Published in final edited form as:

Biol Res Nurs. 2013 July ; 15(3): . doi:10.1177/1099800411436171.

The association between *OPRM1* and *COMT* genotypes and postoperative pain, opioid use, and opioid-induced sedation

Richard A. Henker, PhD, CRNA, FAAN¹, Allison Lewis, BSN, RN², Feng Dai, PhD^{3,4}, William R. Lariviere, PhD³, Li Meng, MD³, Gary S. Gruen, MD⁵, Susan M. Sereika, PhD⁶, Hans Pape, MD⁵, Ivan S. Tarkin, MD⁵, Indira Gowda, RN, BSN⁷, and Yvette P. Conley, PhD⁸

Richard A. Henker: rhe001@pitt.edu; Allison Lewis: abd17@pitt.edu; Feng Dai: fed@pitt.edu; William R. Lariviere: wr12@pitt.edu; Li Meng: limeng@pitt.edu; Gary S. Gruen: ggrien@pitt.edu; Susan M. Sereika: ssereika@pitt.edu; Hans Pape: hcpape@upmc.edu; Ivan S. Tarkin: ist2@pitt.edu; Indira Gowda: indira.gowda@gmail.com; Yvette P. Conley: yconley@pitt.edu

¹Department of Acute/Tertiary Care, University of Pittsburgh School of Nursing

²Department of Nursing, University of Pittsburgh Medical Center

³Department of Anesthesiology, University of Pittsburgh School of Medicine

⁴Yale Center for Analytical Sciences, Yale University School of Public Health

⁵Department of Orthopedic Surgery, University of Pittsburgh School of Medicine

⁶Department of Health and Community Systems, University of Pittsburgh School of Nursing

⁷School of Medicine, University of North Carolina

⁸Department of Health Promotion & Development, University of Pittsburgh School of Nursing

Abstract

Background—Previous studies have associated mu-opioid receptor (*OPRM1*) genotype with pain and analgesia responses in postoperative and patient populations. This study investigates the role of catechol-o-methyltransferase (*COMT*) and *OPRM1* genotypes in acute postoperative pain scores, opioid use, and opioid-induced sedation after surgical procedures for orthopedic trauma in an otherwise healthy patient population.

Methods—Verbal pain / sedation scores, opioid use, and physiologic responses in the immediate postoperative period were examined for association with genetic variants in Caucasians genotyped for *OPRM1* single nucleotide polymorphisms (SNPs) A118G and C17T, and *COMT* SNPs.

Corresponding and Archival Author: Richard A. Henker, PhD CRNA FAAN, 336 Victoria Building, School of Nursing, University of Pittsburgh, Pittsburgh PA 15261, Office: (412)624-3816, Fax: (412)383-7227, rhe001@pitt.edu.

Richard A. Henker, data conduct of study, manuscript preparation

Allison Lewis, data collection

Feng Dai, data analysis, manuscript preparation

William R. Lariviere, manuscript preparation

Li Meng, conduct of study

Gary S. Gruen, conduct of study

Susan M. Sereika, study design

Hans Pape, edu, conduct of study

Ivan S. Tarkin, conduct of study

Indira Gowda, data collection

Yvette P. Conley, study design, manuscript preparation

Results—*OPRM1* A118G genotype was associated with patients' postoperative numerical pain scale (NPS) ratings at 15 min in the postanesthesia care unit (PACU) ($P=0.01$) and patients' sedation scores at 15 min in the PACU ($P=0.02$). *COMT* genotype (rs4818) was associated with opioid consumption in the first 45 min in the PACU ($P=0.04$). NPS ratings at 45 min were also higher in the group of patients with A/A genotype of rs4680 than in patients with the other two genotypes at this SNP ($P=0.03$). Our haplotype trend analysis identified a *COMT* haplotype 'GCGG' significantly associated with NPS at 15 min ($P=0.0013$), amount of opioids consumed in the first 45 min ($P=0.0024$), and heart rate at 45 min in PACU ($P=0.017$).

Conclusions—The results indicate that genetic variations in *COMT* contribute to the acute postoperative pain and analgesia responses and physiologic responses in this group of otherwise healthy postoperative orthopedic trauma patients.

