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Association Between Genetic Polymorphisms of the β_1-Adrenergic Receptor and Sensitivity to Pain and Fentanyl in Patients Undergoing Painful Cosmetic Surgery

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Abstract. Individual differences in the sensitivity to fentanyl, a widely used opioid analgesic, can hamper effective pain treatment. The adrenergic system is reportedly involved in the mechanisms of pain and analgesia. Here, we focused on one of the adrenergic receptor genes, *ADRB1*, and analyzed the influence of single-nucleotide polymorphisms (SNPs) in the *ADRB1* gene on individual differences in pain and analgesic sensitivity. We examined associations between pain and fentanyl sensitivity and the two SNPs, A145G and G1165C, in the human *ADRB1* gene in 216 Japanese patients who underwent painful orofacial cosmetic surgery, including bone dissection. The patients who carried the A-allele of the A145G SNP were more sensitive to cold pressor–induced pain than those who did not carry this allele, especially in male patients. The analgesic effect was significantly less in females who carried the G-allele of the G1165C SNP than the females who did not carry the G-allele. The haplotype analysis revealed a significant decrease in 24-h postoperative fentanyl use in female 145A/1165C haplotype carriers. These results suggest that SNPs in the *ADRB1* gene are associated with individual differences in pain and analgesic sensitivity, and analyzing these SNPs may promote personalized pain treatment in the future.

Keywords: β_1-adrenergic receptor, gene polymorphism, opioid, pain, individual difference

Introduction

The pain response comprises an alarm system that protects individuals from physical threats. However, because excessive pain can markedly increase psychological health problems, including depression and sleeplessness, and decrease health-related quality of life, the pain should be managed and treated appropriately by analgesics. Currently, various pain treatments, including cancer-related pain treatment and postoperative pain management, include opioid analgesics. Opioids are general analgesics that are widely used, but they also induce several side-effects, including dependence, nausea, vomiting, constipation, and respiratory depression. Therefore, opioids should be prescribed with proper doses for individual patients (1). Considering that proper analgesic doses can markedly differ between individuals, interindividual differences in pain and analgesic sensitivity can hamper effective pain treatment (2).

Environmental and genetic factors are the main causes of interindividual differences in pain and analgesic sensitivity. Human genetic studies with monozygotic and dizygotic twins reported that the genetic contributions (heritability, h^2) to back pain, migraine, and sciatica are 0.50, 0.34 – 0.57, and 0.21, respectively (3). To date, studies with genetically manipulated mice have identified many genes that are related to nociceptive and opi-

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oid-induced antinociceptive sensitivity (4, 5). Although genetic polymorphisms, including single-nucleotide polymorphisms (SNPs), and short tandem repeats have been found to be associated with pain and analgesic sensitivity in human studies (6), the underlying mechanisms of interindividual differences in pain and analgesic sensitivity have not yet been sufficiently elucidated.

The adrenergic system is involved in the mechanisms of pain and analgesia. For example, the centrally acting α_2-adrenergic receptor agonist clonidine is used clinically as an analgesic against migraine (7). Nine distinct subtypes of adrenergic receptors have been identified from multiple species: α_{1A}, α_{1B}, α_{1D}, $\alpha_{2A\text{-}2C}$, and $\beta_{1\text{-}3}$ (8). Esmolol, a β_1-adrenergic receptor blocker, reportedly suppressed nociception induced by formalin in rats (9). Additionally, morphine requirements for adequate analgesia in patients who were prescribed esmolol were significantly lower than those in non-prescribed patients, suggesting that β_1-adrenergic receptors are related to morphine-induced analgesia (10). Furthermore, β-adrenergic receptor antagonists, such as propranolol and alprenolol, reportedly directly activate the G-protein pathway, thus decreasing the transmission of spinal pain signals (11).

Microinjections of β-adrenergic receptor antagonists and an α_2-receptor agonist into the bed nucleus of the stria terminalis (BNST) in rats markedly attenuated opioid withdrawal–induced conditioned place aversion (12), suggesting that β receptors are closely related to the opioid system. The BNST also reportedly plays an important role in the generation of the negative affective component of pain and is densely innervated by noradrenergic fibers (13). A report that examined the role of noradrenergic transmission within the BNST found that an intra-ventral BNST injection of the β-adrenergic antagonist timolol dose-dependently attenuated intraplantar formalin–induced conditioned place aversion without reducing nociceptive behaviors (14). Thus, β-adrenergic receptors, whose involvement in the negative affective component of pain has been demonstrated, are also considered likely to affect pain sensitivity.

