these two SNPs on pain-related phenotypes, including postoperative fentanyl requirements, in detail. Furthermore, the effects of these two SNPs on pain-related phenotypes have not been examined in patients who underwent other types of surgery, such as mandibular sagittal split ramus osteotomy. These two types of surgery are quite different. Laparoscopic colectomy primarily involves visceral pain, whereas mandibular sagittal split ramus osteotomy solely involves somatic pain.¹⁹

In the present study, we investigated the effects of these two SNPs, rs958804 and rs7858836, of the *ASTN2* gene on eight and seven pain-related traits in patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy, respectively, by additionally genotyping genomic DNA samples and re-analyzing existing data that were initially obtained in our previous studies.^{8,12,18}

2 | METHODS

We studied two groups of patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy.

2.1 | Pain-related phenotypes in patients who underwent laparoscopy colectomy

We enrolled 351 patients who underwent laparoscopic colectomy for colorectal cancer at Saitama Medical University International Medical Center. The surgical and anesthetic protocols were the same as in our previous study.¹⁸ Briefly, general anesthesia was induced with fentanyl (100 μ g), propofol (1-2 mg/kg), and rocuronium (0.8-1 mg/kg) and maintained with sevoflurane (1.2% end-tidal concentration), remifentanyl (0.2-0.5 μ g/kg/min), and intermittent doses of rocuronium. The average intraoperative dose of remifentanyl was recorded. At the end of surgery, fentanyl (100 μ g) was intravenously administered. If required after emergence from anesthesia, incremental doses of fentanyl (50 μ g) were titrated at 3-min intervals until adequate pain relief was achieved. Intravenous fentanyl patient-controlled analgesia (PCA) with a PCA pump (CADD-Legacy PCA pump, Model 6300; Smiths Medical Japan) was started. The bolus dose of fentanyl on demand and lockout time was set at 20 μ g and 5 min, respectively. Intravenous flurbiprofen (50 mg) or pentazocine (30 mg) was given as a rescue analgesic as required. The cumulative fentanyl doses via PCA 6, 12, and 24 hours postoperatively were normalized to body weight and recorded as 6-hour, 12-hour, and 24-hour postoperative fentanyl use, respectively. The intensity of pain at rest was evaluated using an 11-point numerical rating scale (NRS; 0, no pain; 10, most severe pain imaginable) at each postoperative time point and recorded as pain scores 6, 12, and 24 hours postoperatively. The number of patients who required rescue analgesics within 24 hours postoperatively was recorded. Thus, we examined a total of eight pain-related phenotypes in this group of patients (Table 1).

2.2 | Pain-related phenotypes in patients who underwent mandibular sagittal split ramus osteotomy

We enrolled 362 patients who underwent mandibular sagittal split ramus osteotomy for mandibular prognathism at Tokyo Dental College Suidobashi Hospital. The surgical and anesthetic protocols were the same as in our previous study.⁸ Briefly, before the induction of anesthesia, the patients had an intravenous line placed in their nondominant forearm. The cold pressor-induced pain test was then performed before and 3 minutes after an intravenous bolus injection of fentanyl (2 μ g/kg). Briefly, 0°C ice-cold water was prepared in a 1-L tank. The patient kept their whole dominant hand calm in the ice-cold water and withdrew it as soon as the patient perceived any pain. The baseline latency to pain perception,

TABLE 1 Demographic, genomic, and pain-related variables in patients undergoing laparoscopic colectomy

	Laparoscopic colectomy (n = 350)
Age (y)	63.7 ± 10.5
Sex	Males 217/Females 133
Body height (cm)	160.8 ± 9.2
Body weight (kg)	60.1 ± 11.0
Genotypes of the rs958804 T/C SNP	TT: 144/TC: 162/CC: 44
Genotypes of the rs7858836 C/T SNP	CC:114/CT:174/TT: 62
Average remifentanyl dose(μg/kg/min)	0.2 (0.2-0.3)
6-h postoperative fentanyl use(μg/kg)	4.1 (1.8-5.8)
12-h postoperative fentanyl use(μg/kg)	6.1 (2.9-8.7)
24-h postoperative fentanyl use (μg/kg)	9.6 (5.3-13.7)
11-point NRS pain scores at 6 h	2.6 (1-4)
11-point NRS pain scores at 12 h	2.2 (0-3)
11-point NRS pain scores at 24 h	2.1 (1-3)
Number of patients who required rescue analgesics within 24 h	159/350

Note: Data are shown as Mean ± SD, Median (Interquartile range), or Number.

defined as the duration of immersion of the hand in the ice-cold water, was recorded as PPLpre. A cutoff time of 150 seconds was set to avoid tissue damage. After the hand was fully rewarmed, the patient received 2 μg/kg of intravenous fentanyl. Three minutes after the fentanyl injection, the cold pressor-induced pain test was performed again, and the pain perception latency of the dominant hand was measured as PPLpost. The analgesic effect of fentanyl in the preoperative cold pressor-induced pain test was evaluated as the difference between PPLpost and PPLpre (PPLpost-PPLpre) and the percent maximum possible effect (%MPE): (PPLpost-PPLpre)/(150-PPLpre) × 100.

