OPRM1 and COMT Gene–Gene Interaction Is Associated With Postoperative Pain and Opioid Consumption After Orthopedic Trauma

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Abstract

Background: mu-opioid receptor (*OPRM1*) and catechol-O-methyltransferase (*COMT*) contribute to the neurotransmission pathway of pain. *COMT* affects mu receptor expression and density in the brain. The aim of this study was to explore the *OPRM1* and *COMT* interaction effects on postoperative pain and opioid consumption. **Methods:** This cross-sectional exploratory study used genotype and clinical data from 153 postoperative patients. Using multiple regression analyses, four single-nucleotide polymorphisms of *COMT* (rs6269, rs4633, rs4818, and rs4680), their haplotypes, and diplotypes were considered for their interactions with A118G of *OPRM1* regarding postoperative pain and opioid consumption. **Results:** For opioid consumption, significant interactions were found between *OPRM1* A118G and *COMT* rs4680 ($p = .037$) and between *OPRM1* and *COMT* rs4633 ($p = .037$). Patients having Met158Met of *COMT* rs4680 and AG/GG of *OPRM1* or TT of *COMT* rs4633 and AG/GG of *OPRM1* consumed the largest amount of opioid compared to those having other combinations. For postoperative pain, a significant interaction was found between *OPRM1* and the low pain sensitivity (LPS; GCGG) haplotype of *COMT* ($p = .017$). For patients with no copies of the LPS haplotype, AA of *OPRM1* A118G was significantly associated with higher pain scores compared to the variant AG/GG. However, the opposite direction was observed for patients with at least one copy of the LPS haplotype. **Conclusions:** The interaction of *OPRM1* with *COMT* may contribute to variability in postoperative pain and opioid consumption. Additional larger studies are needed to confirm findings.

Keywords

OPRM1, *COMT*, gene–gene interaction, postoperative pain, postoperative opioid consumption

Inadequate relief of postoperative pain may result in many harmful physiological, psychological, and behavioral consequences that have a significant impact on morbidity and mortality as well as health-care costs (Harsoor, 2011). The highly individualized effect of opioids on patients makes optimal pain management challenging. Researchers have suggested genetic variations as a possible explanation for variation in pain intensity and response to opioids (Tremblay & Hamet, 2010).

Recent systematic reviews and meta-analyses have demonstrated the impact of the A118G single-nucleotide polymorphism (SNP) of mu-opioid receptor (*OPRM1*) on the variability of postoperative pain and responses to opioids (Hwang et al., 2014; Ren et al., 2015). The G variant of *OPRM1* A118G (AG/GG) has been associated with higher pain scores and larger opioid consumption compared to the wild-type A118A (Henker et al., 2013; S. Zhang, Li, & Tan, 2013). Multiple SNPs within the catechol-O-methyltransferase (*COMT*) gene, including rs6269, rs4633,

rs4818, and rs4680 (Val158Met) are also associated with pain sensitivity and opioid efficacy (Diatchenko et al., 2005; F. Zhang et al., 2014). Researchers used these four SNPs of *COMT* to construct three major pain sensitivity haplotypes: low pain sensitivity (LPS; GCGG), average pain sensitivity (APS; ATCA), and

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high pain sensitivity (HPS; ACCG; Diatchenko et al., 2006). The evidence showed that *COMT* haplotypes, rather than a single SNP, better account for the variability in pain sensitivity (Landau, Liu, Blouin, & Carvalho, 2013; Nackley et al., 2006).

The relationships between these genes and pain are complex and might involve interactions among multiple genes (Landau et al., 2013; Reyes-Gibby et al., 2007). The biological associations between *OPRM1* A118G, *COMT*, and pain sensitivity have been well described: *COMT* genetic variants have been found to affect mu receptor (*OPRM1*) expression and density in brain tissue by affecting enkephalin levels, which inversely regulate mu receptor expression (Berthele et al., 2005; Kowarik et al., 2012; Zubieta et al., 2003). Therefore, the interaction between *OPRM1* and *COMT* (*OPRM1* $\times$ *COMT*) may significantly impact pain perception and response to opioids.

Researchers first evaluated the interaction effect of *OPRM1* A118G and *COMT* rs4680 (Val158Met) on opioid dose needed to control cancer pain. They found that carriers of *OPRM1* AA and *COMT* Met/Met genotype require the lowest opioid dose to relieve pain (Reyes-Gibby et al., 2007). However, in another study, women with that genetic combination had the least pain relief after intravenous fentanyl dose during labor and delivery (Landau et al., 2013). De Gregori et al. (2013), meanwhile, did not find a significant effect of interactions between these genes on postoperative opioid consumption. Finally, Yao et al. (2015) found that cancer patients undergoing elective surgery who had *OPRM1* GG and *COMT* Met/Met genotype had the lowest preoperative pain threshold and tolerance. The findings of *OPRM1* and *COMT* interaction studies have, thus, been inconsistent. Furthermore, the variations in measurements of pain outcomes and opioid regimens among those studies make it difficult to compare their findings and draw broader conclusions.

The purpose of the present study was to explore the effects of *COMT* $\times$ *OPRM1* interaction on postoperative pain and opioid consumption in postoperative orthopedic trauma patients. To the best of our knowledge, this is the first study to investigate the interaction effects of four *COMT* SNPs (rs6269, rs4633, rs4818, and rs4680) as well as *COMT* haplotypes and diplotypes with *OPRM1* A118G on postoperative pain and opioid consumption.