The human β_1-adrenergic receptor gene (*ADRB1*) consists of only one exon that contains short untranslated regions. Therefore, polymorphisms of the human *ADRB1* gene are primarily located in the coding region of the gene (15). To date, several *ADRB1* gene polymorphisms have been reported to be associated with clinical traits (16). The A145G SNP (rs1801252) causes an amino acid substitution from serine to glycine at amino acid position 49, which is located at the extracellular *N*-terminus of the β_1-adrenergic receptor. The β_1-adrenergic receptor with 49Gly shows high agonist affinity, adenylyl cyclase activity, and treatment efficacy for ventricular dilation induced by the β_1-adrenergic receptor inverse agonist metoprolol (17, 18). The G1165C SNP (rs1801253) causes a Gly389Arg amino acid substitution at the intracellular *C*-terminus of the β_1-adrenergic receptor. The 389Arg β_1-adrenergic receptor exhibits high adenylyl cyclase activity (19). This SNP has been reported to be associated with hypertension, heart disease, and lipid metabolism (20 – 22). However, no *ADRB1* polymorphisms have been analyzed with regard to their associations with pain and analgesic sensitivity.

The present study focused on the *ADRB1* gene and analyzed the influence of the A145G and G1165C SNPs in the *ADRB1* gene on individual differences in pain and analgesic sensitivity.

Materials and Methods

Patients

Enrolled in the study were 216 healthy patients (American Society of Anesthesiologists Physical Status I, age 16 – 50 years, 78 males and 138 females) who were scheduled to undergo cosmetic orthognathic surgery (mandibular sagittal ramus osteotomy) for mandibular prognathism at Tokyo Dental College Suidoubashi Hospital. Patients with chronic pain, who took pain medication, or had experienced Raynaud's phenomenon were excluded. The study protocol was approved by the Institutional Review Board, Tokyo Dental College, Chiba, Japan and the Institutional Review Board, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. Written informed consent was obtained from all of the patients and parents if required, and the study was conducted in accordance with the Declaration of Helsinki.

Preoperative cold pressor–induced pain test

The patients were premedicated with oral diazepam (5 mg) and oral famotidine (150 mg) 90 min before the induction of anesthesia. The patients had an intravenous (i.v.) line on the forearm on their nondominant side. The temperature in the operating room was maintained at 26°C. The cold pressor–induced pain test was then performed before and 3 min after an i.v. bolus injection of fentanyl at 2 μg/kg as previously described (23, 24). Briefly, the dominant hand was immersed up to the wrist in the ice-cold water. The patients were instructed to keep their hand calm in the ice-cold water and withdraw it as soon as they perceived any pain. All of the patients had the test conducted by the same investigator. The baseline latency to pain perception (PPLpre) was defined as the time of immersion of the hand in the ice water before fentanyl injection. A cut-off time of 150 s was set to avoid tissue damage. The hand was warmed until the sensation of cold was completely abolished. Three minutes after the injection, the pain perception latency of the

dominant hand (PPLpost) was measured again. The analgesic effect of fentanyl in the preoperative cold pressor–induced pain test was evaluated simply as the difference between PPLpost and PPLpre (PPLpost – PPLpre).

Anesthesia and surgery

After the cold pressor–induced pain test ended, general anesthesia was induced with a target-controlled infusion (TCI) of propofol using a TCI pump (TE-317; Terumo, Tokyo). Vecuronium (0.1 mg/kg) was administered to facilitate nasotracheal intubation. After the induction of anesthesia, 10 ml of venous blood was sampled for the preparation of DNA specimens. General anesthesia was maintained with propofol at a target blood concentration of 4 – 6 μg/ml. Vecuronium was administered at a rate of 0.08 mg/kg per hour. The lungs were ventilated with oxygen-enriched air. Local anesthesia was induced on the right side of the mandibular ramus with 8 ml of 2% lidocaine that contained epinephrine (12.5 μg/kg), and mandibular ramus osteotomy was performed on this side. Local anesthesia was then performed on the left side, and mandibular ramus osteotomy was performed. The bilateral mandibular bone segments were fixed in the appropriate position. Whenever systolic blood pressure or heart rate exceeded +20% of the preinduction value during surgery, i.v. fentanyl (1 μg/kg) was administered.

