Novel single nucleotide polymorphism biomarkers to predict opioid effects for cancer pain

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Abstract. There have been few studies on predictive biomarkers that may be useful to select the most suitable opioids to optimize therapeutic efficacy in individual patients with cancer pain. We recently investigated the efficacy of morphine and oxycodone using single nucleotide polymorphisms (SNPs) of the catechol-O-methyltransferase (*COMT*) rs4680 gene as a biomarker (RELIEF study). To explore additional biomarkers that may enable the selection of an appropriate opioid for individual patients with cancer pain, three SNPs were examined: C-C motif chemokine ligand 11 (*CCL11*; rs17809012), histamine N-methyltransferase (*HNMT*; rs1050891) and transient receptor potential V1 (*TRPV1*; rs222749), which were screened from 74 pain-related SNPs. These SNPs, which were identified as being significantly associated with the analgesic effect of morphine, were then used to genotype the 135 patients in the RELIEF study who had been randomized into a morphine group (n=69) or an oxycodone group (n=66). The present study then assessed whether the SNPs could also be used as selective biomarkers to predict which opioid(s) might be the most suitable to provide pain relief for patients with cancer. Oxycodone tended to provide superior analgesic effects over morphine in patients carrying the genotype AA for the *CCL11* rs17809012 SNP (P=0.012 for interaction), suggesting that it could serve as a potential biomarker for personalized analgesic therapy for patients suffering with cancer pain.

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Abbreviations: SNP, single nucleotide polymorphism; COMT, catechol-O-methyltransferase; IR, immediate-release; NRS, Numerical Rating Scale; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential V1; HADS, hospital anxiety and depression scale; SF-MPQ-2, Short-Form McGill Pain Questionnaire-2

Key words: SNP, morphine, oxycodone, genotype, CCL11, cancer pain

Introduction

Opioids are still the most commonly used drugs for cancer pain relief, with oral morphine that is recommended as the first-line drug for moderate-to-severe cancer pain in the international guidelines (1,2), and oxycodone and hydromorphone, both of immediate-release (IR) and of modified-release oral formulations as well as oral methadone that are effective alternatives. Hydromorphone was unavailable until 2017, and methadone requires a special license for use; thus, morphine and oxycodone are the most frequently used opioids in Japan.

Because of its many formulations, such as tablet, liquid medicine and suppository, morphine is superior in terms of its ease of administration, convenience of use in patients undergoing home care, and effectiveness in patients with respiratory distress (3). Furthermore, immediate-release (IR) morphine reaches a high serum concentration more rapidly (Tmax=0·9 h) as compared with oxycodone (Tmax=1·9 h) (4). On the other hand, since the oral bioavailability ratio of oxycodone is higher than that of morphine, it can be safely used in patients with chronic kidney diseases and may also be useful for patients with neuropathic pain (5-7), making selection of the most appropriate opioid more difficult. There is lack of consensus regarding the choice of drug (e.g., morphine or oxycodone) and the dose required to provide quick and potent pain relief in individual patients (3), especially since opioid sensitivity and the associated side effects vary widely among patients.

Numerous studies have proposed that the catechol-O-methyltransferase (*COMT*) 472G→A (rs4680, pVal158Met) single nucleotide polymorphism (SNP) may be a predictive biomarker of the response to morphine treatment (8). In these studies, patients with the GG genotype received the highest dose of morphine (9,10), while those with the AA genotype received the lowest dose (11).

Based on these studies, we performed a randomized controlled trial-the RELIEF study (Trial registration number: UMIN000015579), in which we compared the efficacy of morphine and oxycodone using the *COMT* rs4680 SNP as a biomarker. We randomized total of 140 patients (1:1) into a morphine group (Group M) or an oxycodone group (Group O) and evaluated the patients to determine the ability of pain controllability of the drugs, and found that in Group M, not only patients with the rs4680 GG genotype, but also those with the non-GG (GA/AA) genotype required higher doses of opioids, as compared with Group O (12). The results of this study, together with those of others, imply that the analysis of the *COMT* genotype alone may not be sufficient to adequately individualize opioid therapy (4,13). To explore SNPs other than the *COMT* rs4680 SNP, which may aid in individualizing treatment with morphine or oxycodone, we focused on a few other SNPs that have previously been suggested to be linked to pain sensitivity and/or opioid efficacy. The investigation consisted of two parts: a development study of 94 cases to screen for the candidate SNPs and a validation study including an additional 135 cases from the RELIEF study to validate these SNPs.

Patients and methods

Patients and samples. Two subsets of patients with advanced malignancies were enrolled in the current study (Fig. 1). Our cohorts do not include any families affecting genotype independency. The development study to screen for SNPs was conducted on the patients enrolled in our prospective study performed from 2009 to 2012 at Kindai University Faculty of Medicine and Sakai Hospital; these patients were treated with morphine alone. The characteristics of the patients have been described previously (14-16). Of the original 97 morphine-naïve patients who met inclusion criteria (15), 94 were genotyped as mentioned below, after excluding the remaining 3 patients because of inadequate DNA samples.

The validation study was performed in the 135 patients who fulfilled the second registration due to the criteria of suffering from cancer pain that necessitated daily treatment with opioids, from among the subjects (n=378) who were originally enrolled based on the first registration criteria in RELIEF study, a randomized controlled trial conducted recently by us (Trial registration number: UMIN000015579) (12). The second registration patients were randomized (1:1) based on the *COMT* rs4680 SNP (GG or non-GG) into a morphine group (Group M; n=70) or an oxycodone group (Group O; n=70), in such a way that patients with the GG and non-GG (GA or AA) genotypes were equally distributed in each group. The optimal sample size was calculated as 140 based on our preliminary analysis for the *COMT* rs4680 SNP (14), which has been described in detail in a previous report (12). We finally included 135 cases, after excluding 5 cases (because the data description was incomplete in 1 case and the trial was not underway in time for the genotyping in the remaining 4 cases), reducing the number of subjects in Group M to 69 and that in Group O to 66. The baseline characteristics of these 135 patients are presented in Table I. The inclusion and exclusion criteria for patients were as listed previously (12). CYP inducers such as rifampicin or carbamazepine or inhibitors such as itraconazole or SSRI were not administered to the participants.

