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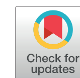

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ABSTRACT

Opioid analgesics are widely used for the treatment of moderate to severe pain. The analgesic effects of opioids are well known to vary among individuals. The present study focused on the genetic factors that are associated with interindividual differences in pain and opioid sensitivity. We conducted a multistage genome-wide association study in subjects who were scheduled to undergo mandibular sagittal split ramus osteotomy and were not medicated until they received fentanyl for the induction of anesthesia. We preoperatively conducted the cold pressor-induced pain test before and after fentanyl administration. The rs13093031 and rs12633508 single-nucleotide polymorphisms (SNPs) near the *LOC728432* gene region and rs6961071 SNP in the *tcag7.1213* gene region were significantly associated with the analgesic effect of fentanyl, based on differences in pain perception latency before and after fentanyl administration. The associations of these three SNPs that were identified in our exploratory study have not been previously reported. The two polymorphic loci (rs13093031 and rs12633508) were shown to be in strong linkage disequilibrium. Subjects with the G/G genotype of the rs13093031 and rs6961071 SNPs presented lower fentanyl-induced analgesia. Our findings provide a basis for investigating genetics-based analgesic sensitivity and personalized pain control.

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1. Introduction

Clinicians often need to consider patients' interindividual differences in pain and the effects of analgesics. Individual differences may be related to various environmental factors, mental factors, and genetic factors. Several studies have reported associations between pain sensitivity and polymorphisms of the genes that encode catechol-*O*-methyltransferase (COMT),[1,2] opioid receptor μ 1 (OPRM1),[3,4] and GTP cyclohydrolase 1 (GCH1),[5,6] among others. Genetic factors reportedly contribute to the differential response to opioids by possibly regulating their pharmacokinetics (metabolizing enzymes and transporters) and pharmacodynamics (receptors and signal transduction).[7] Indeed, genetic polymorphisms of the cyclic adenosine monophophosphate response element binding protein 1 (*CREB1*), calcium voltage-gated channel subunit α1E (*CACNA1E*), dopamine receptor D4 (*DRD4*), adrenoceptor β1 (*ADRB1*), *OPRM1*, and adenosine triphosphate binding cassette subfamily B member 1 (*ABCB1*) genes have been reported to influence the analgesic effects of opioids.[8–12]

Studies that seek to replicate previously reported genetic polymorphisms often report inconsistent or even opposite outcomes because of variable study designs, sample heterogeneity, small sample sizes, phenotype complexity, and the use of different statistical approaches.[13] More research is needed to reveal the basis of interindividual differences in pain and analgesic sensitivity. Thanks to recent advances in genome science, large-scale genotyping has been established, and this technological advancement simplified

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the analysis of genetic factors. More than one million single-nucleotide polymorphisms (SNPs) throughout the genome can be comprehensively analyzed by conducting genome-wide association studies (GWASs). GWASs and other association studies will likely contribute to personalized medical care and pain control, in which opioid sensitivity and the side effects of opioids (e.g., fentanyl) can be predicted based on genetic polymorphisms.[14]

Previous studies that investigated interindividual differences in analgesic sensitivity tended to include subjects who were already in a state of pain (e.g., cancer pain and postoperative pain) when they were treated with analgesic medications. The inclusion of such subjects can cause difficulty in selecting subjects who are in exactly the same condition when evaluating individual differences in drug sensitivity. Patients who are scheduled to undergo mandibular sagittal split ramus osteotomy (SSRO)[15,16] are considered ideal because they do not have pain before surgery. Furthermore, many of them are young, and they are currently unmedicated when they receive fentanyl for the induction of anesthesia. This makes them nearly ideal subjects for examining preoperative pain sensitivity and analgesic effects. Our previous study evaluated the analgesic effect of fentanyl in the preoperative cold pressor-induced pain test. Analgesia was defined as the difference in pain sensitivity before and after fentanyl administration (PPLpost−PPLpre).[17] We identified SNPs that were associated with the analgesic effects of fentanyl in the cold pressor-induced pain test.[17–19]

Previous studies have not performed GWAS to comprehensively investigate genetic polymorphisms that are associated with pain sensitivity (PPLpre) and the analgesic effects of fentanyl in that evaluation (PPLpost-PPLpre). Therefore, the present study investigated genetic polymorphisms that are associated with PPLpre and PPLpost−PPLpre in subjects who were scheduled to undergo SSRO by performing a GWAS. We found several SNPs that were genome-wide significantly associated with the analgesic effects of fentanyl.