After the pain test with fentanyl (2 μg/kg), general anesthesia was induced with a target-controlled infusion (TCI) of propofol using a TCI pump (TE-371, Terumo) and vecuronium (0.1 mg/kg) and maintained with propofol at a target blood concentration of 4-6 μg/mL. Fentanyl (1 μg/kg) was administered repeatedly when systolic blood pressure or heart rate exceeded +20% of the preinduction value, and vecuronium was administered at a rate of 0.08 mg/kg/h. Right and left mandibular ramus osteotomies were performed after local anesthesia with 2% lidocaine with 1:80 000 epinephrine (8 mL) on each side of the surgical field. Finally, the bilateral mandibular bone segments were fixed in appropriate positions.

At the end of surgery, rectal diclofenac sodium (50 mg) and intravenous dexamethasone (6.6 mg) were administered at the request of surgeons to prevent postoperative orofacial edema/swelling. After emergence from anesthesia, intravenous PCA with fentanyl was then started with a CADD-Legacy PCA pump. The bolus dose of fentanyl on demand and lockout time was set at 20 μg and 10 minutes, respectively. Rectal diclofenac sodium

(50 mg) was given as a rescue analgesic as required. The intensity of spontaneous pain was assessed 3 and 24 hours postoperatively using a 100-mm visual analog scale (VAS; 0 mm, no pain; 100 mm, most severe pain imaginable) and recorded as pain scores 3 and 24 hours postoperatively. Intraoperative fentanyl doses and the cumulative fentanyl doses via PCA 24 hours postoperatively, normalized to body weight, were recorded as intraoperative fentanyl use and 24-hour postoperative fentanyl use, respectively. Thus, we examined a total of seven pain-related phenotypes in this group of patients (Table 2).

2.3 | Genotyping of the rs958804 and rs7858836 SNPs in patients who underwent laparoscopic colectomy

Total genomic DNA was extracted from peripheral blood. Genotype data for the rs958804 and rs7858836 SNPs 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) as described in detail in a previous study.¹⁸ The genotyping results for the two SNPs were qualified using a data cleaning process using GenomeStudio with the Genotyping 3.3.7 module (Illumina). In the data cleaning process, markers with a genotype call frequency <0.95, "Cluster sep" (ie, an index of genotype cluster separation) <0.1, or *P* value (*df* = 1) <.001 in the Hardy-Weinberg equilibrium tests were excluded from the subsequent association study. A total of 921 239 SNP markers, including the rs958804 and rs7858836 SNPs, survived the entire quality control filtration process. Linkage disequilibrium (LD) analysis was performed using Haploview 4.1 as appropriate.

TABLE 2 Demographic, genomic, and pain-related variables in patients undergoing mandibular sagittal split ramus osteotomy

	Sagittal split ramus osteotomy (n = 358)
Age (y)	25.9 ± 7.6
Sex	Males 125/Females 233
Body height (cm)	164.7 ± 10.0
Body weight (kg)s	57.6 ± 10.8
Genotypes of the rs958804 T/C SNP	TT: 135/TC: 180/CC: 43
Genotypes of the rs7858836 C/T SNP	CC:104/CT: 191/TT: 63
PPLpre(s)	19.2 (9-23)
PPLpost-PPLpre(s)	26.1 (4.8-35)
%MPE	22.1 (3.4-26.6)
Intraoperative fentanyl use(μg/kg)	4.5 (3.2-5.9)
24-h postoperative fentanyl use(μg/kg)	3.0 (1.0-4.3)
100-mm VAS pain scores at 3 h	31.8 (15-50)
100-mm VAS pain scores at 24 h	27.1(10-41.3)

Note: Data are shown as Mean ± SD, Median (Interquartile range), or Number.



2.4 | Genotyping of the rs958804 and rs7858836 SNPs in patients who underwent mandibular sagittal split ramus osteotomy

Genomic DNA was extracted from whole blood. For the analysis of the rs958804 SNP, genotype data from whole-genome genotyping were used as described in detail in a previous study.⁸ Briefly, whole-genome genotyping was performed using the Infinium assay II with an iScan system (Illumina). The data for the whole-genome genotyped samples were analyzed using BeadStudio or GenomeStudio with the genotyping module 3.3.7 (Illumina) to evaluate the quality of the results. The genotype data from all five BeadChips were merged to analyze all of the samples simultaneously. Thus, only markers that were common to all of the BeadChips were included in the analysis. In the data cleaning process, samples with a genotype call rate <0.95 were excluded from subsequent analyses. As a result, one sample was excluded. Markers with a genotype call frequency <0.95 , "Cluster Sep" <0.1 , or P value ($df = 1$) $<.001$ in the Hardy-Weinberg equilibrium tests were excluded from the subsequent association study. A total of 295 036 SNP markers, including the rs958804 SNP, survived the entire quality control filtration process. Because the genotype data for the rs7858836 SNP were not included in the aforementioned BeadChips for whole-genome genotyping, genotyping of the rs7858836 SNP was additionally performed using the TaqMan allelic discrimination assay (Life Technologies). Linkage disequilibrium analysis was performed using Haploview 4.1 as appropriate.