Titration and classification of the patients. For opioid titration following cancer pain onset, the opioid-naïve patients were administered intermediate release (IR) opioids according to the guidelines for titration (NCCN Guidelines™, Adult Cancer Pain) (2,17) by specialized palliative care physicians. The 97 patients in the development study were administered IR morphine, and the 135 patients in the validation study received either IR morphine or IR oxycodone (groups M or O). We define the day of titration as day 1. The administrations of the minimum standard dose of IR opioids, that is, 5 mg/dose for morphine and 2.5 mg/dose for oxycodone (3.75 mg equivalent of IR morphine) were repeated for dose titration of the patients until the pain decreased by ≥33% on the Numerical Rating Scale (NRS, 0=no pain to 10=maximal pain), or the NRS score decreased to ≤3 from pre- to post-titration on day 1. Patients requiring 10 mg or more of IR morphine, or 7.5 mg or more of IR oxycodone were classified into the high-dose group and patients requiring 5 mg of IR morphine or 5 mg or less of IR oxycodone were classified into the low-dose group. Patients in whom the NRS score did not decrease to ≤3 or by ≥33% on the NRS after treatment on day 1 were categorized into the high-dose group, even if the dose was 5 mg of IR morphine or 5 mg of IR oxycodone (12). The post-titration NRS scores were recorded one or two hours after the final titration in the patients treated with morphine (Tmax=0·9 h) or oxycodone (Tmax=1·9 h), respectively.

Genotyping. Genomic DNA was isolated from the blood samples, as described previously (16). Genotyping of the patients in the validation study was performed for 74 SNPs of 54 genes (Table SI), which were selected based on previous reports linking them to pain sensitivity and/or opioid efficacy (18-26). We focused on both chemokines and cytokines because they have come to be more and more accepted as the major mediators that activate glial cells to interact with neurons, which is emerging as a key mechanism underlying chronic pain (21,22). Several chemokines implicated could be missing from our list if they have no appropriate SNPs within their genes.

Genes encoding transient receptor potential (TRP) ion channels, which represent the major group of molecules involved in nociception and development of pathological pain, were also included as candidate genes (24).

In addition to several well-known molecules modifying the mechanisms of opioid signaling or pain development (20,21,26), novel members likely to be associated with pain modulation such as the oxytocin receptor (OXTR) (23), molecules associated with increased morphine requirement (serine/threonine-protein kinase TAOK3) (19) or with histamine degradation that is thought to be important in nociception at the periphery [histamine N-methyltransferase (HNMT)

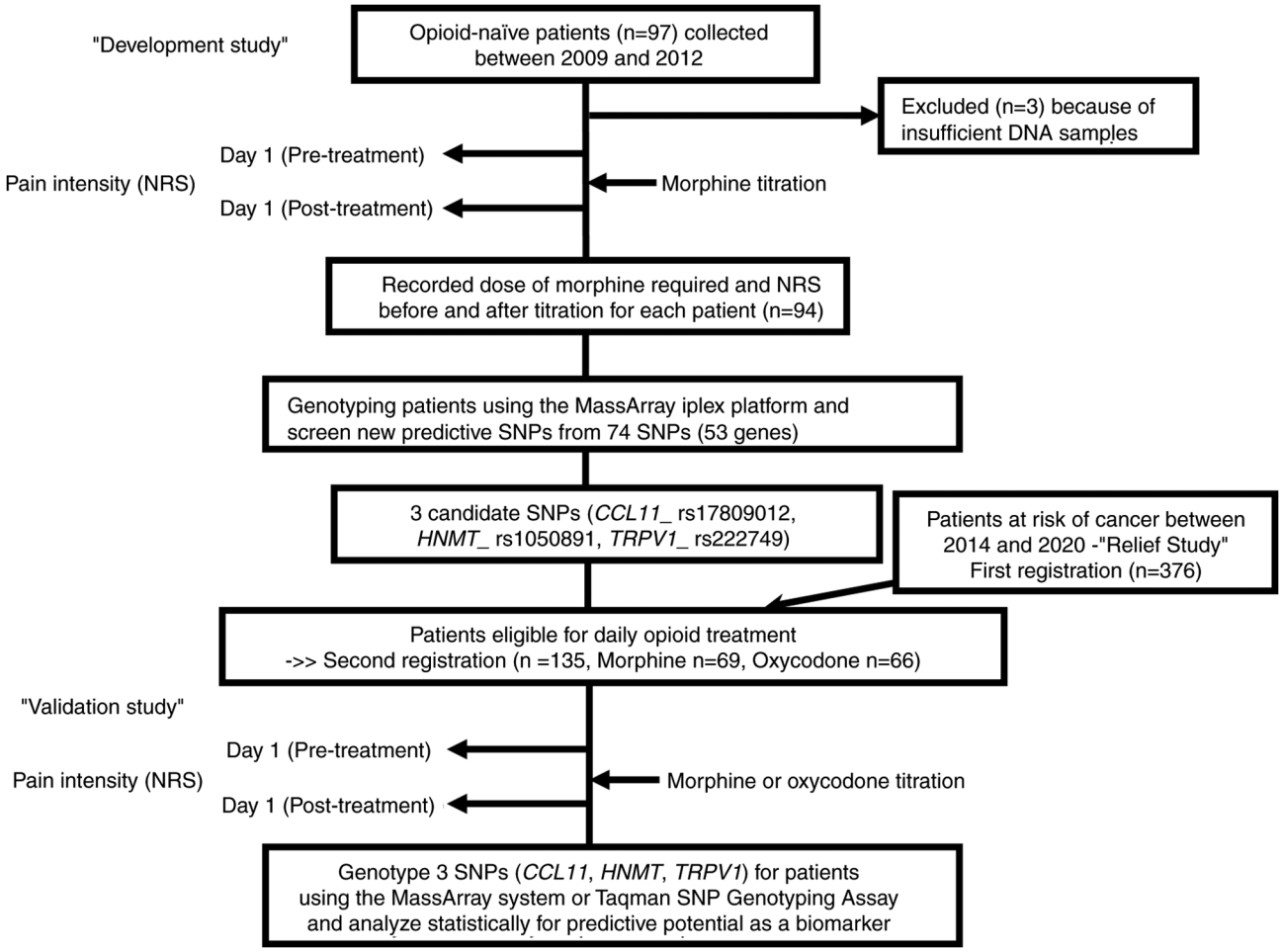

Figure 1. Schematic diagram of the study design. NRS, Numerical Rating Scale; SNP, single nucleotide polymorphism; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential vanilloid 1.

and amiloride-sensitive amine oxidase (AOC1)] (18) were also added to the list (Table SI). Analysis of these genes were conducted in the 94 patients in the first study (development study in Fig. 1), using the MassARRAY iPLEX platform (27). The primer sequences used for the MassARRAY genotyping will be offered on demand. The CYP450 enzymes and the ATP-binding cassette transporters that have been thought to be involved in the metabolism and efflux of opioids, respectively, could also be candidates (26,28). The SNPs of such genes are comprehensively covered in the DMET Platform (29), which we are using to genotype and analyze the same patients elsewhere (in preparation). Before proceeding to the analysis, we performed a quality control check on the data. We eliminated subjects with a genotype call rate of <90%, deviation of the Hardy-Weinberg equilibrium ($P<1x10^{-5}$) (29), or if the minor allele frequency (MAF) was less than 5% (Table SI).