2. Materials and methods

2.1. Ethics statement

The study protocol was approved by the Institutional Review Boards at Tokyo Dental College (Tokyo, Japan) (Approval No. 86), and Tokyo Metropolitan Institute of Medical Science (Tokyo, Japan) (Approval No. 15-6). All of the subjects provided informed, written consent for the genetics studies.

2.2. Subjects

Enrolled in this GWAS were 355 healthy patients (American Society of Anesthesiologists Physical Status I, age 15–52 years, 125 males and 230 females) who were scheduled to undergo SSRO for mandibular prognathism at Tokyo Dental College Suidobashi Hospital. The detailed demographic and clinical data of the subjects were reported in a previous study.[11]

2.3. Preoperative cold pressor-induced pain test

All the patients were premedicated with oral diazepam, 5 mg, and oral famotidine, 150 mg, 90 min before the induction of anesthesia. 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 intravenous (i.v.) bolus injection of fentanyl, 2 μg/kg, as previously described.[20,21] Briefly, crushed ice cubes and cold water were blended 15 min before testing in a 1-L isolated tank, and the mixture was stirred immediately before each test to ensure the uniform distribution of temperature (0 °C) within the tank. The dominant hand was immersed up to the wrist. The patients were instructed to keep their hand calm in the ice-cold water and withdraw it as soon as they perceived any pain. The baseline latency to pain perception, defined as the time of immersion of the hand in the ice water, before the i.v. injection of fentanyl (PPLpre) was recorded. A cut-off point was set at 150 s. The hand was warmed with a hair dryer as soon as it was withdrawn from the ice water until the sensation of cold was completely abolished. We then injected i.v. fentanyl, 2 μg/kg. 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).

2.4. Genotyping procedure and linkage disequilibrium analysis

Genomic DNA was extracted from whole-blood samples as described previously.[11] The DNA concentration was adjusted to 5–50 ng/μl for genotyping an individual SNP or 100 ng/μl for whole-genome genotyping. The procedure for whole-genome genotyping was fundamentally the same as in a previous study.[11] Briefly, whole-genome genotyping was performed using the Infinium assay II and an iScan system (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Five kinds of BeadChips were used to genotype the samples. Approximately 300,000 SNP markers were commonly included in all of the BeadChips, and these markers were considered for our association analyses. After whole-genome genotyping, the data for genotyped samples were analyzed using BeadStudio or GenomeStudio with Genotyping module v3.3.7 (Illumina) to evaluate the quality of the results as described previously.[4]

To secondarily analyze SNPs within and around the *LOC728432* gene region, which includes the most potent SNPs that were selected after the GWAS, genotype data that resulted from whole-genome genotyping as described previously[1] were basically used. To identify the relationships between the SNPs located in the *LOC728432* gene region, linkage disequilibrium (LD) analysis was performed for the SNPs with a minor allele frequency ≥ 0.05 in the genomic position ranging from 87,915,041 to 89,987,572 using Haploview v. 4.22[22] based on the genotype data for 127 of 355 subjects. For the estimation of LD strength between the SNPs, the commonly used r^2 values were pairwise-calculated using the genotype dataset of each SNP. Linkage disequilibrium blocks were defined among the SNPs that showed “strong LD,” based on the default algorithm of Gabriel et al.,[23] in which the upper and lower 95% confidence limits on D′ for a strong LD were set to 0.98 and 0.7, respectively.

2.5. Genome-wide association study

A multistage GWAS was conducted for the patients who underwent painful cosmetic surgery to investigate the association between genetic variations and pain and the analgesic effect of fentanyl. Among the 355 subjects, one subject lacked preoperative clinical data, and another subject did not meet the criteria for quality control in our preliminary analysis. Therefore, a total of 353 subjects were used for our multistage GWAS (118, 117, and 118 subjects for the first-, second-, and final-stage analyses, respectively). The average age was 26.0 years (28 males and 90 females) in the first-stage analysis, 25.5 years (51 males and 66 females) in the second-stage analysis, and 26.2 years (46 males and 72 females) in the final-stage analysis. In our preliminary analysis that used merged markers between different BeadChips with BeadStudio or GenomeStudio, 295,036 SNPs were selected for the analyses.