Materials and Methods

Subjects

For this cross-sectional descriptive study, we used phenotype, demographic, clinical, and genotype data previously obtained for a parent study (Henker et al., 2013). Inclusion criteria for the parent study were age 18–80 years, receipt of general or general-with-regional anesthesia, admission for a single orthopedic surgery, and planned length of surgery of 1–4 hr. Patients were excluded if they had a second trauma site, a history of mental illness, any neurologic conditions, or hepatic or renal disease. Subjects were prospectively enrolled in the parent study after investigators obtained institutional review board approval and written informed consent.

Figure 1. Linkage disequilibrium (LD) graph of *COMT* single-nucleotide polymorphisms (SNPs; left) and haplotype frequency (right). LD was calculated using D' (0 = no disequilibrium; 1 = maximum disequilibrium). The numbers inside the squares are $100 \times D'$. The graph was created using Haploview software. LPS = low pain sensitivity; APS = average pain sensitivity; HPS = high pain sensitivity.

Postoperative Pain and Opioids

Pain was assessed using the Numeric Rating Scale, an 11-point verbal pain response scale that ranges from 0 (*no pain*) to 10 (*pain as bad as I can imagine*). Researcher collected pain scores in the preoperative holding area and then at 45 min after arrival in the post anesthesia care unit (PACU) when the intraoperative anesthetic effects were beginning to wear off and patients had become more alert and able to report pain.

Opioids administered during the PACU stay included fentanyl, hydromorphone, morphine, and meperidine. The amount of opioid administered was converted to intravenous (IV) morphine equivalents, where 100 μ g of fentanyl IV, 1.5 mg of hydromorphone IV, or 75 mg of meperidine IV were equivalent to 10 mg of morphine IV (National Pharmaceutical Council & Joint Commission on Accreditation of Healthcare Organizations, 2006).

Genotyping Data

Researchers used Oragene DNA self-collection kits (Genotek Inc., Ottawa, Ontario, Canada) to collect saliva samples from patients and extracted DNA using the manufacturer's protocol. Saliva is a viable alternative to blood as a source of DNA for epidemiologic studies (Abraham et al., 2012). To ensure accuracy of association results, we assessed the quality of the genotyping data using filters on call rate (>0.95) and repeatability of calls (>0.99) and checks comparing expected homozygosity to observed homozygosity at each marker.

OPRM1 A118G (rs1799971) was genotyped using sequencing. Only two (2%) patients were homozygous for the G118 variant. Therefore, we combined the heterozygotes and homozygous variant in one group and analyzed A118G under only the dominant genetic model. *COMT* SNPs including rs6269, rs4633, rs4818, and rs4680 were genotyped using 5'

Table 1. Clinical and Genetic Characteristics of the Study Sample.

Variable	Descriptive Statistics		
	Total (N = 153)	White (n = 121)	Non-White (n = 31)
Gender (male)	104 (68)	88 (73)	16 (51.6)
Smoking (nonsmoker)	86 (57)	69 (57)	17 (54.8)
Fracture type			
Ankle	56 (39)	44 (38)	12 (41)
Femur	16 (11)	14 (12)	2 (7)
Tibial plateau	30 (21)	25 (22)	5 (17)
Tibia-fibula	35 (24)	25 (25)	10 (35)
Other	7 (5)	7 (6)	0 (0)
Age (years)	38.48 ± 13.13	39.00 ± 12.96	36.45 ± 13.81
Body mass index (kg/m ²)	29.09 ± 7.43	29.18 ± 7.53	28.74 ± 7.17
Surgical time (min)	120.92 ± 59.36	125.83 ± 62.56	101.76 ± 40.14
PACU time (min)	128.99 ± 56.40	130.28 ± 60.79	124.00 ± 34.94
Preoperative pain score	4.56 ± 2.95	4.43 ± 2.79	5.07 ± 3.55
Postoperative pain score at 45 min in PACU	6.48 ± 2.69	6.27 ± 2.66	7.29 ± 2.72
Opioid consumption in OR (mg/kg)	0.40 ± 0.22	0.40 ± 0.21	0.43 ± 0.25
Opioid consumption in the first 45 min in PACU (mg/kg)	0.08 ± 0.07	0.08 ± 0.07	0.08 ± 0.06
Opioid consumption during PACU stay (mg/kg)	0.11 ± 0.09	0.11 ± 0.10	0.10 ± 0.08
OPRM1 A118G			
AA	106 (78)	81 (77)	24 (80)
AG	28 (20)	22 (21)	6 (20)
GG	2 (2)	2 (2)	0
COMT rs4680			
AA	37 (27)	35 (33)	2 (7)
AG	46 (34)	35 (33)	11 (40)
GG	53 (39)	37 (34)	15 (53)
COMT rs4633			
CC	52 (39)	36 (35)	15 (54)
TT	33 (25)	31 (40)	2 (7)
CT	48 (36)	37 (35)	11 (39)
COMT rs4818			
GG	30 (23)	29 (28)	1 (4)
GC	56 (43)	39 (35)	17 (63)
CC	45 (34)	36 (37)	9 (33)
COMT rs6269			
GG	42 (32)	35 (34)	7 (26)
GA	49 (37)	33 (32)	16 (59)
AA	40 (31)	36 (34)	4 (15)
COMT LPS haplotype			
0 copy	52 (39)	42 (40)	10 (36)
At least 1 copy	82 (61)	64 (60)	18 (64)
COMT APS haplotype			
0 copy	63 (47)	46 (44)	17 (61)
At least 1 copy	71 (53)	60 (56)	11 (39)
COMT HPS haplotype			
0 copy	110 (82)	92 (87)	18 (64)
At least 1 copy	24 (18)	14 (13)	11 (36)
COMT diplotype			
Low	77 (50)	61 (50)	16 (52)
High	57 (37)	45 (37)	12 (39)