In the validation study, the 3 candidate SNPs (*CCL11* rs17809012, *HNMT* rs1050891, and *TRPV1* rs222749) which were selected from among the 74 SNPs identified in the development study were analyzed in second registration patients selected from the RELIEF study (n=135) (12), using the MassARRAY iPLEX platform.

Statistical analysis. In the development study, we screened 74 SNPs by estimating the differences in the required dose of morphine at titration (high or low) between the patients with the major genotype (major-allele homozygotes) and the patients with the non-major genotypes (heterozygotes plus minor-allele homozygotes) using Fisher's exact test at a two-tailed significance level of 5%. The odds ratio (OR) and 95% confidence interval (CI) were obtained for each SNP.

For the analysis in the validation study, we characterized the 3 candidate SNPs (*CCL11* rs17809012, *HNMT* rs1050891, and *TRPV1* rs222749) by performing simple regression analyses separately for Group M and Group O. The primary outcome was pain relief. The objective variables examined were the ΔNRS. The ΔNRS was defined as the difference in the NRS score before and after the titration. Therefore, the greater the ΔNRS, the greater the pain relief.

In addition to genotype (major-allele homozygotes or heterozygotes plus minor-allele homozygotes) of the 3 SNPs, independent variables considered were age (<70 or ≥70 years), sex, performance status (ps;1/≥2), pre-NRS (1-10), total scores on the HADS (Hospital Anxiety and Depression Scale) (15), SF-MPQ-2 (Short-Form McGill Pain Questionnaire-2) (30), and the required dose (high or low), of which pre-NRS, HADS, and SF-MPQ-2 were ordinal variables. The differences in the required dose (high or low) of each opioid were estimated using Fisher's exact test for categorical variables or using Mann-Whitney U test for ordinal data.

Table I. Baseline characteristics of the patients.

Item	Number of patients	Morphine_group (n=69) Low dose (n=39)	Morphine_group (n=69) High dose (n=30)	P-value	Oxycodone_group (n=66) Low dose (n=51)	Oxycodone_group (n=66) High dose (n=15)	P-value
Age, n (%)[a]				0.028			1.000
<70 years	62	23 (59.0)	9 (30.0)		23 (45.1)	7 (46.7)	
≥70	73	16 (41.0)	21 (70.0)		28 (54.9)	8 (53.3)	
Sex, n (%)[a]				0.217			0.256
Male	77	21 (53.8)	21 (70)		25 (49.0)	10 (66.7)	
Female	58	18 (46.2)	9 (30)		26 (51.0)	5 (33.3)	
Performance status, n (%)[a,c]				0.805			1.000
0	10	3 (7.7)	0 (0)		3 (5.9)	4 (26.7)	
1	79	22 (56.4)	18 (60)		31 (60.8)	6 (40.0)	
2	33	10 (25.6)	8 (26.7)		13 (25.5)	3 (20.0)	
3	10	3 (7.7)	3 (10)		2 (3.9)	2 (13.3)	
4	4	1 (2.6)	1 (3.3)		2 (3.9)	0 (0)	
Pre-NRS, median (IQR)[b]		5 (3-6)	6 (4-7)	0.032	5 (4-6)	7 (6-8)	0.0007
HADS, median (IQR)[b]		13 (11-21)	14.5 (11-23)	0.682	14 (9-21)	15 (12-22)	0.619
SF-MPQ-2, median (IQR)[b]		30 (17.5-56)	35 (21.5-56)	0.566	42 (21-77)	66 (58-82)	0.015
CCL11, n (%)[a]				0.03			0.78
AA	70	14 (35.9)	19 (63.3)		28 (54.9)	9 (60.0)	
AG/GG	65	25 (64.1)	11 (36.7)		23 (45.1)	6 (40.0)	
HNMT, n (%)[a]				0.81			0.016
AA	75	20 (51.3)	17 (56.7)		25 (49.0)	13 (86.7)	
AG/GG	60	19 (48.7)	13 (43.3)		26 (51.0)	2 (13.3)	
TRPV1, n (%)[a]				0.23			1
CC	71	24 (61.5)	14 (46.7)		25 (49.0)	8 (53.3)	
CT/TT	64	15 (38.5)	16 (53.3)		26 (51.0)	7 (46.7)	

pre-NRS, pre-treatment numerical rating scale; IQR, interquartile range; HADS, Hospital Anxiety and Depression Scale; SF-MPQ-2, Short-Form McGill Pain Questionnaire-2; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential vanilloid 1; Low/High dose, patients requiring low/high dose of opioid. [a]Fisher's exact test was performed. [b]Mann-Whitney U test. [c]Performance status was compared between (0 + 1) and (2 + 3 + 4).

We also analyzed the three SNPs for the overall subject population, in which the variable of 'dose' was omitted because of a different conversion ratio of 3:2 between oral morphine and oral oxycodone formulations due to their incompatible dosage forms, as has been mentioned (12). Instead, 'treatment' (morphine or oxycodone) is added as an independent variable. For the analysis of the overall subject population, a simple regression analysis, together with multiple regression analyses to adjust for confounding variables, were performed. We performed two ways of multiple regression analyses. Either all of the above-mentioned variables or selected variables (pre_NRS, treatment, HADS and SF-MPQ-2) was entered into each of the analyses.

The variance inflation factor (VIF) was used to diagnose multicollinearity problems. A $P<0.05$ was considered as denoting statistical significance. The analyses were performed using the JMP statistical software (v14.2, SAS Institute, Cary, NC, USA).

Results

First screening of SNPs. In our development study conducted on 94 patients, 33% (n=31) of the patients were classified as requiring high-dose morphine while 67% (n=63) were classified into the low-dose morphine group. Correlations of the required dose of morphine with the gene polymorphisms were analyzed using 74 SNPs, without corrections for multiple comparisons. A total of 7 SNPs that did not meet the quality control criterion [deviation from the Hardy-Weinberg equilibrium (n=2), call rate <90% (n=2), and minor allele frequency <5% (n=3)] were not considered in the subsequent analyses (Table SI).