Before the analyses, the quantitative values of PPLpost–PPLpre (s) were natural-log-transformed for approximation to the normal distribution according to the following formula in the case of zero or positive values: Value for analyses = Ln (1 + PPLpost–PPLpre [s; log-transformed]). In the case of negative values, we used the following formula: Value for analyses = -Ln (1 - PPLpost–PPLpre [s; log-transformed]). To explore the association between the SNPs and phenotype, linear regression analyses were conducted in each stage of the analysis, in which PPLpre or PPLpost–PPLpre (s) and the genotype data for each SNP were incorporated as dependent and independent variables, respectively. Additive, dominant, and recessive genetic models were used for the analyses. The male genotypes were excluded from the analysis of X chromosome markers. All of the statistical analyses were performed using gPLINK v. 2.050, PLINK v. 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/; accessed September 24, 2017),[24] and Haploview v. 4.1.[22]

The GWAS procedure is summarized in a previous study.[11] In the first- and second-stage analyses, the SNPs that showed statistical *P*-values less than 0.05 were selected as the candidate SNPs for the next stage analysis. In the final stage, the *Q*-values of the false discovery rate were calculated to correct for multiple testing in addition to the *P*-values based on previous reports.[25,26] The SNPs that showed $Q < 0.05$ in the analysis were considered genome-wide significant. To calculate *Q*-values in the third stage and for all samples combined, Stratified False Discovery Rate (SFDR) software was used (http://www.utstat.toronto.edu/sun/Software/SFDR/index.html; accessed September 24, 2017).[27]

A log quantile–quantile (QQ) *P*-value plot as a result of the GWAS for the combined samples was subsequently drawn as described previously.[11] All of the plots were mostly concordant with the expected line (y = x), especially over the range of $0 < -\log10$ (*P*-value) < 5, indicating no apparent population stratification of the samples used in the study (Supplementary Fig. S1, S2).

The distributions of genotypes of the SNPs for PPLpre and PPLpost–PPLpre that were selected after the three-stage GWAS were checked using the χ^2 test. The absence of significant deviation from the theoretical distribution that is expected from Hardy–Weinberg equilibrium was confirmed for both phenotypes (Supplementary Tables S1, S2). For the statistical analyses, SPSS 19 software (International Business Machines, Armonk, NY, USA) was used. The criterion for significance was set at $P < 0.05$.

3. Results

We first explored the association between genetic variations and pain sensitivity in a total of 353 healthy subjects who were scheduled to undergo SSRO for mandibular prognathism that involved the administration of opioid analgesics.[11] Consequently, five, 11, and five SNPs were selected as the top candidates for PPLpre in the additive, dominant, and recessive models for each minor allele, respectively, after the final stage (Tables 1–3). Additionally, six, seven, and six SNPs were selected as the top candidates for PPLpost–PPLpre in the additive, dominant, and recessive models for each minor allele, respectively, after the final stage (Tables 4–6). The rs13093031 and rs12633508 SNPs that mapped to 3p11.1 showed significant associations with PPLpost–PPLpre after the final stage in the additive model (combined $\beta = -1.096$, nominal $P = 2.57 \times 10^{-7}$ for the rs13093031 SNP; combined $\beta = -1.092$, nominal $P = 2.93 \times 10^{-7}$ for the rs12633508 SNP) and recessive model (combined $\beta = -2.239$, nominal $P = 1.06 \times 10^{-7}$ for the rs13093031 SNP; combined $\beta = -2.240$, nominal $P = 1.10 \times 10^{-7}$ for the rs12633508 SNP; Tables 4 and 6). The rs6961071 SNP that mapped to 7q36.3 showed a significant association with PPLpost–PPLpre for all samples combined in the recessive model (combined $\beta = -1.115$, nominal $P = 2.74 \times 10^{-7}$; Table 6). The rs13093031, rs12633508, and rs6961071 SNPs showed no significant associations with PPLpre after the final stage in the additive model (combined $\beta = -0.004$, nominal $P = 0.96$ for the rs13093031 SNP; combined $\beta = -0.004$, nominal $P = 0.97$ for the rs12633508 SNP; combined $\beta = -0.032$, nominal $P = 0.52$ for the rs6961071 SNP), dominant model (combined $\beta = 0.058$, nominal $P = 0.42$ for the rs13093031 SNP; combined $\beta = 0.062$, nominal $P = 0.39$ for the rs12633508 SNP; combined $\beta = 0.021$, nominal $P = 0.78$ for the rs6961071 SNP), and recessive model (combined $\beta = -0.031$, nominal $P = 0.86$ for the rs13093031 SNP; combined $\beta = -0.032$, nominal $P = 0.85$ for the rs12633508 SNP; combined $\beta = -0.098$, nominal $P = 0.27$ for the rs6961071).