2.5 | Statistical analyses

Parametric, nonparametric, and categorical data are expressed as mean \pm SD, median (interquartile range), and number, respectively. The statistical analysis was performed using SPSS 20.0.0 software (IBM). Because all of the continuous variables that were associated with pain-related phenotypes were non-normally distributed, nonparametric analyses, including the Mann-Whitney U test and Spearman's correlation coefficient, were primarily used to detect possible associations between any of the demographic or genomic variables, including age, sex, and genotypes of the two SNPs, and continuous variables that were associated with the pain-related phenotypes. The χ^2 test was used to detect possible associations between any of the demographic or genomic variables and categorical data that were associated with pain-related phenotypes. When associations between the genotypes and pain-related phenotypes were examined, patients were dichotomized into homozygotes of the major allele and carriers of the minor allele of each SNP, primarily because many pain-related phenotypes significantly differed between homozygotes of the major allele and heterozygotes but not between heterozygotes and homozygotes of the minor allele, especially in patients who underwent mandibular sagittal split ramus osteotomy (Figures S1 and S2), and secondarily because dichotomized data (compared with trichotomized data)

were extremely helpful in simplifying the data analysis, especially when application of the multiple regression analysis was required for data adjustment. When significant associations between genotypes and pain-related phenotypes were detected, we used nonparametric analyses to evaluate whether the genotype groups were controlled by demographic factors (age and sex) that could affect pain-related phenotypes. If required, then we conducted the additional multiple regression analysis by employing SNP genotypes, sex, and/or age as independent covariates after selecting candidate covariates that should be entered into the multivariate regression model using Pearson's correlation coefficient, although the nonparametric distributions of most of our data were unsuitable for the application of such parametric techniques. For analyses of the rs958804 and rs7858836 SNPs, values of $P < .025$ ($0.05/2$) were considered statistically significant when considering multiple testing for these SNPs. Otherwise, values of $P < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Patients who underwent laparoscopic colectomy

One patient was excluded from the analysis because reoperation for hemostasis was performed within 24 hours postoperatively. Thus, a total of 350 patients were included in the analysis. Demographic, genomic, and pain-related data are presented in Table 1.

3.1.1 | Effect of age on pain-related phenotypes

Spearman's and Pearson's correlation coefficients revealed that 6-hour, 12-hour, and 24-hour postoperative fentanyl use generally correlated or tended to correlate with age ($\rho = -0.126$, $P = .0183$, and $r = -0.192$, $P = .0003$ for 6 hours; $\rho = -0.102$, $P = .0569$, and $r = -.171$, $P = .0013$ for 12 hours; $\rho = -0.053$, $P = .3219$, and $r = -.102$, $P = .0565$ for 24 hours, respectively). Other pain-related phenotypes did not correlate or tended to correlate with age (data not shown).

The Mann-Whitney test revealed no significant association between age and dichotomized genotypes of the rs958804 T/C SNP (ie, TT vs TC + CC; $P = .9970$) or between age and dichotomized genotypes of the rs7858836 C/T SNP (ie, CC vs CT + TT; $P = .2778$), indicating that age and genotype could be simultaneously employed as independent covariates in the multiple regression analysis.

3.1.2 | Effect of sex on pain-related phenotypes

The Mann-Whitney and χ^2 tests revealed that none of the pain-related phenotypes were associated with sex (data not shown).

3.1.3 | Relationship between the rs958804 T/C SNP and rs7858836 C/T SNP

In 350 patients, genotypes of the rs958804 T/C SNP were TT, TC, and CC in 144, 162, and 44 patients, respectively, which were in Hardy-Weinberg equilibrium (data not shown). The minor C allele frequency was 35.7%. Genotypes of the rs7858836 C/T SNP were CC, CT, and TT in 114, 174, and 62 patients, respectively, which were in Hardy-Weinberg equilibrium (data not shown). The minor T allele frequency was 42.6%. The LD analysis revealed that the rs958804 T/C and rs7858836 C/T SNPs were located in the same LD block of the *ASTN2* gene, and the r^2 values between these two SNPs were 0.75 (Figure S3), suggesting that the minor C allele of the rs958804 T/C SNP was moderately linked to the minor T allele of the rs7858836 C/T SNP.

3.1.4 | Effect of genotypes of the rs958804 T/C SNP (TT vs TC + CC) on pain-related phenotypes

The Mann-Whitney test revealed that 6-hour, 12-hour, and 24-hour postoperative fentanyl use was lower in patients with the C allele compared with patients with the TT genotype ($P = .0051$, $P = .0028$, and $P = .0019$, respectively; Figure 1A), although the other continuous variables that were associated with pain-related phenotypes, including pain scores 6, 12, and 24 hours postoperatively, were not associated with these genotypes (data not shown). The χ^2 test revealed that the number of patients who required rescue analgesics within 24 hours was lower in patients with the C allele than in patients with the TT genotype (81/206 [39.3%] for TC + CC vs 78/144 [54.2%] for TT, $P = .0061$).

The multiple regression analysis revealed that 6-hour, 12-hour, and 24-hour postoperative fentanyl use was significantly associated with genotypes of the rs958804 SNP, independent of age ($P = .0012$ and $P = .0002$ [6 hours], $P = .0005$ and $P = .0010$ [12 hours], and $P = .0020$ and $P = .0500$ [24 hours] for genotype and age, respectively).

In summary, patients with the minor C allele of the rs958804 T/C SNP required less postoperative PCA fentanyl and required rescue

analgesics less frequently to achieve similar levels of analgesia compared with patients with the TT genotype.