Note. Descriptive statistics are expressed as mean ± SD for continuous variables and n (%) for categorical variables. While the total sample included 153 patients, race was not identified for one patient; consequently, that patient is not included in the descriptive statistics by race in the last two columns. APS = average pain sensitivity; HPS = high pain sensitivity; LPS = low pain sensitivity; OR = operating room; PACU = postanesthesia care unit.

exonuclease Assay-on-Demand TaqMan assays. We used Haploview software (<http://www.broad.mit.edu/mpg/haploview>) to visualize the pairwise linkage disequilibrium (LD) between the selected four *COMT* SNPs measured by Lewontin's D'

(Barrett, Fry, Maller, & Daly, 2005; Figure 1). We designated the *COMT* haplotypes, constructed from the four *COMT* SNPs, as LPS (GCGG, APS (ATCA), and HPS (ACCG; Diatchenko et al., 2006). Then, we coded *COMT* haplotypes into two

Table 2. Genotype and Allele Frequency of *OPRM1* and *COMT* Single-Nucleotide Polymorphisms (SNPs).

Gene and SNP	Chromosome	Position	Alleles			Genotypes			HWE ^a
			A1	A2	MAF	A1/A1	A1/A2	A2/A2	
<i>OPRM1</i> rs1799971	6	154360797	G	A	0.1176	2	28	106	1
<i>COMT</i> rs4680	22	19951271	A	G	0.4412	37	46	53	0.00
rs4633	22	19950235	T	C	0.4286	33	48	52	0.00
rs4818	22	19951207	G	C	0.4427	30	56	45	0.16
rs6269	22	19949952	A	G	0.4924	40	49	42	0.01

Note. A1 = minor allele; A2 = major allele; MAF = minor allelic frequency.

^aHardy-Weinberg Equilibrium (HWE) test *p* value.

levels: having no copies and having at least one copy. Using combinations of these three major haplotypes, we created two diplotypes: LPS (LPS/LPS or LPS/APS) and HPS (APS/APS, HPS/HPS, LPS/HPS, or APS/HPS). Details for sample collection and genotyping procedure were previously described in Henker et al. (2013).

Statistical Analysis

All statistical analyses were preceded by detailed descriptive and exploratory analyses of the data. We assessed Hardy-Weinberg equilibrium (HWE) for all genotyped SNPs using the exact test implemented in the software PLINK (Purcell et al., 2007). Postoperative pain scores at 45 min in PACU and opioid consumption during the PACU stay were the primary outcomes (i.e., the dependent variables) of the multiple linear regression analyses, in which *OPRM1* A118G (under dominant genetic model); the *COMT* SNPs rs6269, rs4633, rs4818, and rs4680 (under additive, dominant, and recessive genetic models); the *COMT* haplotypes (LPS, APS, and HPS); and *COMT* diplotypes (LPS and HPS) were the independent variables.

We investigated the main and interaction effects of the above-mentioned independent variables after adjusting for covariates. For the outcome of opioid consumption, the covariates for which we adjusted included gender, race/ethnicity, age, smoking, fracture type, and operating room (OR) opioid consumption (mg/kg/hr). For postoperative pain outcome, we adjusted for preoperative pain and opioid consumption during the first 45 min in the PACU (mg/kg) in addition to the aforementioned covariates.

We used square root transformation of reflected values of the postoperative pain score for regression analyses to remediate the violation of normality assumption for model residuals. Since the frequencies of alleles and haplotypes may vary among different ancestries, we also conducted a subgroup analysis limited to Caucasian subjects (*n* = 121 of 153). All analyses were carried out using IBM® SPSS® Statistics version 22 (IBM Corp., Armonk, NY), and a two-sided *p* value of <.05 was considered to be statistically significant. Due to the exploratory nature of the current data analyses, we made no multiple comparisons adjustment of the *p* value.

Results

The sample of enrolled postoperative patients (*N* = 153) was mostly male (*n* = 104, 68%), non-Hispanic Caucasian (*n* = 121, 80%), and nonsmokers (*n* = 86, 57%) and had an average age of 38.48 ($\pm$ 13.1) years. Table 1 presents further description of the sample. We assumed that missing observations were missing completely at random. Most missing data were genetic information for *OPRM1* or *COMT* or both (*n* = 28, 18%) genotypes. Three of the four *COMT* SNPs (rs4680, rs4633, and rs6269) displayed significant deviation from HWE, as shown in Table 2. These SNPs were out of equilibrium even after we restricted our analyses to the subset of Caucasian patients. We performed systematic lab review of raw genotype data to examine whether deviations from HWE were due to genotyping error and found no evidence of error. Pairwise linkage disequilibrium values computed by Haploview software showed that all *COMT* SNPs were in strong LD (*D'* > 0.75; Figure 1).

PACU Opioid Consumption

When covariates were entered first in the hierarchical regression model, they significantly predicted postoperative opioid consumption, $F(9, 113) = 2.125, p < .05, R^2 = .15$. Age and OR opioid consumption contributed significantly to the prediction of opioid consumption in the PACU, $\beta = -0.001$ (95% confidence interval: CI [-0.003, 0.000]) and $\beta = 0.14$ (95%CI [0.014, 0.267]), respectively. When *OPRM1* was added to the earlier model, it significantly improved the prediction of opioid consumption, R^2 change = .04, $F(1, 112) = 5.8, p < .05$. Patients with the G allele of *OPRM1* A118G required significantly higher doses of opioid compared to those with wild type, $\beta = 0.46$ (95%CI [-0.008, -0.085]). However, adding *COMT* rs4680, assuming a recessive genetic model (MetMet vs. MetVal/ValVal), did not improve the prediction of opioid consumption, $\beta = 0.01$ (95%CI [-0.027, 0.047]). As the interaction term was added finally to the model, it significantly improved the prediction, R^2 change = .03, $F(1, 110) = 4.4, p < .05$. The entire group of variables significantly predicted opioid consumption, $F(12, 110) = 2.59, p < .01$ (see Table 3 for results of the regression analysis). We found that patients having Met158Met of *COMT* rs4680 and AG/GG of *OPRM1* A118G

Table 3. Regression Analysis Results for Predicting Opioid Consumption (mg/kg) in the Postanesthesia Care Unit (PACU) by *OPRM1* × *COMT*.