In the PPLpre groups, no SNPs were identified as genome-wide significant. The observed *P*-values of 295,036 SNPs in each model for PPLpre and PPLpost–PPLpre, calculated as −log10 (*P*-value), deviated from the expected values from the null hypothesis of a uniform distribution in the QQ plot for the entire sample (Supplementary Fig. S1, S2). The rs13093031 and rs12633508 SNPs were located near the *LOC728432* gene, which encodes interactor of little elongation complex ELL subunit 2 pseudogene 2 (*ICE2P2*). Two alleles of the *LOC728432* gene SNPs (rs13093031 and rs12633508) represented almost one absolute LD block near the *LOC728432* gene region (Fig. 1). The rs6961071 was located near the *tcag7.1213* gene, which is synonymous with *LOC393076* and is a previously annotated uncharacterized gene.

The PPLpost–PPLpre values in subjects with the A/A, A/G, and G/G genotypes of the rs13093031 SNP, reflecting the analgesic effects of fentanyl, were 24.45 ± 2.088 s, 32.09 ± 3.767 s, and 0.400 ± 2.048 s (mean ± standard error of the mean [SEM]), respectively (Fig. 2A). The PPLpost–PPLpre values in subjects with the A/A, A/G, and G/G genotypes of the rs6961071 SNP, were 27.79 ± 3.472 s, 31.04 ± 2.755 s, and 9.205 ± 1.734 s (mean ± SEM), respectively (Fig. 2B).

Table 1
Top candidate SNPs for PPLpre selected from 3-stage GWAS (additive model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	*P*	β	*P*	β	*P*	*Q*	β	*P*	*Q'*	
1	rs1151357	12	130059982	0.48	0.0084	1.135	0.0001	0.2467	0.0490	0.9052	0.4114	0.0000159	0.2604	*GPR133*
2	rs2412504	15	38345356	0.4865	0.0026	0.2428	0.0357	0.3427	0.0352	0.9052	0.3448	0.0000318	0.409	*PAK6*
3	rs1195906	12	130071019	0.4567	0.0123	0.5787	0.0067	0.2307	0.0495	0.9052	0.3624	0.0000454	0.4563	*GPR133*
4	rs3827040	20	43948966	0.3611	0.0489	0.2833	0.0264	0.4302	0.0079	0.5067	0.3466	0.0001002	0.5593	*C20orf165*
5	rs12050748	15	24892817	0.2118	0.0419	0.1937	0.0258	0.1658	0.0290	0.9052	0.1869	0.0002206	0.7891	*GABRA5*

CHR, chromosome number; Position, chromosomal position (bp); Q, Q values for FDR correction in the third stage; Q′, Q values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.

Table 2
Top candidate SNPs for PPLpre selected from 3-stage GWAS (dominant model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	P	β	P	β	P	Q	β	P	Q′	
1	rs7874530	9	83645492	0.4046	0.0093	0.304	0.0140	0.4937	0.0002	0.0724	0.396	0.00000057	0.0917	*FLJ43950*
2	rs2031757	9	83641664	0.3589	0.0147	0.41	0.0012	0.3851	0.0030	0.3101	0.3849	0.000000679	0.0917	*FLJ43950*
3	rs679090	13	76445792	0.3338	0.0115	0.3643	0.0034	0.3468	0.0047	0.3697	0.3422	0.00000211	0.19	*LOC390413*
4	rs2194734	2	162034445	1.981	0.0054	0.8905	0.0031	0.7257	0.0116	0.4047	0.9269	0.00000708	0.248	*TBR1*
5	rs6432689	2	162088006	1.981	0.0054	0.8905	0.0031	0.7257	0.0116	0.4047	0.9269	0.00000708	0.248	*KRT18P46*
6	rs10182681	2	162112698	1.981	0.0054	0.8905	0.0031	0.7257	0.0116	0.4047	0.9269	0.00000708	0.248	*KRT18P46*
7	rs768835	2	162005765	1.981	0.0054	0.8808	0.0035	0.7257	0.0116	0.4047	0.9236	0.00000772	0.248	*TBR1*
8	rs6949736	7	48330883	0.2817	0.0432	0.3025	0.0155	0.4025	0.0008	0.1212	0.3247	0.0000101	0.248	*ABCA13*
9	rs9846242	3	9543136	−0.4359	0.0195	−0.4631	0.0143	−0.4614	0.0162	0.5077	−0.4528	0.0000291	0.5614	*LHFPL4*
10	rs2831306	21	28279043	0.2727	0.0393	0.2623	0.0184	0.2538	0.0320	0.7006	0.2648	0.0001382	0.6796	*C21orf94*
11	rs17394484	10	6776927	−0.2824	0.0382	−0.2303	0.0441	−0.2615	0.0401	0.7016	−0.262	0.0002936	0.801	*LOC439949*

CHR, chromosome number; Position, chromosomal position (bp); *Q*, *Q* values for FDR correction in the third stage; *Q*′, *Q* values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.