3.1.5 | Effect of genotypes of the rs7858836 C/T SNP (CC vs CT + TT) on pain-related phenotypes

The Mann-Whitney test revealed that 6-hour, 12-hour, and 24-hour postoperative fentanyl use was lower in patients with the T allele than in patients with the CC genotype ($P = .0136$, $P = .0033$, and $P = .0017$, respectively; Figure 1B). The other continuous variables that were associated with pain-related phenotypes, including pain scores 6, 12, and 24 hours postoperatively, were not associated with these genotypes (data not shown). The χ^2 test revealed that the number of patients who required rescue analgesics within 24 hours tended to be lower in patients with the T allele than in patients with the CC genotype (98/236 [41.5%] for CT + TT vs 61/114 [53.5%] for CC, $P = .0348$).

The multiple regression analysis revealed that 6-hour, 12-hour, and 24-hour postoperative fentanyl use was significantly associated with genotypes of the rs7858836 C/T SNP, independent of age ($P = .0191$ and $P = .0004$ [6 hours], $P = .0057$ and $P = .0017$ [12 hours], and $P = .0037$ and $P = .0703$ [24 hours] for genotype and age, respectively).

In summary, patients with the minor T allele of the rs7858836 C/T SNP required less postoperative PCA fentanyl and required rescue analgesics less frequently to achieve similar levels of analgesia compared with patients with the CC genotype.

3.2 | Patients who underwent mandibular sagittal split ramus osteotomy

Four patients were excluded from the analysis for the following reasons. Genotype data for the rs958804 T/C SNP were unavailable for one patient. The analgesic effect of fentanyl could not be evaluated because PPLpre exceeded the cutoff point (150 seconds) in one patient. The cold-pressor test was not performed in one patient. Data

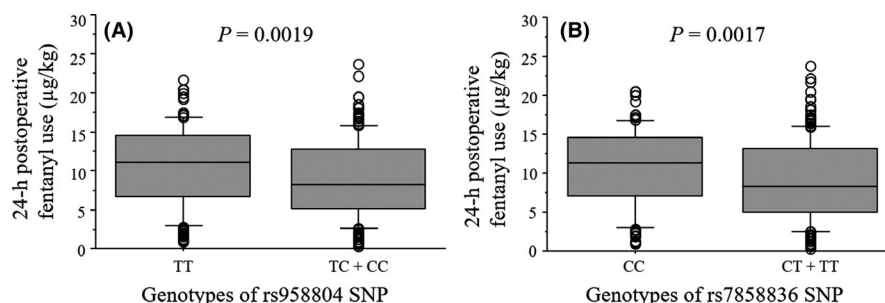


FIGURE 1 Associations (A) between genotypes of the rs958804 SNP (TT, $n = 144$; TC + CC, $n = 206$) and 24-h postoperative fentanyl use and (B) between genotypes of the rs7858836 SNP (CC, $n = 114$; CT + CC, $n = 236$) and 24-h postoperative fentanyl use in 350 patients who underwent laparoscopic surgery. The data are expressed as box and whisker plots. Upper and lower ends of the boxes represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th and 10th percentiles, respectively. Open circles represent outliers. The median is depicted by a solid line in the box

on fentanyl use were missing for one patient. Therefore, a total of 358 patients were included in the analysis. Demographic, genomic, and pain-related data are presented in Table 2. Only one patient required a rescue analgesic (diclofenac sodium) only once. The Mann-Whitney test revealed that 24-hour postoperative fentanyl use was much lower in patients who underwent this surgery compared with patients who underwent laparoscopic colectomy ($P < .0001$; Tables 1 and 2; Figures 1 and 2).

3.2.1 | Effect of age on pain-related phenotypes

Spearman's and Pearson's correlation coefficients revealed that 24-hour postoperative fentanyl use correlated or tended to correlate with age ($\rho = -0.107$, $P = .0427$, and $r = -.089$, $P = .0926$, respectively), although age was not associated or only tended to be associated with other pain-related phenotypes (data not shown).

The Mann-Whitney test, Spearman's correlation coefficient, and Pearson's correlation coefficient revealed a tendency toward an association between age and genotype of the rs958804 T/C SNP (TT vs TC + CC; Mann-Whitney test: $P = .0361$; Spearman's correlation: $\rho = 0.111$, $P = .0361$; Pearson's correlation: $r = .104$, $P = .0482$) and a significant association or tendency toward an association between age and the rs7858836 C/T SNP (CC vs CT + TT; Mann-Whitney test: $P = .0166$; Spearman's correlation: $\rho = 0.127$, $P = .0166$; Pearson's correlation: $r = .114$, $P = .0307$). However, the r values were too low (ie, $r < .8$) to indicate that age and the genotype of these SNPs were mathematically related. Therefore, age and these genotypes could be simultaneously employed as independent covariates in the multiple regression analysis.

3.2.2 | Effect of sex on pain-related phenotypes

The Mann-Whitney test revealed that PPLpost-PPLpre and %MPE were higher in males than in females ($P = .0399$ and $P = .0330$, respectively). Intraoperative and 24-hour postoperative fentanyl use were lower in males than in females ($P = .0068$ and $P = .0305$,

respectively). Other pain-related phenotypes were not associated or tended to be associated with sex (data not shown).

The Mann-Whitney test revealed no significant association between sex and genotype of the rs958804 T/C SNP (TT vs TC + CC; $P = .8437$) or between sex and genotype of the rs7858836 SNP C/T SNP (CC vs CT + TT; $P = .3686$). Therefore, sex and these genotypes could be simultaneously employed as independent covariates in the multiple regression analysis. The Mann-Whitney test revealed that age was significantly higher in females than in males ($P = .0137$), and Spearman's and Pearson's correlation coefficients revealed significant correlations between sex and age ($\rho = 0.130$, $P = .0137$, and $r = .138$, $P = .0087$, respectively), but the r value was too low to indicate that age and sex were mathematically related. Therefore, age and sex could also be simultaneously employed as independent covariates in the multiple regression analysis.