Postoperative pain management

At the end of surgery, rectal diclofenac sodium (50 mg) and i.v. dexamethasone (8 mg) were administered at the request of surgeons to prevent postoperative orofacial edema/swelling. After emergence from anesthesia and tracheal extubation, droperidol (1.25 mg) was administered i.v. to prevent nausea/vomiting, and i.v. patient-controlled analgesia (PCA) with a fentanyl-droperidol combination (2 mg fentanyl and 5 mg droperidol diluted in normal saline in a total volume of 50 ml) commenced using a CADD-Legacy PCA pump (Smiths Medical Japan, Tokyo). The bolus dose of fentanyl on demand and lockout time were set at 20 μg and 10 min, respectively. Continuous background infusion was not used. Droperidol was coadministered with fentanyl to prevent nausea/vomiting because our preliminary study showed a high incidence (up to 30%) of nausea/vomiting with PCA fentanyl in young females. Patient-controlled analgesia was continued for 24 h postoperatively. In cases of treatment-refractory adverse effects or inadequate analgesia, PCA was discontinued, and rectal diclofenac sodium (50 mg) was prescribed as a rescue analgesic as required. The intensity of spontaneous pain was assessed 3 and 24 h postoperatively using a 100-mm visual analog scale (VAS), with 0 mm indicating no pain and 100 mm indicating the worst pain imaginable. Intraoperative fentanyl use and postoperative PCA fentanyl use during the first 24-h postoperative period were recorded. Doses of fentanyl administered intraoperatively and postoperatively were normalized with body weight. Additionally, perioperative fentanyl use was calculated as the sum of intraoperative fentanyl use and postoperative fentanyl use because the analgesic effect of the intermediate-acting opioid fentanyl, administered pre- and intraoperatively, could outlast the duration of surgery and thus affect postoperative fentanyl use, especially in patients who received a large dose of fentanyl intraoperatively. Therefore, in the present study, we considered perioperative fentanyl use an appropriate indicator of fentanyl analgesia in addition to postoperative fentanyl use. Furthermore, 1 mg/kg diclofenac sodium was converted to a fentanyl-equivalent dose of 1 μg/kg according to a dose conversion described previously (25). Because the potent analgesic diclofenac sodium was administered at a uniform dose (50 mg/body) across all of the patients, and individual differences in the weight-adjusted dose of diclofenac sodium could affect postoperative fentanyl use, total perioperative analgesic use was calculated as the sum of perioperative fentanyl use and the fentanyl-equivalent dose of diclofenac sodium.

Genotyping procedures

Total genomic DNA was extracted from peripheral blood samples using standard procedures. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method and direct sequencing were performed to genotype both the A145G and G1165C SNPs in the *ADRB1* gene.

To perform PCR-RFLP to genotype the A145G (rs1801252) SNP, the restriction enzyme Sau96 I (New England Biolabs, Ipswich, MA, USA) and two K157 and K158 primers were used (Table 1). First, PCR was performed in a final volume of 10 μl that contained TaKaRa LA Taq reaction buffer (2 mM magnesium), dideoxyribonucleoside triphosphate (dNTP), 0.5 μM of each primer, 0.5 U LA Taq DNA polymerase (TaKaRa Bio, Tokyo), and 5 – 10 ng extracted genomic DNA as the template. The PCR program was the following: 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 40 s. The amplified DNA fragments were digested by the restriction enzyme at 60°C in a total of 10 μl reaction solution that contained 10 × NEBuffer (200 mM Tris-HCl, pH 7.9, 100 mM $MgCl_2$, 500 mM NaCl, and 10 mM dithiothreitol), 1 U Sau96, and 5 μl PCR product as the substrate. The digestion products were analyzed by electrophoresis using 3% agarose gel and ethidiumbromide staining for visualization under ultraviolet illumination. The appearance of only the 389 base pair (bp) DNA fragment corresponded to the A/A

Table 1. Primers used to genotype the SNPs in the *ADRB1* gene

SNPs	Assay type	Primer No.	Strand	Sequence (5′ to 3′)
A145G (rs1801252)	PCR-RFLP	K157	Forward	GACCTCCCTCTGCGCACCAC
		K158	Reverse	CTGAGGTCCACAGCTCGCAGA
	Sequencing	27pcf-2	Forward	ATTGGCTGCAGGAGCCTGACG
		27pcr-2	Reverse	CTGAGGTCCACAGCTCGCAGA
G1165C (rs1801253)	PCR-RFLP	K159	Forward	ACGCTGGGCATCATCATGGGC
		K166	Reverse	ACATCGTCGTCGTCGTCGTCC
	Sequencing	28pcf-2	Forward	ACGCTGGGCATCATCATGGGC
		28pcr-2	Reverse	TCTCCTCGTTCCCCTGGGAAG

genotype of the loaded sample. The appearance of both the 389 and 190 bp DNA fragments corresponded to the A/G genotype, and the appearance of only the 190 bp DNA fragment corresponded to the G/G genotype.