Table 3
Top candidate SNPs for PPLpre selected from 3-stage GWAS (recessive model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	P	β	P	β	P	Q	β	P	Q′	
1	rs2412504	15	38345356	1.024	0.00148	0.5567	0.01655	0.6634	0.03905	0.9286	0.7223	0.0000116	0.2143	*PAK6*
2	rs1151357	12	130059982	0.9236	0.01061	2.281	0.0001001	0.5255	0.03274	0.9286	0.8208	0.0000143	0.2147	*GPR133*
3	rs1195924	12	130064055	0.8062	0.01311	1.159	0.006174	0.5162	0.02548	0.9286	0.711	0.0000291	0.3407	*GPR133*
4	rs1195906	12	130071019	0.8587	0.01774	1.159	0.006174	0.5162	0.02548	0.9286	0.7211	0.0000412	0.3665	*GPR133*
5	rs1381324	14	24279038	0.846	0.04286	0.8691	0.01255	0.4535	0.03852	0.9286	0.6325	0.0002076	0.7487	*STXBP6*

CHR, chromosome number; Position, chromosomal position (bp); *Q*, *Q* values for FDR correction in the third stage; *Q*′, *Q* values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.

Table 4
Top candidate SNPs for PPLpost-PPLpre selected from 3-stage GWAS (additive model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	P	β	P	β	P	Q	β	P	Q′	
1	rs13093031	3	88941731	−0.9449	0.0074	−0.8982	0.0099	−1.562	0.0002	0.0262[a]	−1.096	0.000000257	0.0361[a]	*LOC728432*
2	rs12633508	3	88897825	−0.9454	0.0077	−0.8852	0.0108	−1.562	0.0002	0.0262[a]	−1.092	0.000000293	0.0361[a]	*LOC728432*
3	rs6715117	2	166729443	−0.9669	0.0494	−0.7182	0.0167	−0.7146	0.0305	0.9569	−0.7456	0.0002488	0.447	*SCN9A*
4	rs440869	14	76761405	−1.677	0.0466	−1.183	0.0279	−1.758	0.0356	0.9569	−1.438	0.0003945	0.5103	*TMEM63C*
5	rs10511452	9	3663041	−0.6998	0.0151	−0.4089	0.0460	−0.472	0.0437	0.9569	−0.4736	0.0005042	0.5103	*RFX3*
6	rs4738858	8	62126253	−1.027	0.0357	−0.5561	0.0384	−0.5583	0.0499	0.9569	−0.5674	0.001728	0.6191	*LOC442389*

CHR, chromosome number; Position, chromosomal position (bp); *Q*, *Q* values for FDR correction in the third stage; *Q*′, *Q* values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.
[a] Significant after FDR correction ($Q < 0.05$ or $Q' < 0.05$).

Table 5
Top candidate SNPs for PPLpost-PPLpre selected from 3-stage GWAS (dominant model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	P	β	P	β	P	Q	β	P	Q′	
1	rs10486603	7	28994918	−5.159	0.0018	−3.625	0.0007	−2.409	0.0131	0.5751	−3.228	0.000000981	0.2628	*CPVL*
2	rs4413160	2	241243535	0.7639	0.0204	0.8102	0.0069	1.035	0.0015	0.2015	0.8677	0.00000235	0.3147	*AQP12B*
3	rs2060190	2	241242674	0.7445	0.0273	0.8466	0.0065	1.037	0.0023	0.2015	0.8741	0.00000412	0.3678	*AQP12B*
4	rs4149316	9	106621128	0.9114	0.0045	0.646	0.0211	0.66	0.0418	0.8901	0.7309	0.0000412	0.7882	*ABCA1*
5	rs2041570	7	31165792	−0.6983	0.0221	−0.5907	0.0366	−0.6472	0.0366	0.8901	−0.6558	0.0001386	0.8734	*ADCYAP1R1*
6	rs2678822	8	96131777	−0.6141	0.0460	−0.6922	0.0141	−0.6267	0.0449	0.8901	−0.645	0.0001963	0.8758	*C8orf38*
7	rs7250773	19	56998359	−0.7744	0.0171	−0.754	0.0253	−0.6829	0.0459	0.8901	−0.7019	0.0002516	0.8758	*FPRL2*

CHR, chromosome number; Position, chromosomal position (bp); *Q*, *Q* values for FDR correction in the third stage; *Q*′, *Q* values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.