The patients were genotyped for the 74 SNPs to compare the high- and low-dose groups for the numbers of patients who were homozygous for the major allele and the numbers of patients with non-major (heterozygous plus homozygous for minor allele) alleles. This analysis showed that 3 SNPs (*TRPV1* rs222749, *CCL11* rs17809012, *HNMT* rs1050891)

Table II. Correlation between genotypes and morphine dose requirement[a].

Gene	SNP	Genotypes	OR (95% CI)[b]	P-value[c]
CCL11	rs17809012	AA // AG + GG	0.32 (0.11-0.85)	0.014
TRPV1	rs222749	CC // CT + TT	3.13 (1.27-7.74)	0.020
HNMT	rs1050891	AA // AG + GG	0.40 (0.16-0.99)	0.050
TRPV1	rs224534	AA // AG + GG	0.44 (0.17-1.07)	0.079
ADRB2	rs1042713	AA // AG + GG	3.22 (0.86-12.1)	0.101
KCNS1	rs734784	AA // AG + GG	2.62 (0.95-7.26)	0.102
IL-1RN	rs2234677	GG // GA + AA	2.80 (0.72-11.3)	0.110
COMT	rs4680	GG // GA + AA	0.47 (0.20-1.14)	0.125
TRPM8	rs17868387	AA // AG + GG	2.39 (0.80-7.09)	0.145
GCH1	rs3783641	TT // TA + AA	0.47 (0.15-1.37)	0.165
AIF1	rs2844475	TT // TC + CC	0.48 (0.19-1.22)	0.165
CCL8	rs1133763	AA // AC + CC	1.96 (0.76-5.17)	0.187

CCL11, C-C motif chemokine ligand 11; TRPV1, transient receptor potential vanilloid 1; HNMT, histamine N-methyltransferase; ADRB2, adrenoceptor beta 2; KCNS1, potassium voltage-gated channel modifier subfamily S member 1; IL-1RN, interleukin 1 receptor antagonist; COMT, catechol-O-methyltransferase; TRPM8, Transient Receptor Potential Melastatin 8; GCH1, GTP cyclohydrolase 1; AIF1, allograft inflammatory factor 1; CCL8, C-C Motif Chemokine Ligand 8; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval. [a]Proportions of patient homozygous for the major allele and patients heterozygous plus homozygous for minor allele were compared between the morphine dose required (high and low), using Fisher's exact test. [b]For OR >1, non-major genotypes are associated with a relatively higher dose requirement. [c]SNPs with a P-value of <0.2 are shown.

out of the 74 SNPs were associated with the morphine dose requirement (P≤0.05), suggesting that these SNPs could be biomarkers to predict the morphine controllability in cancer pain for individual patients (Table II).

Validation study. The 3 candidate SNPs thus extracted in the first screening were then examined in the total of 135 patients enrolled in our validation study. We first determined if these SNPs were strongly linked to the analgesic effect of morphine, as the ability of morphine to relieve the pain was the first selection criterion in the development study. A simple regression analysis for Group M showed that the three SNPs were correlated with the difference in the ΔNRS between the genotype groups. The patients homozygous for the major allele of *CCL11* rs17809012 (AA) or *HNMT* rs1050891 (AA) showed a significantly reduced ΔNRS by 0.63 or 0.48, on average, as compared with patients carrying rs17809012 (AG/GG) or rs1050891 (AG/GG), with P-values of 0.007 and 0.041, respectively (Table III). For the patients who were homozygous for the major allele of *TRPV1* rs222749 (CC), the ΔNRS was higher by 0.43 as compared with that in patients with rs222749 (CT/TT); the P-value was 0.071. Much lower ΔNRS differences were observed between genotype groups for these SNPs in Group O (Table III), confirming that these SNPs are specific biomarkers for morphine-induced analgesia. The difference in the analgesic effect between the *CCL11* genotype groups was also shown to be particularly pronounced for morphine, by plotting the post-titration NRS against the pre-titration NRS (Fig. S1A). Regardless of the opioids that were used in the overall subject population, these SNPs appeared to affect the ΔNRS, although the differences were statistically insignificant in the simple regression model (Table SII). However, multiple linear regression model to adjust for age, sex, ps,pre-NRS, treatment, genotypes, total scores on the HADS and SF-MPQ-2 showed that significant ΔNRS differences were observed between genotype groups of *CCL11* rs17809012 and *HNMT* rs1050891 (0.26 and 0.30, with P-values of 0.038 and 0.021, respectively), and there seemed no strong confounding variables, with all the VIF values evenly low (<1.5) (Table SIII, multiple regression model 1). We also chose independent variables that were likely to directly influence on pain sensitivity (and thus the ΔNRS) such as pre NRS, treatment, HADS and SF-MPQ-2 (15,30). Multiple linear regression model using these variables with the three genotypes showed that the ΔNRS differences between genotypes for the two SNPs remained significant (0.27 and 0.29, with P-values of 0.034 and 0.022 for *CCL11* rs17809012 and *HNMT* rs1050891, respectively; (Table SIII, multiple regression model 2).

Predictive factors for opioid selection. Next, we examined the genotype-treatment interactions for ΔNRS. A forest plot was drawn based on the estimate (relative risk) with its 95% CI in 2 categorical groups for each variable (Fig. 2). Better efficacy was observed in Group O than in Group M in patients homozygous for the major alleles of *CCL11* or *HNMT*, and in patients heterozygous or homozygous for minor alleles of the *TRPV1* genotype. A significant P-value was detected for the interaction (0.012) between the *CCL11* genotype and treatment (Table SIV and Fig. 2). The Group M patients with *CCL11*(AA), *HNMT*(AA), and *TRPV1*(CT/TT) showed reduced least square means (LSM) of the ΔNRS as compared to patients with other genotype and treatment combinations (Table IV). The superiority of one opioid over another in terms of the analgesic effect was also shown to be reversed between the two *CCL11* genotype groups by plotting the post-titration NRS against the pre-titration NRS, oxycodone being the better treatment

Table III. Simple regression analyses for determinants of the ΔNRS on day 1 in the Morphine and Oxycodone groups.