Interaction Variable and Sample	<i>n</i>	β	95%CI	<i>p</i> value	<i>sr</i> ²
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (dominant)					
Total	123	0.003	[−0.077, 0.083]	.940	<.001
Caucasians	96	−0.030	[−0.131, 0.071]	.554	.003
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (recessive)					
Total	123	0.093	[0.006, 0.179]	.037	.031
Caucasians	96	0.108	[0.017, 0.200]	.021	.047
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (additive)					
Total	123	0.073	[−0.027, 0.172]	.150	.015
Caucasians	96	0.052	[−0.059, 0.164]	.353	.007
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (dominant)					
Total	120	0.015	[−0.069, 0.099]	.726	<.001
Caucasians	93	−0.019	[−0.133, 0.094]	.733	.001
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (recessive)					
Total	120	0.097	[0.006, 0.189]	.037	.033
Caucasians	93	0.102	[0.006, 0.199]	.038	.039
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (additive)					
Total	120	0.087	[−0.019, 0.193]	.105	.019
Caucasians	93	0.059	[−0.067, 0.185]	.352	.008
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (dominant)					
Total	119	−0.044	[−0.126, 0.037]	.284	.009
Caucasians	93	−0.058	[−0.153, 0.036]	.223	.014
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (recessive)					
Total	119	0.020	[−0.085, 0.126]	.702	.001
Caucasians	93	0.089	[−0.033, 0.211]	.150	.020
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (additive)					
Total	119	−0.017	[−0.133, 0.098]	.765	<.001
Caucasians	93	0.027	[−0.104, 0.158]	.679	.002
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (dominant)					
Total	118	−0.075	[−0.160, 0.010]	.082	.023
Caucasians	92	−0.070	[−0.163, 0.023]	.136	.021
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (recessive)					
Total	118	−0.027	[−0.114, 0.060]	.537	.003
Caucasians	92	0.050	[−0.052, 0.153]	.332	.009
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (additive)					
Total	118	−0.073	[−0.176, 0.029]	.160	.015
Caucasians	92	−0.009	[−0.122, 0.104]	.877	<.001
<i>OPRM1</i> A118G × <i>COMT</i> LPS haplotype (GCGG)					
Total	123	−0.047	[−0.125, 0.030]	.230	.011
Caucasians	96	−0.059	[−0.149, 0.031]	.194	.015
<i>OPRM1</i> A118G × <i>COMT</i> APS haplotype (ATCA)					
Total	123	0.009	[−0.068, 0.086]	.818	<.001
Caucasians	96	−0.015	[−0.1060, .077]	.750	<.001
<i>OPRM1</i> A118G × <i>COMT</i> HPS haplotype (ACCG)					
Total	123	0.001	[−0.095, 0.098]	.982	<.001
Caucasians	96	−0.073	[−0.191, 0.045]	.222	.013
<i>OPRM1</i> A118G × <i>COMT</i> diplotypes					
Total	123	0.071	[−0.006, 0.148]	.070	.024
Caucasians	96	0.047	[−0.040, 0.135]	.285	.010

Note. APS = average pain sensitivity; HPS = high pain sensitivity; LPS = low pain sensitivity; *sr*² = squared semipartial correlation. In addition to the *OPRM1* × *COMT* interaction, models included the main effects of both *OPRM1* and *COMT*, gender, race, age, smoking, fracture type, and operating room opioid consumption.

required higher total dose of opioid to relieve pain compared to other combinations, $\beta = 0.093$ (95%CI [0.006, 0.179]; see Figure 2 for a representation of the estimated total opioid dose adjusted to the covariates). *OPRM1* A118G × *COMT* rs4680 accounted uniquely for 3.1% of the total PACU opioid consumption variance. *COMT* rs4633, which has a strong LD with rs4680, showed a similar interaction effect with *OPRM1*

A118G on opioid consumption, with patients having TT of *COMT* rs4633 and AG/GG of *OPRM1* A118G consuming higher doses of opioids compared to other combinations (opioid consumption for these patients would have the same pattern as that displayed in Figure 1 for *COMT* rs4680).

We ran a similar regression model for the other two SNPs of *COMT* (rs4818 and rs6269), *COMT* haplotypes and diplotypes.

Figure 2. The interaction effect of *OPRM1* A118G $\times$ *COMT* rs4680 on postanesthesia care unit (PACU) opioid consumption for the total sample. Graphs display the *OPRM1* $\times$ *COMT* interaction effect for the typical participant in our sample: Caucasian, male, with ankle fracture, aged 39.9 years, and operating room (OR) opioid consumption of 0.233mg/kg/hr.