Keywords

OPRM1; *COMT*; pain; opioid use; sedation

Introduction

Factors associated with variability in responses to opioids include age, (Likar et al., 2008) gender, (Cepeda & Carr, 2003) and previous opioid use (Fillingim et al., 2005; Patanwala, Jarzyna, Miller, & Erstad, 2008; Paulson, Minoshima, Morrow, & Casey, 1998). Genetic variants have been suggested as an additional factor contributing to individual variability in responses to opioids (Chou, Wang et al., 2006; Chou, Yang et al., 2006; Reyes-Gibb et al., 2007). Variability in genotypes for the mu opioid receptor gene *OPRM1* on chromosome 6q 24–25 has been evaluated to determine if single nucleotide polymorphisms (SNPs) account for individual differences in postoperative pain. Patients undergoing abdominal hysterectomy (Chou, Wang et al.), Cesarean section, (Sia et al., 2008), or total knee arthroplasty (Chou, Yang et al.) have been found to have differences in opioid use and pain scores associated with genotype for the A118G SNP of *OPRM1*. Other SNPs located in *OPRM1* such as C17T, T1793A, A1320G and C11T have been associated with substance dependence (Lotsch, Skarke, Liefhold, & Geisslinger, 2004) but have not been evaluated in postoperative patients for association with opioid requirements.

Genetic variants of the gene for catechol-O-methyltransferase, *COMT* on chromosome 22q11.2 have also been identified as contributing to the variability in pain and responses to opioids. Kim, Lee, Rowan, Brahim, and Dionne (2006) found an association between a *COMT* SNP rs740603 and maximum postoperative pain rating. In addition, the *COMT* Val158Met polymorphism (rs4680) has been associated with greater opioid requirements in cancer patients (Rakvag et al., 2008; Reyes-Gibb et al.). Haplotype analysis of *COMT* SNPs, rs5746849, rs740603, rs6269, rs2239393, and rs4818 by Rakvag et al. has shown that in Caucasian population carriers of the most frequent haplotype needed less morphine than non-carriers; however, no significant differences in patient symptoms such as pain, fatigue and nausea, were found between carriers and non-carriers.

One of the concerns when giving opioids particularly to the opioid naïve patient is opioid-induced sedation (OIS). Lentschener and colleagues (Lentschener, Tostivint, White, Gentili, & Ozier, 2007) found a high incidence of OIS in postoperative patients. OIS is a considerable concern because it is a precursor to opioid induced respiratory depression and few risk factors for OIS have been identified (Pattinson, 2008). Recent studies by Chou and colleagues (Chou, Wang et al.; Chou, Yang et al.) did not find an association between the *OPRM1* A118G SNP and sedation scores.

Patients under the effects of anesthesia cannot provide an indication of pain to anesthesia providers. Instead, pain is assessed by evaluating autonomic nervous system responses of heart rate and respiratory rate during surgery. Identification of genotypes that help predict postoperative pain and OIS prior to surgical intervention would improve and provide safer treatment of postoperative pain. The purpose of this pilot study was to explore the relationship of *OPRM1* and *COMT* genotypes with postoperative pain reports, opioid use and OIS in the immediate postoperative period in patients after surgical procedures for orthopedic trauma.

Methods

Subjects

After institutional review board approval and written informed consent was obtained from subjects, we prospectively enrolled 79 Caucasian adult subjects having surgery for isolated orthopedic trauma of the upper or lower extremities. Patients' demographic data including gender, race, ethnicity, age, weight, smoking, anatomic site of repair, length of surgical time, length of operating room time and PACU time were collected. Subjects were excluded for multiple fracture sites, other traumatic injuries (e.g., head, thoracic, abdominal), opioid use 6 months prior to injury, depression, neuropathy, liver disease, kidney disease, recreational drug use in the last 6 months, or a history of alcohol abuse (consistently greater than 3 drinks/day).

Anesthesia Management

Subjects received either general anesthesia alone (n = 66) or general anesthesia with regional anesthesia (n = 13). Regional techniques included sciatic (n = 9), lumbar plexus (n = 2), combination sciatic, femoral and popliteal fossa blocks (n = 1) and one unknown. Subjects received 2–4 mg midazolam prior to anesthesia induction. For induction, patients usually received propofol although some received sodium thiopental. Muscle relaxants used during induction included succinylcholine and/or rocuronium. Inhaled anesthetic agents used during the maintenance phase included isoflurane, sevoflurane, or desflurane.

Pain Assessment

Pain was assessed using an 11-point verbal response numeric pain scale (NPS) with ratings from 0 ("no pain") to 10 ("pain as bad as I can imagine") Subjects were taught how to use the scale prior to surgery and subjects were asked to verbally report a pain score in the preoperative holding area, and then within the first 15 min and at 45 min after arrival in the PACU. Inter rater verification of scoring was completed on the first 3–5 patients by data collectors.