3.2.3 | Relationship between the rs958804 T/C SNP and rs7858836 C/T SNP

In 358 patients, genotypes of the rs958804 SNP were TT, TC, and CC in 135, 180, and 43 patients, respectively, which were in Hardy-Weinberg equilibrium (data not shown). The minor C allele frequency was 37.2%. Genotypes of the rs7858836 SNP were CC, CT, and TT in 104, 191, and 63 patients, respectively, which were in Hardy-Weinberg equilibrium (data not shown). The LD analysis revealed that the rs958804 T/C and rs7858836 C/T SNPs were located in the same LD block of the *ASTN2* gene, and the r^2 value between these two SNPs were 0.71, again suggesting that the minor C allele of the rs958804 T/C SNP was moderately linked to the minor T allele of the rs7858836 C/T SNP (Figure S4).

3.2.4 | Effect of genotypes of the rs958804 SNP (TT vs TC + CC) on pain-related phenotypes

The Mann-Whitney test revealed that PPLpost-PPLpre and %MPE tended to be higher in patients with the C allele of the rs958804

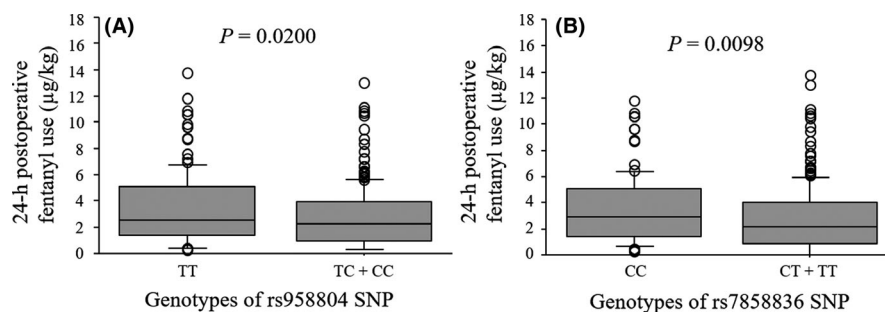


FIGURE 2 Associations (A) between genotypes of the rs958804 SNP (TT, $n = 135$; TC + CC, $n = 223$) and 24-h postoperative fentanyl use and (B) between genotypes of the rs7858836 SNP (CC, $n = 104$; CT + CC, $n = 254$) and 24-h postoperative fentanyl use in 358 patients who underwent mandibular sagittal split ramus osteotomy. The data are expressed as box and whisker plots. Upper and lower ends of the boxes represent the 75th and 25th percentiles, respectively. Whiskers represent the 90th and 10th percentiles, respectively. Open circles represent outliers. The median is depicted by a solid line in the box



SNP than in patients with the TT genotype ($P = .0504$ and $P = .0434$, respectively), and 24-hour postoperative fentanyl use was lower ($P = .0200$; Figure 2A), and pain scores 3 hours postoperatively tended to be lower ($P = .0910$) in patients with the C allele than in patients with the TT genotype, although other pain-related phenotypes, including PPLpre, did not tend to be associated with genotype.

The multiple regression analysis revealed that 24-hour postoperative fentanyl use was significantly associated with genotypes of the rs958804 SNP, independent of age and sex ($P = .0162$, $P = .0977$, and $P = .0904$ for genotype, age, and sex, respectively). PPLpost-PPLpre and %MPE were associated with sex ($P = .0139$ and $P = .0126$, respectively) and tended to be associated with genotype ($P = .0589$ and $P = .0737$, respectively).

In summary, PPLpost-PPLpre and %MPE tended to be higher, 24-hour postoperative fentanyl use was significantly lower, and pain score 3 hours postoperatively tended to be lower in patients with the minor C allele of the rs958804 T/C SNP than in patients with the TT genotype, although PPLpre was not associated with these genotypes. These findings suggested that although sensitivity to cold pressor-induced pain, indicated by PPLpre, was unaffected by genotype, the analgesic effect of fentanyl, indicated by PPLpost-PPLpre and %MPE, tended to be higher in patients with the C allele than in patients with the TT genotype. Patients with the C allele required significantly less fentanyl postoperatively to achieve similar or better levels of postoperative analgesia compared with patients with the TT genotype.

3.2.5 | Effect of genotypes of the rs7858836 SNP (CC vs CT + TT) on pain-related phenotypes

The Mann-Whitney test revealed that 24-hours postoperative fentanyl use was lower in patients with the T allele than in patients with the CC genotype ($P = .0098$; Figure 2B). The other pain-related phenotypes, including pain scores 3 and 24 hours postoperatively, were not associated with these genotypes (data not shown).

The multiple regression analysis revealed that 24-hour postoperative fentanyl use tended to be associated with genotypes of the rs7858836 SNP, age, and sex ($P = .0277$, $P = .0962$, and $P = .0766$, respectively), indicating that 24-hour postoperative fentanyl use could be associated with genotype, independent of age and sex.

In summary, patients with the T allele of the rs7858836 SNP required less fentanyl postoperatively to achieve similar levels of analgesia compared with patients with the CC genotype.