However, none of their main effects or their interactions with *OPRM1* contributed significantly to the prediction of opioid consumption, although *COMT* rs6269 $\times$ *OPRM1* and *COMT* diplotypes $\times$ *OPRM1* demonstrated near-significant trends ($.05 \leq p < .10$) with p values of .08 and .07, respectively (Table 3). Restricting the regression analyses to Caucasian subjects did not change the direction or the statistical significance for the interaction effects of either *OPRM1* A118G $\times$ *COMT* rs4680 or *OPRM1* A118G $\times$ *COMT* rs4633 on PACU opioid consumption (data not shown).

Postoperative Pain in the PACU

When covariates were entered first in the hierarchical regression model, they significantly predicted postoperative pain scoring, $F(11, 109) = 3.651$, $p < .001$, $R^2 = .269$. Race and total opioid consumption in the first 45 min in the PACU contributed significantly to the prediction of postoperative pain with $\beta = -0.42$ (95% CI $[-0.808, -0.028]$) and $\beta = -4.44$ (95% CI $[-6.796, -2.074]$), respectively. Adding the main effects for each of *OPRM1* and the LPS haplotype of *COMT* to the earlier model did not significantly improve the prediction, R^2 change = .00, $F(1, 108) = .02$, $p = .90$ and R^2 change = .02, $F(1, 107) = 3.13$, $p = .08$, respectively. However, adding their interaction term significantly improved the prediction, R^2 change = .04, $F(1, 106) = 5.84$, $p < .05$. The entire group of variables significantly predicted pain scoring, $F(14, 106) = 3.68$, $p < .001$. *OPRM1* A118G $\times$ *COMT* LPS haplotype accounted uniquely for 3.7% of the total postoperative pain variance. We found that postoperative pain ratings varied across levels of the *COMT* LPS haplotype. For patients with no copies of the LPS haplotype, the wild type of *OPRM1* A118G (AA) was significantly associated with higher pain scores compared to the variant (AG/GG). For patients who had at least one copy of the LPS haplotype, AA of *OPRM1* A118G was significantly associated with lower pain score compared with those having the variant (AG/GG), $\beta = -0.93$ (95% CI

$[-1.686, -0.166]$; see Table 4 for details of the regression model and Figure 3, which represents the estimated pain score adjusted to the covariates).

Adding *COMT* rs4818, assuming a dominant genetic model (CC vs. GC/GG), to the regression model with the covariates and *OPRM1* A118G significantly improved the prediction of postoperative pain, R^2 change = .04, $F(1, 103) = 5.2$, $p < .05$. Patients with one or two minor alleles G (GC and GG) had significantly higher postoperative pain scores compared to patients with CC, $\beta = 0.39$ (95% CI $[0.050, 0.725]$). However, *COMT* rs4818 $\times$ *OPRM1* demonstrated a trend near significance ($.05 \leq p < .10$), $p = .06$.

The main effects of each of *COMT* rs4633, assuming a recessive genetic model (TT vs. CT/CC), and *COMT* diplotypes were significantly associated with postoperative pain, $\beta = -0.389$ (95% CI $[-0.759, -0.019]$) and $\beta = -0.32$ (95% CI $[-0.636, -0.005]$), respectively. We found that patients with the HPS *COMT* diplotypes reported significantly higher postoperative pain levels compared to patients with the LPS diplotypes, and those with two minor alleles T (TT) of *COMT* rs4633 reported significantly higher postoperative pain levels at 45 min in the PACU compared to patients with CT or CC. However, neither *COMT* rs4633 $\times$ *OPRM1* nor *COMT* diplotypes $\times$ *OPRM1* significantly improved the prediction of postoperative pain, R^2 change = .02, $F(1, 106) = 2.69$, $p = .10$ and R^2 change = .00, $F(1, 103) = 0.6$, $p = .44$, respectively.

When we restricted the analyses to Caucasian subjects, none of *OPRM1* A118G $\times$ *COMT* SNPs, haplotypes, or diplotypes were significantly associated with postoperative pain. However, the interaction effects for *OPRM1* A118G $\times$ *COMT* LPS, *OPRM1* A118G $\times$ *COMT* HPS, and *OPRM1* A118G $\times$ *COMT* diplotypes were near significant ($p = .070$, $p = .058$, $p = .050$, respectively).

Discussion

The aim of this pilot study was to provide sufficient evidence for a larger study to be undertaken to further investigate the interaction effects of *OPRM1* and *COMT* on postoperative pain and response to opioids. The study was underpowered for drawing firm conclusions about *OPRM1* $\times$ *COMT* interaction effects. However, it does provide initial evidence that *OPRM1* $\times$ *COMT* affects postoperative pain and response to opioids. Patients with combined Met158Met of *COMT* rs4680 and AG/GG of *OPRM1* A118G consumed more opioid compared to patients with other combinations. Consistent with previous research (De Gregori et al., 2013; Reyes-Gibby et al., 2007), we also found that carriers of the *OPRM1* AA and *COMT* Met/Met genotype required the lowest opioid dose compared to other combinations. Regarding postoperative pain, in the present sample having at least of one copy of the LPS haplotype and being homozygous for the wild-type allele of *OPRM1* A118G was associated with lower postoperative pain scores compared to the variant AG/GG. Although *OPRM1* $\times$ *COMT* explained only a small proportion of the variance in postoperative pain and opioid consumption, our findings provide

Table 4. Regression Analysis Results for Predicting Postoperative Pain Ratings by *OPRM1* × *COMT*.