Opioid Use

Opioids administered during the first 45 min after admission to the PACU included fentanyl, hydromorphone, morphine, and meperidine. Time and amount of opioids administered during the first 45 min in the PACU were recorded from the PACU record. If the subject received opioids via patient controlled analgesia, study personnel recorded the amount the patient received in the first 45 min in the PACU. The amount of opioid administered was converted to morphine equivalents. One hundred µg fentanyl was converted to 10 mg of morphine, hydromorphone 1.5 mg was converted to 10 mg of morphine, and 75 mg of meperidine was converted to 10mg of morphine (Berry, 2006). The amount of opioids received by patients is reported as the absolute amount of opioid and the amount of opioid per kg of body weight.

OIS measurement

Sedation scores were collected at 15 and 45 min after entering the PACU using the UPMC-Presby Sedation Scale. In this scale, 0 = none (alert), 1 = mild (occasionally drowsy, easy to arouse), 2 = moderate (frequently drowsy; easy to arouse), 3 = severe (somnolent; difficulty to arouse), S = sleep (normal sleep, easy to arouse), U = unable to assess. Nineteen subjects were missing sedation scores and were excluded from the genetic association analysis.

Heart Rate and Respiratory Rate

Heart rate (beats/minute) and respiratory rate (breaths/minute) were collected at 15 and 45 min of the patients' admission to the PACU. Heart rate was collected from the ECG rate on a Phillips monitor (Model MP50, Andover Maryland); respiratory rate was collected based on ECG electrode plethysmography. In practice, heart rate, blood pressure, and tachypnea are used as indicators of pain of patients by anesthesia providers.

Genotyping

Saliva samples were collected during the subject's hospital stay using the Oragene DNA self-collection kit from DNA Genotek Incorporated (Ottawa, Ontario, Canada). This collection kit contains a vial for saliva collection where upon closing of the vial the saliva mixes with a stabilization buffer. DNA was extracted from the saliva/buffer combination utilizing the protocol and reagents for extraction supplied with the Oragene kit.

Three polymorphisms within *OPRM1* were genotyped. These include A118G (rs1799971), G24A (rs1799973) and C17T (rs1799972). Results for only 2 of the polymorphisms are reported due to lack of variability for G24A in our sample. Because genotyping these polymorphisms is not amenable to certain genotype collection methodologies due to their close proximity to one another, the decision was made to genotype them using sequencing. This decision was facilitated by the fact that both variants are within 101 bp in exon 1 and therefore could be evaluated using one sequencing reaction. The forward primer (5'-TCAGTACCATGGACAGCAG-3') and reverse primer (5'-GGAGTAGAGGGCCATGAT-3') were used in a PCR reaction with an annealing temperature of 59°C. The PCR products were cleaned using exoSAP reagents (USBiochemicals, Cleveland, OH) and sequenced using the reverse primer and Big Dye Cycle Sequencing reagents (Applied Biosystems Inc., ABI, Foster City, CA). The sequencing products were electrophoresed using an ABI377 automated sequencer (ABI) and data viewed and genotypes assigned using Sequencer software (Gene Codes Corporation, Ann Arbor, MI).

Four SNPs, rs6269, rs4633, rs4818, and rs4680, were chosen to evaluate *COMT* based on previously identified haplotypes (Diatchenko et al., 2005). *COMT* genotypes have been proposed to affect opioid responses due to opioid effects on production of catecholamines in dopaminergic neurons in the brain (Furst, 1990; Zubieta et al., 1996). All *COMT* SNPs were genotyped using 5' exonuclease Assay-on-Demand TaqMan assays (ABI). Amplification and genotype assignments were conducted using the ABI7000 and SDS 2.0 software (ABI). For all genotyping conducted for this research, double-masked genotyping assignments were made for each variant, compared and each discrepancy addressed using raw data or by re-genotyping.

Statistical Analysis

Pain scores, heart rate, respiratory rate, sedation scores and opioid use in the immediate postoperative period were treated as dependent variables and assessed for normality using the Shapiro-Wilk test. Variables that deviated from this assumption were transformed into normal scores for association analysis.