4 | DISCUSSION

In the present study, we examined the effects of two SNPs, rs958804 T/C and rs7858836 C/T, of the *ASTN2* gene in patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy. In patients who underwent laparoscopic colectomy, patients with the minor C allele of the rs958804 T/C SNP

required less PCA fentanyl and less rescue analgesics to achieve similar levels of postoperative analgesia compared with patients with the TT genotype. Patients with the minor T allele of the rs7858836 C/T SNP required less PCA fentanyl and less rescue analgesics to achieve similar levels of postoperative analgesia compared with patients with the CC genotype. In patients who underwent mandibular sagittal split ramus osteotomy, patients with the minor C allele of the rs958804 T/C SNP required less PCA fentanyl to achieve similar or better levels of postoperative analgesia compared with patients with the TT genotype. Patients with the minor T allele of the rs7858836 C/T SNP required less PCA fentanyl to achieve similar levels of postoperative analgesia compared with patients with the CC genotype.

Therefore, the major findings of the present study that were common among patients who underwent the two different types of surgery were that patients who carried the minor alleles of the rs958804 T/C and rs7858836 C/T SNPs, which were located in the same LD block of the *ASTN2* gene, required less PCA fentanyl to achieve similar levels of postoperative analgesia compared with homozygotes of the major alleles of these SNPs. These two types of surgery have substantially different characteristics. Laparoscopic colectomy primarily involves visceral pain, and mandibular sagittal split ramus osteotomy solely involves somatic pain.¹⁹ Consequently, patients who underwent mandibular sagittal split ramus osteotomy required much less postoperative fentanyl compared with patients who underwent laparoscopic colectomy. Despite such differences between the two types of surgery, the effects of these two SNPs on postoperative PCA fentanyl requirements were common to the two surgical procedures. To our knowledge, no previous studies have addressed the importance of the rs958804 and rs7858836 SNPs. The present study is the first to demonstrate significant effects of these SNPs on postoperative PCA fentanyl requirements.

The mechanisms that underlie lower PCA fentanyl dose requirements in patients who carried minor alleles of these SNPs are unclear. In the present study, however, PPLpost-PPLpre and %MPE were used as indicators of the analgesic effect of fentanyl on cold pressor-induced pain, which tended to be higher in patients with the minor C allele of the rs958804 SNP than in patients who did not carry this allele. PPLpre was used as an indicator of sensitivity to cold pressor-induced pain, which did not differ between patients with these different genotypes. Therefore, the effect of the rs958804 T/C SNP on the analgesic effect of fentanyl, rather than its effect on sensitivity to pain, might be associated with lower postoperative fentanyl requirements in patients with the minor allele of this SNP.

The *ASTN2* gene encodes the vertebrate-specific membrane protein astrotactin 2. Together with astrotactin 1, astrotactin 2 plays a key role in glial-guided neuronal migration during development of the laminar architecture of cortical regions of the mammalian brain.^{23,24} Some researchers reported that certain variants of the *ASTN2* gene might be associated with the development of neuropsychiatric disorders, such as autism spectrum disorder, attention-deficit/hyperactivity disorder, anxiety, obsessive-compulsive disorder, schizophrenia, and Alzheimer's disease,²⁵⁻²⁷ although others did not find such associations between *ASTN2* gene variants and neuropsychiatric disorders.²⁸



A substantial number of studies have shown or suggested interactions between these neuropsychiatric disorders and alterations of opioid system function.^{29–32} To date, however, no study has investigated possible direct associations between variants of the *ASTN2* gene and alterations of opioid system function. The present study found significant effects of SNPs of the *ASTN2* gene on opioid analgesia. The presence of minor alleles of the rs958804 T/C and rs7858836 C/T SNPs of the *ASTN2* gene consistently reduced fentanyl requirements for analgesia after two different types of surgery, possibly by enhancing the analgesic effect of fentanyl. The molecular biological mechanisms that underlie such effects of these SNPs on pain-related traits should be elucidated in future studies.

In conclusion, we investigated the effects of two SNPs, rs958804 T/C and rs7858836 C/T, which are located in the same LD block of the *ASTN2* gene, on pain-related phenotypes in two groups of patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy. We found that these SNPs consistently reduced fentanyl requirements for postoperative analgesia, possibly by enhancing the analgesic effect of fentanyl. Although the molecular biological mechanisms that underlie these effects of these SNPs need to be elucidated in future studies, our findings provide valuable information for personalized pain control after painful surgical procedures.

ACKNOWLEDGEMENTS

We thank Michael Arends for editing the manuscript. We are grateful to the volunteers for their participation in the study and anesthesiologists and surgeons for collecting the clinical data. This work was supported by grants from the Japan Society for the Promotion of Science (JSPS) KAKENHI (no. 22790518, 23390377, 24790544, 26293347, JP16H06276 [AdAMS], 17H04324, 17K08970, 17K09052, 18K08829, 20K09259, and 20K07774), Ministry of Health, Labour, and Welfare (MHLW) of Japan (no. H26-Kakushintekigan-ippa-060), Japan Agency for Medical Research and Development (AMED; no. JP19ek0610011 and JP19dk0307071), Smoking Research Foundation (Tokyo, Japan), and Japan Research Foundation for Clinical Pharmacology (JRFPC). The funding agencies had no role in the study design, data collection or analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTEREST

Kazutaka Ikeda has received support from Asahi Kasei Pharma Corporation for a project that is unrelated to this research and speaker's and consultant's fees from MSD KK, VistaGen Therapeutics, Inc, Atheneum Partners Otsuka Pharmaceutical Co. Ltd., Taisho Pharmaceutical Co. Ltd., Eisai, Daiichi-Sankyo, Inc, Sumitomo Dainippon Pharma, Japan Tobacco, Inc, and Nippon Chemiphar.