	Morphine (n=69)				Oxycodone (n=66)			
Variable	β	t-value	Partial regression coefficient (95% CI)	P-value	β	t-value	Partial regression coefficient (95% CI)	P-value
Age	0.07	0.55	0.13 (-0.34 to 0.60)	0.585	0.03	0.26	0.05 (-0.34 to 0.45)	0.796
Sex	0.01	0.08	0.02 (-0.47 to 0.50)	0.939	-0.11	-0.91	-0.18 (-0.57 to 0.21)	0.366
Performance status	-0.10	-0.81	-0.20 (-0.68 to 0.29)	0.421	0.22	1.81	0.37 (-0.04 to 0.78)	0.075
Pre-NRS	0.55	5.34	0.53 (0.33 to 0.73)	<0.0001	0.60	5.97	0.47 (0.31 to 0.63)	<0.0001
HADS score	0.09	0.73	0.02 (-0.04 to 0.08)	0.469	0.16	1.27	0.03 (-0.02 to 0.08)	0.209
SF-MPQ-2 total score	0.23	1.92	0.02 (-0.00 to 0.03)	0.059	0.28	2.32	0.01 (0.002 to 0.02)	0.024
Dose	-0.21	-1.73	-0.41 (-0.88 to 0.06)	0.09	-0.15	-1.24	-0.29 (-0.75 to 0.18)	0.221
Genotype								
CCL11	-0.32	-2.79	-0.63 (-1.07 to -0.17)	0.007	0.09	0.70	0.14 (-0.26 to 0.53)	0.486
HNMT	-0.25	-2.09	-0.48 (-0.94 to -0.02)	0.041	-0.03	-0.2	-0.04 (-0.44 to 0.36)	0.840
TRPV1	0.22	1.84	0.43 (-0.04 to 0.89)	0.071	0.05	0.42	0.08 (-0.31 to 0.48)	0.673

ΔNRS, difference in the numerical rating scale before and after titration; CI, confidence interval; Pre-NRS, pre-treatment numerical rating scale; HADS, Hospital Anxiety and Depression Scale; SF-MPQ-2, Short-Form McGill Pain Questionnaire-2; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential vanilloid 1; β standardized partial regression coefficient for '<70' (Age), 'male' (Sex), '0 and 1' (Performance status), 'low' (Dose), 'AA' (*CCL11*), 'AA' (*HNMT*) and 'CC' (*TRPV1*).

Table IV. LSMs of ΔNRS for patients in terms of their treatment and genotype interactions.

Variable[a]	Group[b]	LSM	Standard error	95% confidence interval
CCL11*treatment	AA+morphine	2.33	0.30	1.74-2.93
	AG/GG+morphine	3.58	0.29	3.01-4.16
	AA+oxycodone	3.43	0.29	2.87-4.00
	AG/GG+oxycodone	3.16	0.32	2.52-3.79
HNMT*treatment	AA+morphine	2.54	0.29	1.97-3.11
	AG/GG+morphine	3.50	0.31	2.88-4.11
	AA+oxycodone	3.28	0.29	2.71-3.84
	AG/GG+oxycodone	3.36	0.33	2.70-4.02
TRPV1*treatment	CC+morphine	3.37	0.29	2.80-3.94
	CT/TT+morphine	2.52	0.32	1.89-3.14
	CC+oxycodone	3.39	0.31	2.78-4.00
	CT/TT+oxycodone	3.23	0.31	2.62-3.84

LSM, least square means; ΔNRS, difference in the numerical rating scale before and after titration; [a]'*' means interaction between gene (genotype) and treatment (opioid); [b]'+' means 'and', e.g. AA+morphine means patients with genotype AA treated with morphine; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential vanilloid 1.

for patients with *CCL11*_AA and morphine for patients with AG/GG (Fig. S1A). Such interaction between opioid and genotype was not observed for the *HNMT* SNP (Fig. S1B).

Discussion

In the current study, we explored predictive biomarkers for selecting the most suitable opioid for treatment of cancer pain. From our development study that included 94 patients, three (*TRPV1* rs222749, *CCL11* rs17809012, *HNMT* rs1050891) out of 74 SNPs were selected as new biomarker candidates for predicting analgesic response to morphine. The subsequent validation study confirmed these SNPs as being involved in the analgesic effect of morphine in Group M, but not in that of oxycodone. Such discrepancy in the effects of the SNPs between these drugs could also be anticipated from the notion that morphine and oxycodone exert their antinociceptive effects through distinct opioid receptor populations (31,32) despite the structural and functional similarities between the two opioids (33).

Multiple regression analysis of data from the 135 patients in the validation study suggested the *CCL11* and *HNMT* genotypic variants as the major determinants of the choice of