Interaction Variable and Sample	<i>n</i>	β	95%CI	<i>p</i> value	<i>sr</i> ²
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (dominant)					
Total	121	0.269	[−0.572, 1.111]	.527	.003
Caucasian	95	0.073	[−0.914, 1.060]	.884	<.001
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (recessive)					
Total	121	0.444	[−0.436, 1.324]	.319	.007
Caucasian	95	0.421	[−0.501, 1.342]	.366	.007
<i>OPRM1</i> A118G × <i>COMT</i> rs4680 (additive)					
Total	121	0.501	[−0.530, 1.532]	.337	.006
Caucasian	95	0.361	[−0.779, 1.501]	.531	.003
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (dominant)					
Total	118	0.359	[−0.537, 1.254]	.429	.004
Caucasian	92	0.342	[−0.750, 1.434]	.534	.004
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (recessive)					
Total	118	0.359	[−0.559, 1.276]	.440	.004
Caucasian	92	0.419	[−0.526, 1.364]	.380	.007
<i>OPRM1</i> A118G × <i>COMT</i> rs4633 (additive)					
Total	118	0.512	[−0.592, 1.616]	.360	.006
Caucasian	92	0.626	[−0.621, 1.872]	.321	.009
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (dominant)					
Total	117	−0.755	[−1.543, 0.033]	.060	.024
Caucasian	92	−0.486	[−1.390, 0.420]	.289	.010
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (recessive)					
Total	117	0.498	[−1.569, 0.572]	.358	.006
Caucasian	92	−0.574	[−1.751, 0.604]	.335	.008
<i>OPRM1</i> A118G × <i>COMT</i> rs4818 (additive)					
Total	117	−0.939	[−2.079, 0.200]	.105	.018
Caucasian	92	−0.818	[−2.130, 0.494]	.218	.014
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (dominant)					
Total	116	−0.500	[−1.297, 0.296]	.216	.010
Caucasian	91	−0.640	[−1.470, 0.188]	.128	.020
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (recessive)					
Total	116	−0.507	[−1.353, 0.338]	.237	.009
Caucasian	91	−0.504	[−1.413, 0.406]	.274	.010
<i>OPRM1</i> A118G × <i>COMT</i> rs6269 (additive)					
Total	116	−0.691	[−1.686, 0.305]	.172	.012
Caucasian	91	−0.504	[−1.413, 0.406]	.274	.010
<i>OPRM1</i> A118G × <i>COMT</i> LPS haplotype (GCGG)					
Total	121	−0.926	[−1.686, −0.166]	.017	.037
Caucasian	95	−0.794	[−1.660, 0.0670]	.070	.023
<i>OPRM1</i> A118G × <i>COMT</i> APS haplotype (ATCA)					
Total	121	0.288	[−0.510, 1.0850]	.476	.003
Caucasian	95	0.080	[−0.8110, 0.971]	.858	<.001
<i>OPRM1</i> A118G × <i>COMT</i> HPS haplotype (ACCG)					
Total	121	0.596	[−0.372, 1.563]	.225	.010
Caucasian	95	1.110	[−0.040, 2.261]	.058	.031
<i>OPRM1</i> A118G × <i>COMT</i> diplotypes					
Total	121	0.637	[−0.132, 1.407]	.103	.017
Caucasian	95	0.831	[−0.001, 1.662]	.050	.033

Note. In addition to the *OPRM1* × *COMT* interaction, models included the main effects of both *OPRM1* and *COMT*, gender, race in total sample, age, smoking, fracture type, preoperative pain, operation room opioid consumption, and opioid consumption during the first 45 min in PACU. APS = average pain sensitivity; HPS = high pain sensitivity; LPS = low pain sensitivity; *sr*² = squared semipartial correlation.

preliminary data that are essential for establishing the associations between gene interactions and postoperative pain and response to opioids and suggest the need for future examination among a larger sample.

A to G substitution in position 118 of the *OPRM1* gene results in less messenger RNA (mRNA) expression in brain

tissues of G allele carriers (Y. Zhang, Wang, Johnson, Papp, & Sadee, 2005). Moreover, the G118 has a secondary structure alteration that affects its expression and translation into functional protein. Given this finding, others have explored the genotype–phenotype relationship and have found that carriers of the G allele experienced more pain and needed higher opioid

Figure 3. The interaction effect of *OPRM1* × *COMT* low pain sensitivity (LPS) haplotype on postoperative pain ratings at 45 min in the postanesthesia care unit (PACU) for the total sample. Graphs display the *OPRM1* × *COMT* interaction effect for the typical participant in our sample: Caucasian, male, with ankle fracture, aged 38.9 years, operating room (OR) opioid consumption of 0.246mg/kg/hr and opioid consumption during the first 45 min in the PACU of .08 mg/kg. The pain variable was reflected and transformed using square root to meet the assumption of normality.

dose to achieve adequate pain control compared to those with the wild-type A118A (Sia et al., 2013; Tan et al., 2009). In agreement with this previous research, in the present study, we also found that patients who were homozygous for the variant G needed higher opioid dose to achieve adequate postoperative pain control compared to those who were homozygous for the wild-type A118A.

Researchers have found that “synonymous silent” SNPs of the *COMT* gene could affect mRNA stability and consequently COMT protein expression and enzymatic activity (Nackley et al., 2006). Thus, these SNPs might also influence pain sensitivity. Consistent with those findings, in the present study, we found that the two synonymous SNPs of *COMT*, rs4633 and rs4818, were significantly associated with postoperative pain in the PACU. TT of rs4633 and AG/GG of rs4818 were associated with high pain sensitivity.