Hardy-Weinberg equilibrium (HWE; e.g., both allele and genotype frequencies in a population remain constant) was assessed for all genotypes in all available samples using the exact test implemented in Plink (Purcell et al., 2007; Wigginton, Cutler, & Abecasis, 2005). The Haploview software (<http://www.broad.mit.edu/mpg/haploview>) was used to compute the pair-wise linkage disequilibrium (LD) between the selected four *COMT* SNPs measured by Lewontin's D (e.g., D/D_{max} , proportion of observed LD out of maximum possible LD) and to show LD block graphically (Barrett, Fry, Maller, & Daly, 2005). In a visually appealing and interactive interface, Haploview provides researchers a one-stop solution for common marker quality statistics, LD information, haplotype blocks (segments of strong LD within which there is little evidence for ancestral recombination events) and single marker association statistics in a user-friendly format.

Multiple linear regression analysis was used to evaluate the association of each SNP with a dependent variable under additive, dominant and recessive genetic models. In addition to each single SNP, effects of several covariates including age, gender, anesthesia plan (general vs. general with regional block), type of fracture, and smoking on the dependent variable were investigated using stepwise selection procedure, with a significance level (or P -value) of 0.05 to keep a covariate in the final regression model.

COMT haplotypes were constructed using the SAS/Genetics module (SAS version 9.2; Cary, NC, USA) that implements a Bayesian method that is fairly robust against deviations from HWE for genotypes. All haplotypes with less than 1% of haplotype frequency were first combined into a rare-haplotype category (unique haplotype $\geq 1\%$ itself treated as a single category). A haplotype trend regression (HTR) analysis, (Zaykin et al., 2002) in which each haplotype category was treated an independent predictor, was performed to model the effect of haplotypes on our traits of interest under additive, dominant and recessive models after adjustment for covariates using the same stepwise selection procedure in single SNP analysis.

Because this was intended as a pilot genetic association study, analyses were restricted to Caucasian patients that underwent surgical procedures for orthopedic trauma, and no formal correction for multiple testing was applied. Nevertheless, we discussed the potential effects of multiple testing adjustments accordingly when we tried to interpret and generalize our association results.

Results

Demographic and clinical characteristics of all study subjects are shown in Table 1. The sample consisted of 79 Caucasian subjects, 18 to 70 years of age (mean 39; standard deviation, SD 13) with mostly lower extremity fractures (ankle/tibia/fibula). Average surgical time for subjects was 124 min (SD = 58) and the average amount of time recovering in the PACU 129 min (SD = 66). Minor allele frequency (MAF), P -value of the HWE test, and total number of three different genotypes for every SNP are reported in Table 2. For the A118G (rs1799971) SNP in *OPRM1*, the MAF of allele G was 0.14 and there were two individuals in the GG group that were combined with the GA group ($n = 15$) and compared with the AA group ($n = 51$) in association analyses. For C17T (rs1799972), there was no individual with the homozygous genotype TT (MAF of T is 0.007), only one individual with the genotype TC was observed and another 67 individuals had homozygous genotype CC. Therefore, no comparison among the three genotype groups was performed for this SNP. Two *COMT* SNPs rs4633 and rs4680 were found to deviate from HWE with P -values 0.004 and 0.03, respectively. A systematic lab-review of raw genotype data did not identify problematic genotyping issues to cause the SNPs to be out of HWE.

Single marker analysis

The distribution of different measurements including NPS scores, sedation scores, and opioid consumptions for each genotype of *OPRM1* A118G rs1799971 and *COMT* rs6269, rs4633, rs4818 and rs4680 was plotted in Figure 1. Assuming a dominant genetic model of minor allele G (equivalent to a two-sample comparison of group means between the AA +AB group and the BB group in Figure 1), *OPRM1* rs1799971 was found modestly associated with patients' postoperative NPS pain ratings at 15 min upon arrival in PACU ($P = 0.01$), being adjusted for significant ($P < 0.05$) covariates age, anesthesia plan and weight. Assuming the same dominant model, rs1799971 was also modestly associated with patients' sedation score at 45 min ($P = 0.02$), being adjusted for covariate anesthesia plan. These were consistent to the data that the common homozygous genotype group (BB in Figure 1) appeared to have higher NPS scores at 15 min and lower sedation scores at 45 min than two other genotype groups that contain rare allele.

As shown in Figure 1, the *COMT* SNP rs4818 was modestly associated with the amount of opioids patients consumed in the PACU during the first 45 min ($P = 0.04$) after adjustment of significant covariates anesthesia plan and weight, assuming a recessive model for allele G. Patients who were G/G homozygotes for rs4818 consumed less opioids than patients in the other two genotypic groups. The patients' postoperative NPS at 15 and 45 min in the rare A/A genotype group of the non-synonymous rs4680 (Val158Met) were higher than those in the heterozygous A/G group and homozygous G/G group ($P = 0.05$ and 0.03 , respectively).