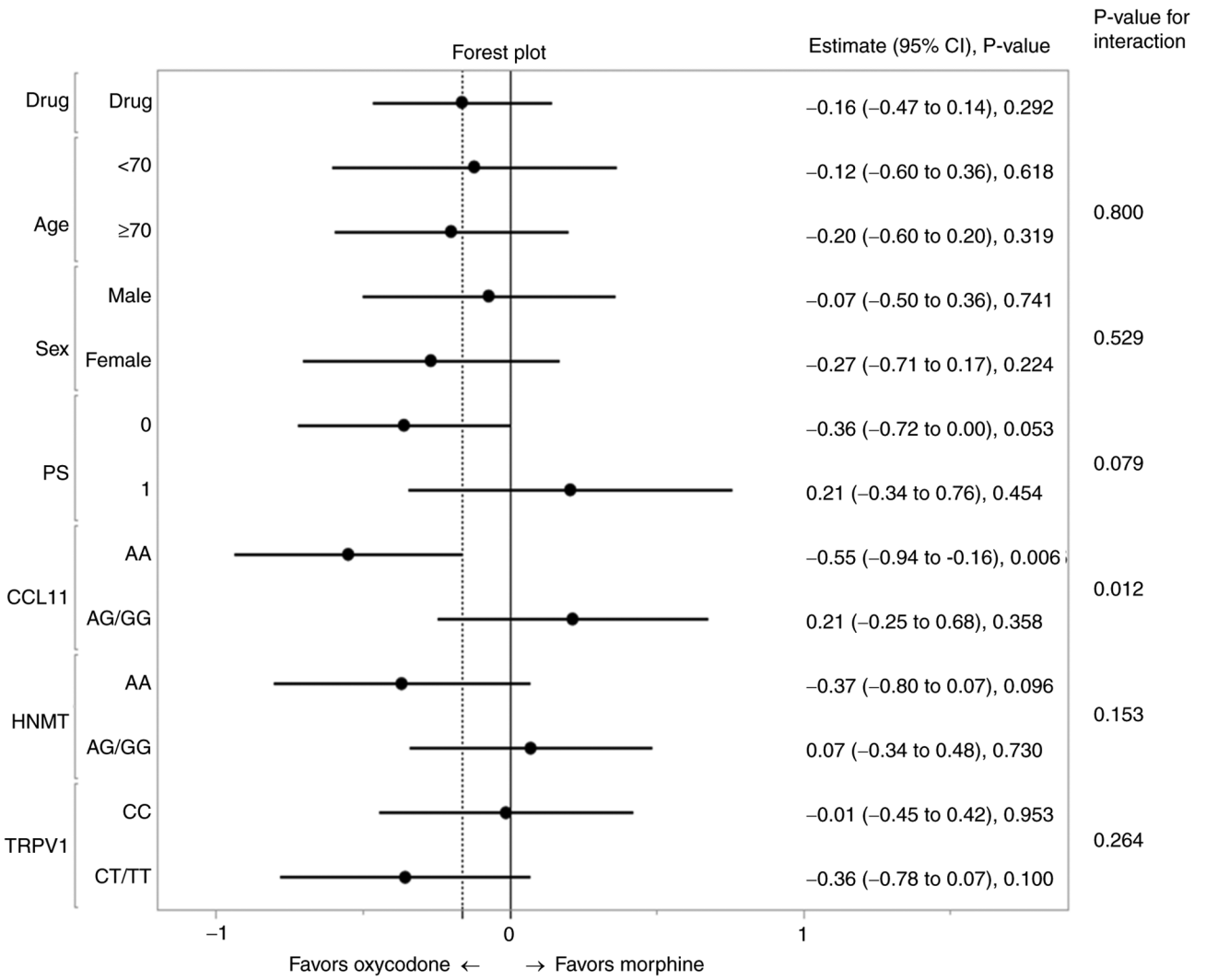

Figure 2. A forest plot comparing treatments for ΔNRS according to age, sex, PS and the 3 SNPs. The dotted line represents the regression coefficient (estimate) for treatment in the overall subject population. CI, confidence interval; PS, performance status; CCL11, C-C motif chemokine ligand 11; HNMT, histamine N-methyltransferase; TRPV1, transient receptor potential vanilloid 1.

opioid. Further analysis of these SNPs revealed a significant interaction with the treatment effect for the *CCL11* genotype. The partial regression coefficient for the interaction term (*CCL11**treatment) was -0.38, which was a significantly large value compared with that of the *CCL11* (-0.24) or the treatment (-0.17) alone (Table SIV). The least square means (LSM) of the ΔNRS calculated for patients with AA+morphine (patients with AA treated with morphine) and patients with AG/GG+morphine were 2.33 and 3.59, while those with AA+oxycodone and AG/GG+oxycodone were 3.43 and 3.16, respectively (Table IV). The patients with AA treated with morphine showed a considerably reduced ΔNRS, suggesting that oxycodone should be administered to patients with the AA genotype of *CCL11* to ease pain with an additional ~1.0 reduction in the post-titration NRS. This procedure may be more critical for patients with high pre-NRS scores who require immediate analgesia.

Existence of relationships has been reported between *CCL11* rs17809012 (also known as eotaxin1) with inflammatory-related diseases, such as asthma (34), fibromyalgia (25), and even ischemic stroke (35) and schizophrenia (36). Zhang *et al* demonstrated that expression of this chemokine caused by inflammation in various sites throughout the body amplifies and prolongs the inflammatory condition, leading to fibromyalgia, a chronic pain syndrome (25). The SNP rs17809012 is located in the *CCL11* promoter region, and a significantly higher mRNA expression level was observed for the A allele than the G allele (25,34). These data suggest that subjects with rs17809012 AA are more responsive to pathological inflammation than those with the AG/GG genotype. Moreover, contribution of the C-C chemokine receptors (CCRs) to the pathogenesis of neuropathic pain has recently been reported (37,38). Upregulation of CCL11, one of the endogenous ligands of CCR3, binds and activates CCR3 in the neurons or microglia to produce neuropathic pain (38). Cancer pain can be a mixture of nociceptive and less morphine-responsive neuropathic pain (39). Oxycodone has been reported to provide clinically meaningful relief in patients with neuropathic pain (5-7), and this may be more applicable to those with the rs17809012 AA genotype for *CCL11*, who tend to have pains with more neuropathic properties that are refractory to morphine treatment.

Our study had some limitations. First, we conducted genotypic analysis for 74 SNPs that were carefully selected from among pain- and/or opioid-related genes. However, the heterogeneous causes of cancer pain cannot be expected to be covered by these SNPs. Some other related SNPs identified more recently could be also candidates. Such additional examples include SNPs of *CCL2, CCL4, CCL7, CCL24, CCL26, CXCL2, CXCL10, CXCR3, CXCR4, IL-2, IL-4,* and *IL-8* (37,40,41), which remain to be investigated in the future.

Second, we screened the SNP candidates for our development study only from among patients who were treated with morphine. It would be desirable to also identify SNPs specifically associated with oxycodone sensitivity, which, in combination with the present results, may help in optimization of the pain treatment. Further analysis is needed with a larger number of patients treated with oxycodone.

Third, analyses of SNPs normally use models assuming dominant inheritance (major-allele homozygotes vs. heterozygotes plus minor-allele homozygotes) and recessive inheritance (major-allele homozygotes plus heterozygotes vs. minor-allele homozygotes), but we only used the former model. Our sample size was too small to include a sufficient number of minor-allele homozygotes for every SNP. We may have missed some latent candidate SNPs because of the small sample size.

Fourth, we omitted the variable of 'dose' when analyzing the total subject population due to the incompatible dosage forms between oral morphine and oral oxycodone formulations. This precluded us from comparing the opioids for their quantitative effects on pain relief, which might not be unreasonable given that the opioid for each patient is selected before the dose.

In conclusion, this is an extension of the RELIEF study to analyze the correlations between some SNPs and the efficacy (or suitability) of opioid drugs in patients with cancer pain. We identified three SNPs as biomarkers, and found that the *CCL11* rs17809012 SNP, in particular, was highly correlated with the pain controllability in patients treated with morphine or oxycodone. Further studies using larger sample sizes are needed to analyze and confirm the individual as well as synergistic effects of these SNPs. Measurements of the serum concentrations of the candidate proteins (i.e. CCL11 or HNMT) in patients and/or biochemical analyses of these molecules in cultured cells or animals may be expected to pave the way for the development of personalized pain management in cancer patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YF, HM, YC, JTs, AK, KNi and KNa designed the study. YF performed the experiments and collected the data. HM, JTs, TY, KS, MN, RS, CM, YO, KT, HH, MT, TO, NT, KH, TT and JTa collected the clinical data. YF, HM, YC, JTs and TY analyzed and interpreted the data. YF and HM drafted the manuscript. YF, HM, YC, JTs, AK, KNi and KNa revised the manuscript critically. YF, HM and JTs confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted according to the guidelines of The Declaration of Helsinki and the Japanese ethical guidelines for clinical research, and was approved by the Ethical Committee of Kindai University Faculty of Medicine (approval no. 26-130). Written informed consent was obtained from all participants involved in the study.