Recent genetic studies focused on investigating haplotype reconstruction have suggested that combinations/interactions of SNPs within haplotypes lead to synergistic effects on the resultant protein and might also result in functional consequences that differ from the independent effects of those SNPs (Diatchenko et al., 2005). For instance, both LPS and HPS haplotypes of *COMT* include the Val allele of *COMT* rs4680 (Val158Met). However, they significantly differ in the resultant protein and enzyme activity. Individuals with the LPS haplotype catabolize catecholamine at 11.4 times the rate of those with the HPS haplotype and experience decreased pain sensitivity (Diatchenko et al., 2006). This finding suggests that the interactions of multiple SNPs within a *COMT* rs4680 haplotype determine the functional outcomes of regarding the COMT enzyme rather than a single SNP making that determination. In the present study, our main effect analysis findings showed that *COMT* diplotypes were significantly associated with postoperative pain while the single SNP rs4680 was not.

Our findings on postoperative pain changed after we restricted the analyses to our self-reported Caucasian subgroup. The interaction of *OPRM1* and the LPS *COMT* haplotype was not significant in Caucasians; however, the interaction effect was similar to that for the total sample. *OPRM1* × ACCG and *OPRM1* × diplotypes showed a trend toward significance ($.05 \leq p < .10$) that we did not observe for the total sample. None of *COMT* rs4818, *COMT* rs4633, or *COMT* diplotypes were significantly associated with outcome variables in the Caucasian-only sample. The changes in our findings might be partly due to reduced sample size or the variation in the allele and haplotype frequencies among the different racial populations even though we adjusted for race in our regression analyses. The G allele frequencies of *OPRM1* A118G vary across racial and ethnic groups, from 12%–20% in Caucasians to 1%–4% in African Americans and 19%–24% in Hispanics (Hastie et al., 2012). The *COMT* allele frequencies and haplotype structures and frequencies also vary among the different racial groups (Beuten, Payne, Ma, & Li, 2006; Gabriel et al., 2002). Moreover, in one study, researchers found that COMT enzyme activity was significantly higher in African Americans compared to Caucasians (McLeod, Fang, Luo, Scott, & Evans, 1994). However, the differences in our findings for Caucasians when compared to the total sample could also be explained by other factors such as random errors due to sampling or the sampling distributions for the two different sample sizes.

The present study had several limitations. First, while our sample size is larger than those that prior researchers have reported for studies on the genetics of pain sensitivity (Kolesnikov, Gabovits, Levin, Voiko, & Veske, 2011; Landau et al., 2013), it is still relatively small for a general genetic study of a common phenotype. However, even with this small sample size, it appears that significant associations may exist. Second, the multiple testing we conducted may increase the probability of a Type I error. Third, three of the *COMT* SNPs were out of HWE, which may affect the generalizability of our findings. It is possible that the deviation from HWE was due to the distinctive characteristics of our sample, which may not be completely representative of the general population. We tested for HWE for an SNP (rs7948340) that is unrelated to pain/injury among our sample and found that it did not significantly deviate from HWE. Despite violations of HWE, *COMT* SNPs are genetically related (i.e., in strong LD) and they collectively influence the biological process of pain. Finally, we did not include other *OPRM1* SNPs such as C17 T in the analysis because we found no homozygous genotype of rare alleles due to our small sample size.

Conclusions

The present study was the first to investigate the interaction effects of four *COMT* SNPs (rs6269, rs4633, rs4818, and rs4680) and their haplotypes and diplotypes with *OPRM1* A118G on postoperative pain and opioid consumption. Our findings do not provide confirmatory evidence of the existence

of significant interaction effects on these outcomes; however, they do provide initial information that improves our current understanding of the genetic contributions to pain and suggests that *OPRM1* $\times$ *COMT* may contribute to variability in post-operative pain and response to opioids. Future studies with larger sample sizes are needed to confirm our findings. Ultimately, these findings may help to establish personalized pain management that allows clinicians to achieve effective pain control and minimize the risk of opioids' adverse effects.