To perform PCR-RFLP to genotype the G1165C (rs1801253) SNP, the restriction enzyme Mva I (TaKaRa Bio) and two K159 and K166 primers were used (Table 1). First, PCR was performed in a final volume of 10 μl that contained TaKaRa LA Taq reaction buffer (2 mM magnesium), dNTP, 1 μM of each primer, 0.5 U LA Taq DNA polymerase (TaKaRa Bio), and 5 – 10 ng extracted genomic DNA as the template. The PCR program was the following: 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 90 s. The amplified DNA fragments were digested by the restriction enzyme at 60°C in a total of 10 μl reaction solution that contained 10 × K buffer (100 mM Tris-HCl, pH 8.5, 100 mM $MgCl_2$, 1000 mM KCl, and 10 mM dithiothreitol), 0.002 U Mva I, and 5 μl PCR product as the substrate. The digestion products were analyzed by electrophoresis using 3% agarose gel and ethidiumbromide staining for visualization under ultraviolet illumination. The appearance of both the 280 and 52 bp DNA fragments corresponded to the C/C genotype of the loaded sample. The appearance of all three 142/138, 52, and 280 bp DNA fragments corresponded to the C/G genotype, and the appearance of both the 142/138 and 52 bp DNA fragments corresponded to the G/G genotype.

For direct sequencing to confirm the genotypes of the A145G and G1165C SNPs in the *ADRB1* gene, PCR was first performed in a final volume of 10 μl that contained TaKaRa LA Taq reaction buffer (2 mM magnesium), dNTP, 0.2 μM of each primer, 0.5 U LA Taq DNA polymerase (TaKaRa Bio), and 5 – 10 ng extracted genomic DNA as the template. The PCR program was the following: 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 40 s. Following the cycle sequencing reaction according to the manufacturer's instructions with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies Japan, Tokyo) and two primers of 27pcf-2 and 27pcr-2 for rs1801252 and two primers of 28pcf-2 and 28pcr-2 for rs1801253 (Table 1), purification of the PCR products was performed. Afterwards, the DNA sequences of the fragments were determined using an ABI PRISM 3100 Genetic Analyzer automated sequencer (Life Technologies Japan), and the genotypes of the A145G and G1165C SNPs were analyzed using Lasergene software v.7.0 (DNAstar, Madison, WI, USA).

Statistical analyses

Parametric and nonparametric data are expressed as the mean ± S.D. and median (interquartile range), respectively. The statistical analysis was performed using SPSS v.12.0 for Windows (IBM Japan, Tokyo). In the present study, none of the clinically measured endpoints that were related to pain sensitivity (i.e., PPLpre) or fentanyl analgesia (i.e., PPLpost – PPLpre) were normally distributed. Therefore, nonparametric analyses, including the Kruskal-Wallis *H*-test and Mann-Whitney *U*-test, were used to detect possible associations between any of the clinical or genomic parameters (e.g., sex, age, and genotypes of the two screened SNPs) and clinical endpoints related to pain sensitivity or the analgesic effects of fentanyl. PLINK v.1.01 (http://pngu.mgh.harvard.edu/purcell/plink) (26), integrated with gPLINK v.2.049, and Haploview v.4.0 (27) were used for haplotype-specific tests to examine the combinational effects of alleles of the A145G and G1165C SNPs on pain and fentanyl sensitivity using linear regression analyses. In all of the statistical tests, the criterion for significance was $P < 0.05$.

Results

All 216 Japanese patients who enrolled in the study completed the study. The patients' clinical data are summarized in Table 2. Rescue analgesics were required in

Table 2. Patient demographic and clinical data

Age (years)	25.5 ± 7.4	(16 – 50)
Male/Female	78/138	
Body weight (kg)	58.2 ± 10.9	(38 – 128)
Height (cm)	164.7 ± 9.0	(143 – 190)
PPLpre (s)	14 [9, 24]	(3 – 150)
Analgesic effect (PPLpost – PPLpre) (s)	12 [5.0, 37.8]	(−17 to +143)
24-h postoperative fentanyl use (μg/kg)	2.4 [1.1, 4.1]	(0 – 13.8)
VAS pain score at 24 h (mm)	24.0 [10.0, 41.5]	(0 – 83)
VAS pain score at 3 h (mm)	25.5 [13.3, 48.0]	(0 – 90)
Perioperative fentanyl use (μg/kg)	6.2 [4.9, 8.1]	(0.8 – 16.3)
Perioperative analgesic use (converted to fentanyl-equivalent dose) (μg/kg)*	7.1 [5.8, 9.0]	(1.8 – 17.3)

The data are expressed as numbers, mean ± S.D. (range), or median [interquartile range]. *Perioperative analgesic use is the sum of perioperative fentanyl use and dose of diclofenac sodium converted to the PCA fentanyl-equivalent dose.