Patient consent for publication

The publication of data was approved in writing by all patients.

Competing interests

Dr Tsurutani reports research fundings from Daiichi Sankyo, Eisai, Taiho, Chugai, Nihonkayaku, Eli Lilly, Pfizer and MSD outside the submitted work. Dr Hayashi reports honoraria from Amgen K.K., AstraZeneca K.K., Boehringer Ingelheim Japan Inc., Bristol-Myers Squibb Co. Ltd., Chugai Pharmaceutical Co. Ltd., Daiichi Sankyo Co. Ltd., Eli Lilly Japan K.K., Janssen Pharmaceutical K.K., Kyorin Pharmaceutical Co. Ltd., Merck Biopharma Co. Ltd., MSD K.K., Novartis Pharmaceuticals K.K., Ono Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd. and Takeda Pharmaceutical Co. Ltd., and research funding from AstraZeneca K.K., Astellas Pharma Inc., MSD K.K., Ono Pharmaceutical Co. Ltd., Nippon Boehringer Ingelheim Co. Ltd., Novartis Pharma K.K., Pfizer Japan Inc., Bristol-Myers Squibb Co. Ltd., Eli Lilly Japan K.K., Chugai Pharmaceutical Co. Ltd., Daiichi Sankyo Co. Ltd., Merck Serono Co. Ltd., Merck Biopharma Co. Ltd., Takeda Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd., SymBio Pharmaceuticals Ltd., AbbVie Inc., inVentiv Health Japan, ICON Japan K.K., Gritstone Oncology Inc., Parexel International Corp., Kissei Pharmaceutical Co. Ltd., EPS Corp., Syneos Health, Pfizer R&D Japan G.K., A2 Healthcare Corp., Quintiles Inc./IQVIA Services Japan K.K., EP-CRSU

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References

1. Fallon M, Giusti R, Aielli F, Hoskin P, Rolke R, Sharma M and Ripamonti C; ESMO Guidelines Committee: Management of cancer pain in adult patients: ESMO clinical practice guidelines. Ann Oncol 29 (Suppl 4): iv166-iv191, 2018.
2. Swarm RA and Dans M: NCCN frameworks for resource stratification of nccn guidelines: Adult cancer pain and palliative care. J Natl Compr Canc Netw 16: 628-631, 2018.
3. Caraceni A, Hanks G, Kaasa S, Bennett MI, Brunelli C, Cherny N, Dale O, De Conno F, Fallon M, Hanna M, *et al*: Use of opioid analgesics in the treatment of cancer pain: Evidence-based recommendations from the EAPC. Lancet Oncol 13: e58-e68, 2012.
4. Owusu Obeng A, Hamadeh I and Smith M: Review of opioid pharmacogenetics and considerations for pain management. Pharmacotherapy 37: 1105-1121, 2017.
5. Coluzzi F, Caputi FF, Billeci D, Pastore AL, Candeletti S, Rocco M and Romualdi P: Safe use of opioids in chronic kidney disease and hemodialysis patients: Tips and tricks for non-pain specialists. Ther Clin Risk Manag 16: 821-837, 2020.
6. Kalso E and Vainio A: Morphine and oxycodone hydrochloride in the management of cancer pain. Clin Pharmacol Ther 47: 639-646, 1990.
7. Watson CPN, Moulin D, Watt-Watson J, Gordon A and Eisenhoffer J: Controlled-release oxycodone relieves neuropathic pain: A randomized controlled trial in painful diabetic neuropathy. Pain 105: 71-78, 2003.
8. Bell GC, Donovan KA and McLeod HL: Clinical implications of opioid pharmacogenomics in patients with cancer. Cancer Control 22: 426-432, 2015.
9. Rakvåg TT, Klepstad P, Baar C, Kvam TM, Dale O, Kaasa S, Krokan HE and Skorpen F: The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain 116: 73-78, 2005.
10. Rakvåg TT, Ross JR, Sato H, Skorpen F, Kaasa S and Klepstad P: Genetic variation in the catechol-O-methyltransferase (COMT) gene and morphine requirements in cancer patients with pain. Mol Pain 4: 64, 2008.
11. Reyes-Gibby CC, Shete S, Rakvåg T, Bhat SV, Skorpen F, Bruera E, Kaasa S and Klepstad P: Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. Pain 130: 25-30, 2007.
12. Matsuoka H, Tsurutani J, Chiba Y, Fujita Y, Sakai K, Yoshida T, Nakura M, Sakamoto R, Makimura C, Ohtake Y, *et al*: Morphine versus oxycodone for cancer pain using a catechol-O-methyltransferase genotype biomarker: A multicenter, randomized, open-label, phase III clinical trial (RELIEF Study). Oncologist 28: 278-e166, 2023.
13. Crews KR, Monte AA, Huddart R, Caudle KE, Kharasch ED, Gaedigk A, Dunnenberger HM, Leeder JS, Callaghan JT, Samer CF, *et al*: Clinical pharmacogenetics implementation consortium guideline for CYP2D6, OPRM1, and COMT genotypes and select opioid therapy. Clin Pharmacol Ther 110: 888-896, 2021.
14. Matsuoka H, Arao T, Makimura C, Takeda M, Kiyota H, Tsurutani J, Fujita Y, Matsumoto K, Kimura H, Otsuka M, *et al*: Expression changes in arrestin β 1 and genetic variation in catechol-O-methyltransferase are biomarkers for the response to morphine treatment in cancer patients. Oncol Rep 27: 1393-1399, 2012.
15. Matsuoka H, Yoshiuchi K, Koyama A, Makimura C, Fujita Y, Tsurutani J, Sakai K, Sakamoto R, Nishio K and Nakagawa K: Expectation of a decrease in pain affects the prognosis of pain in cancer patients: A prospective cohort study of response to morphine. Int J Behav Med 24: 535-541, 2017.
16. Matsuoka H, Makimura C, Koyama A, Fujita Y, Tsurutani J, Sakai K, Sakamoto R, Nishio K and Nakagawa K: Prospective replication study implicates the catechol-O-methyltransferase $Val^{158}Met$ polymorphism as a biomarker for the response to morphine in patients with cancer. Biomed Rep 7: 380-384, 2017.
17. Swarm R, Abernethy AP, Anghelescu DL, Benedetti C, Blinderman CD, Boston B, Cleeland C, Coyle N, Deleon-Casasola OA, Eilers JG, *et al*: Adult cancer pain. J Natl Compr Canc Netw 8: 1046-1086, 2010.
18. Agúndez JAG, Luengo A, Herráez O, Martínez C, Alonso-Navarro H, Jiménez-Jiménez FJ and García-Martín E: Nonsynonymous polymorphisms of histamine-metabolising enzymes in patients with Parkinson's disease. Neuromolecular Med 10: 10-16, 2008.
19. Cook-Sather SD, Li J, Goebel TK, Sussman EM, Rehman MA and Hakonarson H: TAOK3, a novel genome-wide association study locus associated with morphine requirement and postoperative pain in a retrospective pediatric day surgery population. Pain 155: 1773-1783, 2014.
20. Foulkes T and Wood JN: Pain genes. PLoS Genet 4: e1000086, 2008.
21. Ji RR, Berta T and Nedergaard M: Glia and pain: Is chronic pain a gliopathy? Pain 154 (Suppl 1): S10-S28, 2013.
22. Ramesh G, MacLean AG and Philipp MT: Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm 2013: 480739, 2013.
23. Russo R, D'Agostino G, Raso GM, Avagliano C, Cristiano C, Meli R and Calignano A: Central administration of oxytocin reduces hyperalgesia in mice: Implication for cannabinoid and opioid systems. Peptides 38: 81-88, 2012.
24. Sousa-Valente J, Andreou AP, Urban L and Nagy I: Transient receptor potential ion channels in primary sensory neurons as targets for novel analgesics. Br J Pharmacol 171: 2508-2527, 2014.
25. Zhang Z, Feng J, Mao A, Le K, La Placa D, Wu X, Longmate J, Marek C, St Amand RP, Neuhausen SL and Shively JE: SNPs in inflammatory genes CCL11, CCL4 and MEFV in a fibromyalgia family study. PLoS One 13: e0198625, 2018.
26. Světlík S, Hronová K, Bakhouche H, Matoušková O and Slanař O: Pharmacogenetics of chronic pain and its treatment. Mediators Inflamm 2013: 864319, 2013.
27. Gabriel S, Ziaugra L and Tabbaa D: SNP genotyping using the sequenom MassARRAY iPLEX platform. Curr Protoc Hum Genet Chapter 2: Unit 2.12, 2009.
28. Matic M, de Wildt SN, Tibboel D and van Schaik RHN: Analgesia and opioids: A pharmacogenetics shortlist for implementation in clinical practice. Clin Chem 63: 1204-1213, 2017.