AUTHOR CONTRIBUTIONS

RI, DN, KF, HS, KI, and MH conceived and designed the experiments. RI, DN, and JH performed the experiments. RI, DN, KN, and MH analyzed the data. DN and JH contributed reagents/materials/analysis

tools. RI, DN, HS, KI, and MH wrote the paper. RI, KF, TI, TM, MT, HN, AK, HS, KI, and MH collected clinical data and DNA.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEW BOARD

The protocol for this research project was approved by suitably constituted Ethics Committees of the institutions (Committee of Tokyo Metropolitan Institute of Medical Science, approval no. 18-16[1] and 19-12[1]; Committee of Tokyo Dental College, approval no. 812; Committee of Saitama Medical University International Medical Center, approval no. 09-089) and conformed with provisions of the Declaration of Helsinki.

INFORMED CONSENT

Informed consent was obtained from all subjects and/or their guardians.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article (Data S1).

ORCID

Kazutaka Ikeda  <https://orcid.org/0000-0001-8342-0278>

REFERENCES

- Ikeda K, Ide S, Han W, Hayashida M, Uhl GR, Sora I. How individual sensitivity to opiates can be predicted by gene analyses. *Trends Pharmacol Sci.* 2005;26(6):311–7.
- Coulbault L, Beaussier M, Verstuyft C, Weickmans H, Dubert L, Tregouet D, et al. Environmental and genetic factors associated with morphine response in the postoperative period. *Clin Pharmacol Ther.* 2006;79(4):316–24.
- Nishizawa D, Ikeda K. Genome-wide association studies and human opioid sensitivity. In: Preedy VR editor. *The neuropathology of drug addictions and substance misuse: volume 1. Foundations of understanding, tobacco, alcohol, cannabinoids and opioids.* Academic Press, 2016; 909–21.
- Angst MS, Phillips NG, Drover DR, Tingle M, Galinkin JL, Christians U, et al. Opioid pharmacogenomics using a twin study paradigm: methods and procedures for determining familial aggregation and heritability. *Twin Res Hum Genet.* 2010;13(5):412–25.
- Angst MS, Phillips NG, Drover DR, Tingle M, Ray A, Swan GE, et al. Pain sensitivity and opioid analgesia: a pharmacogenomic twin study. *Pain.* 2012;153(7):1397–409.
- Hayashida M, Nagashima M, Satoh Y, Katoh R, Tagami M, Ide S, et al. Analgesic requirements after major abdominal surgery are associated with *OPRM1* gene polymorphism genotype and haplotype. *Pharmacogenomics.* 2008;9(11):1605–16.
- Nishizawa D, Nagashima M, Katoh R, Satoh Y, Tagami M, Kasai S, et al. Association between *KCNJ6* (*GIRK2*) gene polymorphisms and postoperative analgesic requirements after major abdominal surgery. *PLoS One.* 2009;4(9):e7060.
- Fukuda K, Hayashida M, Ide S, Saita N, Kokita Y, Kasai S, et al. Association between *OPRM1* gene polymorphisms and fentanyl sensitivity in patients undergoing painful cosmetic surgery. *Pain.* 2009;147(1–3):194–201.
- Lotsch J, Geisslinger G, Tegeder I. Genetic modulation of the pharmacological treatment of pain. *Pharmacol Ther.* 2009;124(2):168–84.