Table 3. Associations between genotypes of the *ADRB1* SNP and sensitivity to pain and analgesics (Kruskal-Wallis *H*-test)

	A145G					
	AA	AG	GG	AA/AG/GG		
	median [range]			*P* value		
				All Patients	Male	Female
N	154	58	4			
PPLpre (s)	15.0 [9.0, 23.3]	11.0 [7.8, 23.8]	34.5 [18.0, 86.3]	0.051†	0.056†	0.411
Analgesic effect (PPLpost – PPLpre) (s)	12.0 [4.8, 37.0]	11.0 [5.0, 40.5]	41.5 [10.3, 95.3]	0.508	0.858	0.590
24-h postoperative fentanyl use (μg/kg)	2.3 [1.2, 4.1]	3.0 [1.0, 4.3]	1.9 [0.4, 8.7]	0.753	0.619	0.119
VAS pain score at 24 h (mm)	23.0 [9.0, 40.0]	25.5 [16.3, 51.0]	19.0 [3.8, 32.0]	0.176	0.926	0.088
	G1165C					
	CC	CG	GG	CC/CG/GG		
	median [range]			*P* value		
				All Patients	Male	Female
N	128	74	14			
PPLpre (s)	14.0 [9.0, 24.0]	14.0 [8.8, 24.3]	16.5 [9.0, 23.5]	0.907	0.766	0.852
Analgesic effect (PPLpost – PPLpre) (s)	16.5 [5.0, 40.8]	8.0 [3.8, 26.5]	18.0 [8.5, 37.8]	0.114	0.743	0.048**
24-h postoperative fentanyl use (μg/kg)	2.3 [1.0, 4.1]	2.7 [1.5, 4.1]	2.1 [0.4, 6.6]	0.382	0.962	0.343
VAS pain score at 24 h (mm)	24.0 [10.0, 42.0]	25.0 [12.5, 48.0]	21.0 [3.8, 34.3]	0.320	0.916	0.141

The data are expressed as the number and median [interquartile range]. **$P < 0.05$, †$P < 0.1$.

one of the patients. The genotype distributions of the two SNPs in the patients are shown in Table 3. These genotype frequencies were in Hardy-Weinberg equilibrium.

In the analysis of PPLpre, which reflects sensitivity to pain, the Kruskal-Wallis *H*-test revealed tendencies toward differences among the A145G SNP genotype subgroups in all of the patients and males ($P = 0.051$ and 0.056, respectively; Table 3). The Mann-Whitney *U post hoc* test revealed that PPLpre was significantly less and tended to be less in patients with the A-allele of the A145G SNP compared with patients without this allele in all of the patients and males, respectively ($P = 0.032$ and 0.096, respectively; Table 4), indicating that the patients who carried the A-allele of A145G SNP were more sensitive to cold pressor–induced pain than those who did not carry this allele, especially in male patients.

Table 4. Associations between genotypes of the *ADRB1* SNP and sensitivity to pain and analgesics (Mann-Whitney *U*-test)