Table 6
Top candidate SNPs for PPLpost-PPLpre selected from 3-stage GWAS (recessive model).

Rank	SNP	CHR	Position	1st stage		2nd stage		Final stage			Combined			Related gene
				β	P	β	P	β	P	Q	β	P	Q'	
1	rs13093031	3	88941731	−1.834	0.0083	−1.882	0.0062	−3.232	0.000095	0.0151[a]	−2.239	0.000000106	0.0132[a]	*LOC728432*
2	rs12633508	3	88897825	−1.834	0.0086	−1.882	0.0062	−3.232	0.000095	0.0151[a]	−2.24	0.00000011	0.0132[a]	*LOC728432*
3	rs6961071	7	155667462	−1.481	0.0001	−0.7394	0.0362	−1.078	0.00943	0.7497	−1.115	0.000000274	0.0219[a]	*tcag7.1213*
4	rs960434	7	31259425	−0.9735	0.0169	−0.9586	0.0263	−1.164	0.009007	0.7497	−1.051	0.0000186	0.2628	*NEUROD6*
5	rs6597458	7	154545784	0.8264	0.0444	0.8583	0.0131	0.7209	0.04573	0.8536	0.8114	0.0001424	0.3917	*LOC644697*
6	rs440869	14	76761405	−3.353	0.0454	−2.498	0.0206	−3.592	0.03171	0.8536	−2.944	0.0002854	0.4577	*TMEM63C*

CHR, chromosome number; Position, chromosomal position (bp); Q, Q values for FDR correction in the third stage; Q', Q values for FDR correction for combined all samples; Related gene, the nearest gene from the SNP site.
[a] Significant after FDR correction ($Q < 0.05$ or $Q' < 0.05$).