10. Candiotti KA, Yang Z, Morris R, Yang J, Crescimone NA, Sanchez GC, et al. Polymorphism in the interleukin-1 receptor antagonist gene is associated with serum interleukin-1 receptor antagonist concentrations and postoperative opioid consumption. *Anesthesiology*. 2011;114(5):1162–8.
11. Moriyama A, Nishizawa D, Kasai S, Hasegawa J, Fukuda K, Nagashima M, et al. Association between genetic polymorphisms of the β 1-adrenergic receptor and sensitivity to pain and fentanyl in patients undergoing painful cosmetic surgery. *J Pharmacol Sci*. 2013;121(1):48–57.
12. Aoki Y, Nishizawa D, Kasai S, Fukuda K, Ichinohe T, Yamashita S, et al. Association between the variable number of tandem repeat polymorphism in the third exon of the dopamine D4 receptor gene and sensitivity to analgesics and pain in patients undergoing painful cosmetic surgery. *Neurosci Lett*. 2013;542:1–4.
13. Ide S, Nishizawa D, Fukuda K, Kasai S, Hasegawa J, Hayashida M, et al. Association between genetic polymorphisms in $Ca_v2.3$ (R-type) Ca^{2+} channels and fentanyl sensitivity in patients undergoing painful cosmetic surgery. *PLoS One*. 2013;8(8):e70694.
14. Nishizawa D, Fukuda K, Kasai S, Hasegawa J, Aoki Y, Nishi A, et al. Genome-wide association study identifies a potent locus associated with human opioid sensitivity. *Mol Psychiatry*. 2014;19(1):55–62.
15. Nishizawa D, Fukuda KI, Kasai S, Ogai Y, Hasegawa J, Sato N, et al. Association between *KCNJ6* (*GIRK2*) gene polymorphism rs2835859 and post-operative analgesia, pain sensitivity, and nicotine dependence. *J Pharmacol Sci*. 2014;126(3):253–63.
16. Ide S, Nishizawa D, Fukuda K, Kasai S, Hasegawa J, Hayashida M, et al. Haplotypes of *P2RX7* gene polymorphisms are associated with both cold pain sensitivity and analgesic effect of fentanyl. *Mol Pain*. 2014;10:75.
17. Aoki Y, Nishizawa D, Hasegawa J, Kasai S, Yoshida K, Koukita Y, et al. Association between the rs1465040 single-nucleotide polymorphism close to the transient receptor potential subfamily C member 3 (*TRPC3*) gene and postoperative analgesic requirements. *J Pharmacol Sci*. 2015;127(3):391–3.
18. Mieda T, Nishizawa D, Nakagawa H, Tsujita M, Imanishi H, Terao K, et al. Genome-wide association study identifies candidate loci associated with postoperative fentanyl requirements after laparoscopic-assisted colectomy. *Pharmacogenomics*. 2016;17(2):133–45.
19. Amano K, Nishizawa D, Mieda T, Tsujita M, Kitamura A, Hasegawa J, et al. 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. *J Pain*. 2016;17(10):1126–34.
20. Aoki Y, Nishizawa D, Yoshida K, Hasegawa J, Kasai S, Takahashi K, et al. Association between the rs7583431 single nucleotide polymorphism close to the activating transcription factor 2 gene and the analgesic effect of fentanyl in the cold pain test. *Neuropsychopharmacol Rep*. 2018;38(2):86–91.
21. Ohka S, Nishizawa D, Hasegawa J, Takahashi K, Nakayama K, Ebata Y, et al. Association between rs2275913 single-nucleotide polymorphism of the interleukin-17A gene and perioperative analgesic use in cosmetic orthognathic surgery. *Neuropsychopharmacol Rep*. 2018;38(2):67–74.
22. Yokoshima Y, Sumitani M, Nishizawa D, Nagashima M, Ikeda K, Kato R, et al. Gamma-aminobutyric acid transaminase genetic polymorphism is a candidate locus for responsiveness to opioid analgesics in patients with cancer pain: an exploratory study. *Neuropsychopharmacol Rep*. 2018;38(4):175–81.
23. Wilson PM, Fryer RH, Fang Y, Hatten ME *Astn2*, a novel member of the astrotactin gene family, regulates the trafficking of *ASTN1* during glial-guided neuronal migration. *J Neurosci*. 2010;30(25):8529–40.
24. Chang H, Smallwood PM, Williams J, Nathans J. Intramembrane proteolysis of astrotactins. *J Biol Chem*. 2017;292(8):3506–16.
25. Lionel AC, Tammimies K, Vaags AK, Rosenfeld JA, Ahn JW, Merico D, et al. Disruption of the *ASTN2/TRIM32* locus at 9q33.1 is a risk factor in males for autism spectrum disorders, ADHD and other neurodevelopmental phenotypes. *Hum Mol Genet*. 2014;23(10):2752–68.
26. Arioka Y, Kushima I, Kubo H, Mori D, Ozaki N. Induced pluripotent stem cells derived from a schizophrenia patient with *ASTN2* deletion. *Stem Cell Res*. 2018;30:81–4.
27. Velez JI, Lopera F, Creagh PK, Pineros LB, Das D, Cervantes-Henriquez ML, et al. Targeting neuroplasticity, cardiovascular, and cognitive-associated genomic variants in familial Alzheimer's disease. *Mol Neurobiol*. 2019;56(5):3235–43.
28. Freitag CM, Lempp T, Nguyen TT, Jacob CP, Weissflog L, Romanos M, et al. The role of *ASTN2* variants in childhood and adult ADHD, comorbid disorders and associated personality traits. *J Neural Transm*. 2016;123(8):849–58.
29. Moschinski K, Kuske S, Andrich S, Stephan A, Gnass I, Sirsch E, et al. Drug-based pain management for people with dementia after hip or pelvic fractures: a systematic review. *BMC Geriatr*. 2017;17(1):54.
30. Pellissier LP, Gandia J, Laboute T, Becker JAJ, Le Merrer J. μ Opioid receptor, social behaviour and autism spectrum disorder: reward matters. *Br J Pharmacol*. 2018;175(14):2750–69.
31. Clark SD, Abi-Dargham A. The role of dynorphin and the kappa opioid receptor in the symptomatology of schizophrenia: a review of the evidence. *Biol Psychiatry*. 2019;86(7):502–11.
32. Di Liberto D, D'Anneo A, Carlisi D, Emanuele S, De Blasio A, Calvaruso G, et al. Brain opioid activity and oxidative injury: different molecular scenarios connecting celiac disease and autistic spectrum disorder. *Brain Sci*. 2020;10(7):437.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Inoue R, Nishizawa D, Hasegawa J, et al. Effects of rs958804 and rs7858836 single-nucleotide polymorphisms of the *ASTN2* gene on pain-related phenotypes in patients who underwent laparoscopic colectomy and mandibular sagittal split ramus osteotomy. *Neuropsychopharmacol Rep*. 2021;00:1–9. <https://doi.org/10.1002/npr2.12159>