	A145G		
	AA, AG (median [range])		
	All Patients	Male	Female
N	212	75	137
PPLpre (s)	14 [3, 150]	14 [4, 150]	14 [3, 130]
Analgesic effect (PPLpost – PPLpre) (s)	11.5 [−17, 143]	22 [−9, 143]	9 [−17, 140]
24-h postoperative fentanyl use (μg/kg)	2.407 [0, 13.818]	2 [0, 10.556]	2.593 [0, 13.818]
VAS pain score at 24 h (mm)	24.5 [0, 83]	27 [0, 83]	23 [0, 80]
	GG (median [range])		
	All Patients	Male	Female
N	4	3	1
PPLpre (s)	34.5 [14, 102]	39 [14, 102]	30
Analgesic effect (PPLpost – PPLpre) (s)	41.5 [2, 111]	48 [2, 111]	35
24-h postoperative fentanyl use (μg/kg)	1.876 [0, 10.909]	1.569 [0, 2.182]	10.909
VAS pain score at 24 h (mm)	16.5 [9, 65]	23 [9,65]	10
	AA, AG/GG (*P* value)		
	All Patients	Male	Female
PPLpre (s)	0.032**	0.096†	0.183
Analgesic effect (PPLpost – PPLpre) (s)	0.301	0.594	0.359
24-h postoperative fentanyl use (μg/kg)	0.744	0.336	0.095†
VAS pain score at 24 h (mm)	0.833	0.938	0.393
	G1165C		
	CC (median [range])		
	All Patients	Male	Female
N	128	47	81
PPLpre (s)	14 [3, 110]	17 [4, 110]	13 [3, 103]
Analgesic effect (PPLpost – PPLpre) (s)	16.5 [−10, 140]	23 [−9, 133]	12 [−1, 140]
24-h postoperative fentanyl use (μg/kg)	2.264 [0, 13.818]	1.786 [0, 10.508]	2.4 [0, 13.818]
VAS pain score at 24 h (mm)	24 [0, 83]	25 [0, 83]	22 [0, 80]
	CG, GG (median [range])		
	All Patients	Male	Female
N	88	31	57
PPLpre (s)	14.5 [4, 150]	13 [5, 150]	15 [4, 130]
Analgesic effect (PPLpost – PPLpre) (s)	9.5 [−17, 143]	18 [−1, 143]	6 [−17, 135]
24-h postoperative fentanyl use (μg/kg)	2.692 [0, 11.852]	2.264 [0, 10.556]	2.8 [0, 11.852]
VAS pain score at 24 h (mm)	25 [0, 78]	28 [0, 59]	24 [0, 78]
	CC/CG, GG (*P* value)		
	All Patients	Male	Female
PPLpre (s)	0.955	0.523	0.613
Analgesic effect (PPLpost – PPLpre) (s)	0.104	0.755	0.021$^{\#\#}$
24-h postoperative fentanyl use (μg/kg)	0.209	0.890	0.160
VAS pain score at 24 h (mm)	0.961	0.686	0.643

The data are expressed as the number and median [interquartile range]. **$P < 0.05$, compared with subjects who carried the A-allele in A145G. $^{\dagger}P < 0.1$, compared with subjects who carried the A-allele in A145G. $^{\#\#}P < 0.05$, compared with subjects who did not carry the G-allele in G1165C.

In the analysis of PPLpost – PPLpre, which reflects the preoperative analgesic effect, the Kruskal-Wallis *H*-test revealed significant differences among the G1165C SNP genotype subgroups in females ($P = 0.048$; Table 3). The Mann-Whitney *U post hoc* test revealed that the analgesic effect was significantly less in the subjects who carried the G-allele of the G1165C SNP than in the subjects who did not carry the G-allele in females ($P = 0.021$; Table 4).

However, none of the other clinical traits examined were significantly associated with either the A145G SNP or G1165C SNP in the initial Kruskal-Wallis *H*-tests (Table 3).

To examine the associations between combinations of the *ADRB1* SNPs and clinical parameters, an association analysis was performed with haplotypes that consisted of the A145G and G1165C SNPs in the *ADRB1* gene. As shown in Table 5, a significant decrease was found for the 145A/1165C haplotype in 24-h postoperative fentanyl use in females ($R^2 = 0.036$, $P = 0.026$; Table 5). Furthermore, the subjects who carried the 145A/1165G haplotype showed a tendency toward a decrease in the analgesic effect (PPLpost – PPLpre; $R^2 = 0.022$, $P = 0.080$; Table 5) in female subjects. The association between the 145G/1165C haplotype and a high VAS pain score at 24 h tended to be significant in all of the patients and females ($R^2 = 0.014$, $P = 0.078$, and $R^2 = 0.023$, $P = 0.073$, respectively; Table 5).

Discussion

In the present study, the A145G SNP was associated with pain sensitivity, and the G1165C SNP was associated with fentanyl sensitivity in females, although inconsistency was found in the interpretation of the haplotype and single polymorphism analysis results. The results suggested that these *ADRB1* polymorphisms may contribute to individual differences in the sensitivity to pain and analgesics.