Declaration of Conflicting Interests

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References

- Abraham, J. E., Maranian, M. J., Spiteri, I., Russell, R., Ingle, S., Luccarini, C., ... Caldas, C. (2012). Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. *BMC Medical Genomics*, 5, 19.
- Barrett, J. C., Fry, B., Maller, J., & Daly, M. J. (2005). Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics*, 21, 263–265.
- Berthele, A., Platzer, S., Jochim, B., Boecker, H., Buettner, A., Conrad, B., ... Toelle, T. R. (2005). *COMT* Val108/158Met genotype affects the mu-opioid receptor system in the human brain: Evidence from ligand-binding, G-protein activation and preproenkephalin mRNA expression. *Neuroimage*, 28, 185–193.
- Beuten, J., Payne, T. J., Ma, J. Z., & Li, M. D. (2006). Significant association of catechol-O-methyltransferase (*COMT*) haplotypes with nicotine dependence in male and female smokers of two ethnic populations. *Neuropsychopharmacology*, 31, 675–684.
- De Gregori, M., Garbin, G., De Gregori, S., Minella, C. E., Bugada, D., Lisa, A., ... Ranzani, G. N. (2013). Genetic variability at *COMT* but not at *OPRM1* and *UGT2B7* loci modulates morphine analgesic response in acute postoperative pain. *European Journal of Clinical Pharmacology*, 69, 1651–1658.
- Diatchenko, L., Nackley, A. G., Slade, G. D., Bhalang, K., Belfer, I., Max, M. B., ... Maixner, W. (2006). Catechol-o-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. *Pain*, 125, 216–224.
- Diatchenko, L., Slade, G. D., Nackley, A. G., Bhalang, K., Sigurdsson, A., Belfer, I., ... Maixner, W. (2005). Genetic basis for individual variations in pain perception and the development of a chronic pain condition. *Human Molecular Genetics*, 14, 135–143.
- Gabriel, S. B., Schaffner, S. F., Nguyen, H., Moore, J. M., Roy, J., Blumenstiel, B., ... Altshuler, D. (2002). The structure of haplotype blocks in the human genome. *Science*, 296, 2225–2229.
- Harsoor, S. (2011). Emerging concepts in post-operative pain management. *Indian Journal of Anaesthesia*, 55, 101–103.
- Hastie, B. A., Riley, J. L., III, Kaplan, L., Herrera, D. G., Campbell, C. M., Virtusio, K., ... Fillingim, R. B. (2012). Ethnicity interacts with the *OPRM1* gene in experimental pain sensitivity. *Pain*, 153, 1610–1619.
- Henker, R. A., Lewis, A., Dai, F., Lariviere, W. R., Meng, L., Gruen, G. S., ... Conley, Y. P. (2013). The associations between *OPRM1* and *COMT* genotypes and postoperative pain, opioid use, and opioid-induced sedation. *Biological Research for Nursing*, 15, 309–317.
- Hwang, I. C., Park, J. Y., Myung, S. K., Ahn, H. Y., Fukuda, K., & Liao, Q. (2014). *OPRM1* A118G gene variant and postoperative opioid requirement: A systematic review and meta-analysis. *Anesthesiology*, 121, 825–834.
- Kolesnikov, Y., Gabovits, B., Levin, A., Voiko, E., & Veske, A. (2011). Combined catechol-O-methyltransferase and mu-opioid receptor gene polymorphisms affect morphine postoperative analgesia and central side effects. *Anesthesia and Analgesia*, 112, 448–453.
- Kowarik, M. C., Einhauser, J., Jochim, B., Buttner, A., Tolle, T. R., Riemenschneider, M., ... Berthele, A. (2012). Impact of the *COMT* Val(108/158)Met polymorphism on the mu-opioid receptor system in the human brain: Mu-opioid receptor, met-enkephalin and beta-endorphin expression. *Neuroscience Letters*, 506, 214–219.
- Landau, R., Liu, S. K., Blouin, J. L., & Carvalho, B. (2013). The effect of *OPRM1* and *COMT* genotypes on the analgesic response to intravenous fentanyl labor analgesia. *Anesthesia and Analgesia*, 116, 386–391.
- McLeod, H. L., Fang, L., Luo, X., Scott, E. P., & Evans, W. E. (1994). Ethnic differences in erythrocyte catechol-O-methyltransferase activity in Black and White Americans. *Journal of Pharmacology and Experimental Therapeutics*, 270, 26–29.
- Nackley, A. G., Shabalina, S. A., Tchivileva, I. E., Satterfield, K., Korchynski, O., Makarov, S. S., ... Diatchenko, L. (2006). Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science*, 314, 1930–1933.
- National Pharmaceutical Council & Joint Commission on Accreditation of Healthcare Organizations. (2006). *Pain: Current understanding of assessment, management, and treatments*. Washington, DC: National Pharmaceutical Council.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., ... Sham, P. C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81, 559–575.
- Ren, Z. Y., Xu, X. Q., Bao, Y. P., He, J., Shi, L., Deng, J. H., ... Lu, L. (2015). The impact of genetic variation on sensitivity to opioid analgesics in patients with postoperative pain: A systematic review and meta-analysis. *Pain Physician*, 18, 131–152.
- Reyes-Gibby, C. C., Shete, S., Rakvag, T., Bhat, S. V., Skorpen, F., Bruera, E., ... Klepstad, P. (2007). Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: *OPRM1* and *COMT* gene. *Pain*, 130, 25–30.
- Sia, A. T., Lim, Y., Lim, E. C., Ocampo, C. E., Lim, W. Y., Cheong, P., & Tan, E. C. (2013). Influence of mu-opioid receptor variant

- on morphine use and self-rated pain following abdominal hysterectomy. *Journal of Pain*, 14, 1045–1052. doi:10.1016/j.jpain.2013.03.008
- Tan, E. C., Lim, E. C., Teo, Y. Y., Lim, Y., Law, H. Y., & Sia, A. T. (2009). Ethnicity and OPRM variant independently predict pain perception and patient-controlled analgesia usage for postoperative pain. *Molecular Pain*, 5, 32. doi:10.1186/1744-8069-5-32
- Tremblay, J., & Hamet, P. (2010). Genetics of pain, opioids, and opioid responsiveness. *Metabolism*, 59, S5–S8.
- Yao, P., Ding, Y. Y., Wang, Z. B., Ma, J. M., Hong, T., & Pan, S. N. (2015). Effect of gene polymorphism of *COMT* and *OPRM1* on the preoperative pain sensitivity in patients with cancer. *International Journal of Clinical and Experimental Medicine*, 8, 10036–10039.
- Zhang, F., Tong, J., Hu, J., Zhang, H., Ouyang, W., Huang, D., . . . Liao, Q. (2014). *COMT* gene haplotypes are closely associated with postoperative fentanyl dose in patients. *Anesthesia and Analgesia*, 120, 933–940.
- Zhang, S., Li, S., & Tan, X. (2013). [Human micro-opioid receptor A118G polymorphism affects epidural patient-controlled analgesia with fentanyl]. *Nan Fang Yi Ke Da Xue Xue Bao*, 33, 309–311.
- Zhang, Y., Wang, D., Johnson, A. D., Papp, A. C., & Sadee, W. (2005). Allelic expression imbalance of human mu opioid receptor (*OPRM1*) caused by variant A118G. *Journal of Biological Chemistry*, 280, 32618–32624.
- Zubieta, J. K., Heitzeg, M. M., Smith, Y. R., Bueller, J. A., Xu, K., Xu, Y., . . . Goldman, D. (2003). *COMT* val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science*, 299, 1240–1243. doi:10.1126/science.1078546299/5610/1240