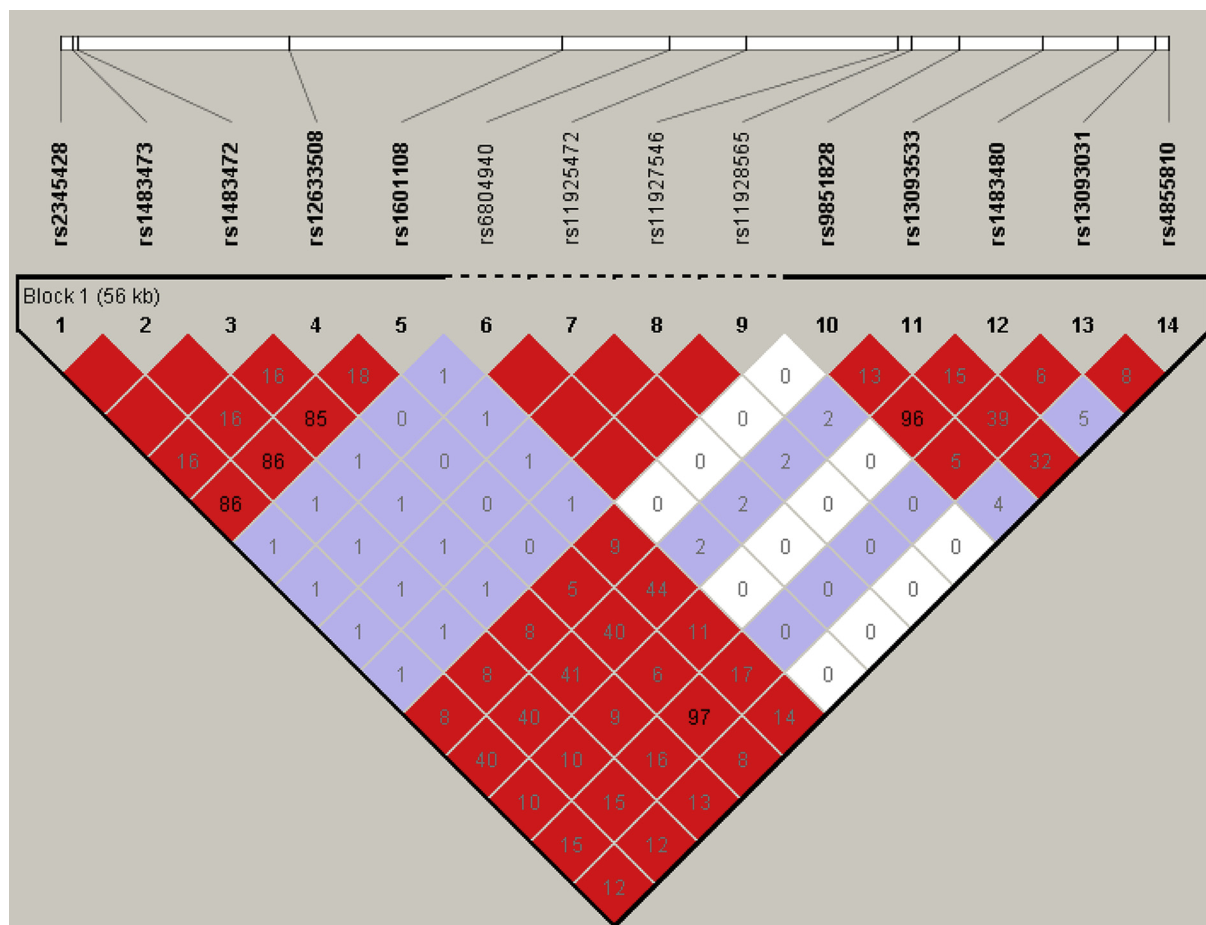

Fig. 1. Linkage disequilibrium plot for the *LOC728432* gene region. Numbers in the diamonds represent percentages of r^2 values for all SNP pairs and were calculated from the genotyped data for the orthognathic surgery samples. Blank squares represent $r^2 = 100\%$. The rs13093031 SNP showed almost absolute linkage disequilibrium with the rs12633508 SNP, indicating similar results of association analyses obtained for these SNPs.

4. Discussion

Analgesic effects can be evaluated as differences in the severity of pain in standardized pain tests before and after analgesic administration.[17] We and other groups have reported several SNPs that are associated with the sensitivity to opioids, such as fentanyl.[17–19,28,29] To our knowledge, no studies have explored associations between genetic polymorphisms and analgesic effects in a GWAS. We conducted a GWAS, and three candidate SNPs (rs13093031, rs12633508, and rs6961071) were selected. Subjects with the G/G genotype of the rs13093031 and rs6961071 SNPs presented lower analgesic sensitivity to fentanyl. These SNPs have not been previously shown to be associated with opioid sensitivity, thus demonstrating the novelty of our findings.

Two SNPs (rs13093031 and rs12633508) showed significant associations with PPLpost−PPLpre and were in strong LD with each other. These two SNPs are located in the flanking region near the 5′ untranslated region of a pseudogene, *ICE2P2*. Although no pseudogene has been previously reported to be associated with opioid sensitivity, our findings suggest the possibility that the *ICE2P2* pseudogene is associated with opioid sensitivity by interfering with endo-siRNAs (esiRNAs) that regulate particular genes. Pseudogenes may function as gene regulators through the generation of esiRNAs.[30] Future studies