A145G SNP

PPLpre in patients who carried the A-allele of A145G (rs1801252) in the β_1-adrenergic receptor gene was significantly lower than in patients who did not carry the A-allele (Table 4), suggesting that the A-allele is associated with high sensitivity to pain. β-Adrenergic receptors are closely related to opioids (12), and the β_1-adrenergic receptor is reported to be especially involved in the negative affective component of pain (14). The A145G SNP (rs1801252) causes an amino acid substitution. β_1-Adrenergic receptors with 49Gly reportedly have high agonist affinity, cyclic adenosine monophosphate (cAMP) activity, and avidity to metoprolol, an inverse agonist (17). Furthermore, the effects of β-adrenergic receptor antagonists on heart failure are significantly higher in patients who carry the G-allele than in patients who do not carry the G-allele (17, 18). These reports suggest that β_1-adrenergic receptors with 49Gly possess higher sensitivity to β_1 agonists and antagonists compared with those receptors with 49Ser.

Table 5. Association of *ADRB1* haplotypes composed of the A145G/G1165C SNPs with sensitivity to pain and analgesics

			Beta			R^2			Stat			*P* value		
Phenotype	Haplotype	Frequency	All Patients	Male	Female	All Patients	Male	Female	All Patients	Male	Female	All Patients	Male	Female
PPLpre (s)	AG	0.236	0.54	1.16	0.13	2.55E-04	8.35E-04	2.01E-05	0.23	0.25	0.05	0.816	0.802	0.958
	GC	0.153	−0.40	−0.27	−1.28	9.38E-05	3.51E-05	1.21E-03	−0.14	−0.05	−0.41	0.887	0.959	0.685
	AC	0.611	−0.22	−0.70	0.54	5.30E-05	4.13E-04	4.26E-04	−0.11	−0.18	0.24	0.915	0.860	0.810
Analgesic effect (PPLpost – PPLpre) (s)	AG	0.236	−4.30	1.32	−7.91	5.58E-03	4.55E-04	2.23E-02	−1.09	0.18	−1.76	0.276	0.854	0.080†
	GC	0.153	7.84	11.25	3.11	1.22E-02	2.53E-02	2.02E-03	1.62	1.40	0.53	0.106	0.167	0.600
	AC	0.611	−0.70	−7.34	5.28	1.82E-04	1.90E-02	1.15E-02	−0.20	−1.21	1.26	0.844	0.232	0.211
24-h postoperative fentanyl use (μg/kg)	AG	0.236	0.41	0.22	0.53	1.01E-02	3.11E-03	1.63E-02	1.47	0.49	1.50	0.142	0.628	0.135
	GC	0.153	0.02	−0.51	0.53	2.47E-05	1.30E-02	9.67E-03	0.07	−1.00	1.15	0.942	0.319	0.251
	AC	0.611	−0.34	0.13	-0.73	8.78E-03	1.42E-03	3.58E-02	−1.38	0.33	−2.25	0.170	0.744	0.026**
VAS pain score at 24 h (mm)	AG	0.236	−1.75	−2.70	−1.16	2.72E-03	6.40E-03	1.22E-03	−0.76	−0.70	−0.41	0.446	0.486	0.684
	GC	0.153	4.96	2.48	6.62	1.44E-02	4.11E-03	2.34E-02	1.77	0.56	1.81	0.078†	0.577	0.073†
	AC	0.611	−1.23	0.60	−2.36	1.66E-03	4.23E-04	5.85E-03	−0.60	0.18	−0.89	0.552	0.858	0.373

Association of the haplotype composed of the A145G/G1165C SNPs. Frequency, haplotype frequency; Beta, regression coefficient; R^2, coefficient of determination; Stat, *t*-statistic. **$P < 0.05$, †$P < 0.1$.

The internalization and desensitization of β_1-adrenergic receptors with 49Gly are reported to be significantly enhanced compared with β_1-adrenergic receptors with 49Ser (17, 18), suggesting that the signal intensity via β_1-adrenergic receptors with 49Gly is decreased after chronic agonist treatment. Considering that β_1-adrenergic receptors are chronically activated by endogenous norepinephrine, the signal intensity via β_1-adrenergic receptors may be decreased in subjects with 49Gly.

Altogether, in patients who do not carry the A-allele, the downregulation of β_1-adrenergic receptors may increase, and receptor function in the BNST may decrease, leading to decreased pain, especially the negative affective component of pain. However, a possible limitation of the present study was that we were not able to separately measure the sensory and emotional aspects of pain. Future studies should measure these parameters independently.

Additionally, the frequency of the A-allele of the A145G SNP is high (16). The number of patients who did not carry the A-allele in the present study was only four, suggesting that the detection power was low. Future studies with a larger number of patients may be necessary to corroborate the present results.