29. Shiotani A, Murao T, Fujita Y, Fujimura Y, Sakakibara T, Nishio K and Haruma K: Novel single nucleotide polymorphism markers for low dose aspirin-associated small bowel bleeding. PLoS One 8: e84244, 2013.
30. Matsuoka H, Tsurutani J, Chiba Y, Fujita Y, Terashima M, Yoshida T, Sakai K, Otake Y, Koyama A, Nishio K and Nakagawa K: Selection of opioids for cancer-related pain using a biomarker: A randomized, multi-institutional, open-label trial (RELIEF study). BMC Cancer 17: 674, 2017.
31. Nakamura A, Hasegawa M, Minami K, Kanbara T, Tomii T, Nishiyori A, Narita M, Suzuki T and Kato A: Differential activation of the μ-opioid receptor by oxycodone and morphine in pain-related brain regions in a bone cancer pain model. Br J Pharmacol 168: 375-388, 2013.
32. Nielsen CK, Ross FB, Lotfipour S, Saini KS, Edwards SR and Smith MT: Oxycodone and morphine have distinctly different pharmacological profiles: Radioligand binding and behavioural studies in two rat models of neuropathic pain. Pain 132: 289-300, 2007.
33. Klepstad P, Fladvad T, Skorpen F, Bjordal K, Caraceni A, Dale O, Davies A, Kloke M, Lundström S, Maltoni M, *et al*: Influence from genetic variability on opioid use for cancer pain: A European genetic association study of 2294 cancer pain patients. Pain 152: 1139-1145, 2011.
34. Chang HS, Kim JS, Lee JH, Cho JI, Rhim TY, Uh ST, Park BL, Chung IY, Park CS and Shin HD: A single nucleotide polymorphism on the promoter of eotaxin1 associates with its mRNA expression and asthma phenotypes. J Immunol 174: 1525-1531, 2005.
35. Liang C, Ni G, Ma J, Liu H, Mao Z, Sun H and Zhang X: Impact of tag single nucleotide polymorphisms (SNPs) in CCL11 gene on risk of subtypes of ischemic stroke in xinjiang han populations. Med Sci Monit 23: 4291-4298, 2017.
36. Kang WS, Kim YJ, Park HJ, Kim SK, Paik JW and Kim JW: Association of CCL11 promoter polymorphisms with schizophrenia in a Korean population. Gene 656: 80-85, 2018.
37. Pawlik K, Piotrowska A, Kwiatkowski K, Ciapała K, Popiolek-Barczyk K, Makuch W and Mika J: The blockade of CC chemokine receptor type 1 influences the level of nociceptive factors and enhances opioid analgesic potency in a rat model of neuropathic pain. Immunology 159: 413-428, 2020.
38. Pawlik K, Ciechanowska A, Ciapała K, Rojewska E, Makuch W and Mika J: Blockade of CC chemokine receptor type 3 diminishes pain and enhances opioid analgesic potency in a model of neuropathic pain. Front Immunol 12: 781310, 2021.
39. Lovo EE, Moreira A, Cruz C, Carvajal G, Barahona KC, Caceros V, Blanco A, Mejias R, Alho E and Soto T: Radiomodulation in mixed, complex cancer pain by triple target irradiation in the brain: A preliminary experience. Cureus 14: e25430, 2022.
40. Richards GC, Lluka LJ, Smith MT, Haslam C, Moore B, O'Callaghan J and Strong J: Effects of long-term opioid analgesics on cognitive performance and plasma cytokine concentrations in patients with chronic low back pain: A cross-sectional pilot study. Pain Rep 3: e669, 2018.
41. Zhou TQ, Gao HY, Guan XH, Yuan X, Fang GG, Chen Y and Ye DW: Chemokines and their receptors: Potential therapeutic targets for bone cancer pain. Curr Pharm Des 21: 5029-5033, 2015.