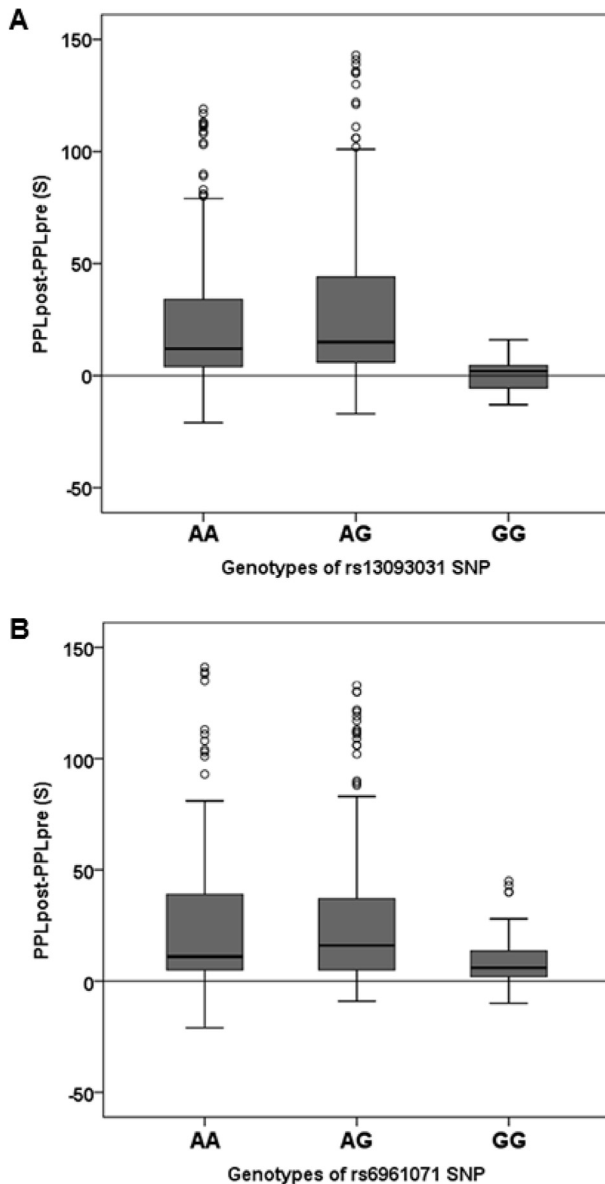

Fig. 2. Association between the analgesic effects of fentanyl in the cold pressor-induced pain test (PPLpost−PPLpre) and genotypes of the (A) rs13093031 SNP and (B) rs6961071 SNP. Comparisons were made between three genotype groups of each SNP: rs13093031 (AA: $n = 227$; AG: $n = 117$; GG: $n = 16$) and rs6961071 (AA: $n = 110$; AG: $n = 177$; GG: $n = 67$). The data are expressed as box and whisker plots. The upper and lower ends of the boxes represent the 75th and 25th percentiles. Whiskers represent the 90th and 10th percentiles. Filled circles represent outliers. The median is depicted by a solid line in the box.

should investigate whether the *ICE2P2* pseudogene affects individual differences in opioid sensitivity.

The present multistage GWAS identified rs6961071 as a potent SNP that is associated with PPLpost-PPLpre. The rs6961071 SNP is in the intron region of the *tcag7.1213* gene, based on the annotation file that was supplied by the BeadChip manufacturer. The *tcag7.1213* gene, also called *LOC393076*, is not currently annotated in the National Center for Biotechnology Information database (http://hapmap.ncbi.nlm.nih.gov/index.html.ja; September 24, 2017). No study has reported the influence of the rs6961071 SNP on these gene functions. However, some reports suggest that the intron region can affect gene function and expression.[31] Our results suggest the possibility that the rs6961071 SNP in the intron region may influence opioid sensitivity.

We investigated PPLpost−PPLpre and found a range of differences in analgesic sensitivity. A total of 26 subjects had negative PPLpost−PPLpre values (12, five, and nine subjects in the first-, second-, and final-stage analyses, respectively), suggesting that these participants may have experienced opioid-induced hyperalgesia.

In our previous report,[11] several SNPs were associated with the sensitivity to opioid analgesics for postoperative pain in subjects who underwent SSRO, although the associations between most of these SNPs and PPLpost−PPLpre were not even nominally significant ($P \geq 0.05$; data not shown). In the present study, we focused on thermal stimulation and identified different SNPs that may be associated with the sensitivity to opioid analgesics in the same cohort, although the associations between these SNPs and postoperative analgesia were not even nominally significant ($P \geq 0.05$; data not shown). Various factors, including differences in the types and degrees of pain and amount of fentanyl, may have resulted in different candidate SNPs that were identified in the present and previous studies.

Higher pain sensitivity can result from the long-term use of opioid analgesics. Regular users of opioids are more sensitive to pain than regular users of non-opioid analgesics.[32] The United States and Canada have a growing population of long-term opioid users for non-cancer pain.[33,34] Consequently, opioid misuse and addiction are ongoing and rapidly evolving public health issues.[35] In contrast, in Japan, few people use opioid analgesics over the long-term. In the present study, we chose 355 healthy subjects who did not have chronic pain and did not generally use analgesics. Thus, our findings of several genome-wide significantly associated SNPs were not confounded by possible changes in opioid sensitivity in long-term opioid users.

In conclusion, based on our results, analgesic sensitivity may be predicted by the identification of genotypes of genetic polymorphisms, which may ultimately lead to improvements in the personalized treatment of pain. The present multistage GWAS found that the rs13093031, rs12633508, and rs6961071 SNPs were associated with the analgesic effects of opioids. Two polymorphic loci (rs13093031 and rs12633508) were in strong LD. These SNPs are located near the *ICE2P2* pseudogene, whereas the rs6961071 SNP is located in the *tcag7.1213* gene region. The functions of these genes remain to be fully characterized.

Conflict of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jphs.2018.02.002.

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