G1165C SNP

PPLpost – PPLpre in female patients who carried the G-allele decreased compared with those who did not carry the G-allele in the G1165C SNP (rs1801253; Table 4), suggesting that female patients who carry the G-allele in the G1165C SNP have decreased sensitivity to fentanyl.

The G1165C SNP induces an amino acid substitution from glycine to arginine at position 389 of the β_1-adrenergic receptor. The 389Arg variant of the β_1-adrenergic receptor showed higher cAMP activation and [^{35}S]guanosine triphosphate-γS binding affinity induced by isoproterenol than the 389Gly variant (19). In a transgenic mouse study, dobutamine-induced cAMP activation was significantly higher in 389Arg-expressing mice than in 389Gly-expressing mice (28).

In the present study, β_1-adrenergic receptor–mediated signaling transduction was reduced in females who carried the G-allele compared with those who did not carry the G-allele, suppressing the expression of the negative affective component of pain and resulting in a reduction of the analgesic effect of fentanyl.

Sex differences have been reported in pain sensitivity and BNST volume (i.e., females > males) (29 – 31). A previous report demonstrated that the selective inhibitory effect of a pure androgen on luteinizing hormone pulse frequency was effectively antagonized by opioid receptor blockade (32). Therefore, sex hormones may affect endogenous opioid pathways, which may lead to sex differences in the sensitivity to pain and fentanyl. In the present study, the significant difference in the effect of fentanyl between genotypes only in female patients may be resulted from these sex characteristics.

Additionally, the β_1-adrenergic agonist dobutamine increased hepatic arterial flow (33), and plasma alfentanil clearance reportedly depends on hepatic plasma flow (34). Therefore, greater cAMP-induced activation of 389Arg-type β_1-adrenergic receptors may increase hepatic blood flow, which would increase the metabolism of opioids and shorten their analgesic effect, thus increasing perioperative opioid analgesic requirements.

The number of male patients analyzed in the present study was considerably smaller than the number of females (78 males, 138 females). Therefore, bias in the number of patients may have influenced the statistical power for each sex and led to the differences observed in the present study. Additionally, confirming the significant trend results (namely, $P < 0.1$) will be necessary in future studies.

Haplotype analyses

A haplotype analysis was conducted to examine the effect of combinations of the related alleles of the two polymorphisms. The analysis revealed that carrying the AC haplotype in females was significantly associated with fewer doses of postoperative analgesic requirements (Table 5). The results of the preceding analyses of the involvement of the two SNPs showed that postoperative analgesic doses tended to be fewer in patients who carried the A-allele of A145G than those who did not carry the A-allele in females (Table 4). Furthermore, postoperative analgesic doses were fewer in female patients who carried the homozygous C-allele of G1165C than those who did not carry the homozygous C-allele, although the difference was not significant (Table 4).

Considering these results, one may suggest the possibility that carrying both the A-allele in the A145G SNP and the C-allele in the G1165C SNP (i.e., the AC haplotype) might have led to a significant decrease in postoperative analgesic requirements in female patients because of the effect of both polymorphisms (Table 4).

The preoperative analgesic effects of fentanyl were greater in female patients who did not carry the G-allele in the G1165C SNP than in those who carried the G-allele (Table 4). Therefore, female patients who carried the AC haplotype needed smaller doses of postoperative analgesics compared with those who carried the other haplotypes as a result of more effective basal analgesic effects, suggesting consistency in the interpretation of the results of the individual SNP and haplotype analyses.

Given the functional change of the A145G SNP de-

scribed above, patients who carried the A-allele might be more sensitive to the negative affective component of pain and thus needed greater doses of analgesics than those who did not carry the A-allele. However, the results of the haplotype analyses were difficult to interpret from this perspective because the patients who carried the AC haplotype, including the A-allele, needed smaller doses of analgesics. The A145G results were apparently inconsistent, but the G1165C results were consistent. Taken together, we may conclude that G1165C plays a more important role than A145G in nociceptive pain and its treatment with opioids.

Concluding remarks

The results of the present study suggested that the A145G SNP was associated with the sensitivity to pain, and the G1165C SNP was associated with the sensitivity to fentanyl only in females. Therefore, these SNPs in the *ADRB1* gene are considered to be associated with individual differences in pain and analgesics, suggesting that the analysis of SNPs can serve to promote personalized pain treatment. Identifying genotypes and haplotypes of *ADRB1* gene polymorphisms may provide valuable information to better modulate individual analgesic dosages required to achieve satisfactory pain control in the future.

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