Haplotype analysis

Pairwise analysis by the Haploview software showed that three *COMT* SNPs, rs6269, rs4633, and rs4818, are in strong LD ($D > 0.80$) and they are in low LD with rs4680 (Figure 2). Although physical distance is a major factor for LD estimation, LD is also known affected by other factors such as sample size, allele frequency, type of polymorphism, and genomic location of markers (Zaykin et al., 2002). This might explain why four *COMT* SNPs, spanning only 1319 bp in physical distance, are not in a single LD block in the current study (Figure 2). One thing to note is that the Haploview software uses a two marker expectation-maximization (EM) to estimate the maximum-likelihood values of the four gamete frequencies to calculate D . Since two of four SNPs are not in HWE, the values of LD and structure of LD patterns shown in Figure 2 might not be accurate representations. That is why we later used a Bayesian method (implemented in SAS/Genetics) that is robust to HWE departure to construct the haplotypes for HTR analysis. In view of their close physical distance and as an attempt to replicate previous studies, we still inferred the haplotypes using all four SNPs together. Seven haplotypes with a frequency of greater than 2% were identified, representing 94.2% of all haplotypes observed in this study (Figure 2). Our HTR analysis identified a common haplotype 'GCGG' (frequency = 12.1%) that was significantly associated with patients' postoperative pain scores at 15 min ($P = 0.0013$), the amount of opioids patients consumed in the PACU for the first 45 min ($P = 0.0024$), and patients' postoperative heart rate for the first 45 min ($P = 0.017$). Carriers of one unit of this 'GCGG' haplotype was associated with an increase in pain score of 1.73, an increase of 2.01 mg in opioid consumption, and an increase of 1.46 (beats/minute) in heart rate, relative to the mean change for non-carriers in the entire study population.

Discussion

Published genetic studies of *COMT* and *OPRM1* focus on their possible associations with pain phenotypes (Diatchenko et al., 2006; Reyes-Gibb et al.). No previous study investigated the association between genetic variants of *COMT* with OIS in postoperative patients and published studies of *OPRM1* failed to detect association between OIS and *OPRM1*

polymorphisms (Chou, Wang et al.; Chou, Yang et al., 2006; Sia et al.). One novel finding of our study is that genetic variability of *OPRM1* SNP A118G (rs1799971) is associated with individual differences in OIS in the immediate postoperative recovery period (i.e., in PACU). Patients carrying AG and GG genotypes for rs1799971 showed lower sedation scores 15 min after admission to the PACU than patients with AA genotype, albeit only significantly prior to correction. This suggests that the minor G allele of rs1799971 may contribute to a decrease in sedation score in carriers, and thus merits further investigation in larger and additional postoperative patient populations.

Variability in the amount of post-operative opioid (e.g., intrathecal or PCA morphine within 24 hour) used in patients having undergone knee arthroplasty, abdominal hysterectomy, and Caesarean section has been shown to be associated with *OPRM1* A118G genotypes with GG genotype carriers generally consuming more opioid than GG non-carriers (Chou, Wang et al.; Chou, Yang et al.; Sia et al.). We did not detect an association between opioid consumption and A118G; however, we only considered opioid consumption up to 45 min in PACU, while other studies measured opioid consumption over 24 and 48 h. For example, Chou, Wang and colleagues showed GG carriers consumed more morphine at 24 h than non-carriers, but they did not identify a statistically significant difference for morphine consumption at 48 h. Therefore, our current finding cannot rule out the possibility of a potential association between A118G and opioid if we extended our measurement to 24 or 48 h as shown in their study. Another critical difference between the current study and previous studies is the higher frequency of the GG genotype for A118G in Asian populations (Chou, Wang et al.; Chou, Yang et al.; Sia et al.) compared to the Caucasian population investigated here. Our study had only 1 subject with the GG genotype for A118G compared to 11% and 23% of subjects in two separate studies by Chou, Wang and colleagues. Similar differences in genotype frequency by ancestry have also been reported in studies related to substance abuse (Tan et al., 2003).

The C17T SNP rs1799972 has been associated with substance abuse but to our knowledge has not been evaluated in postoperative patients. Variants for C17T are more frequently found in blacks or African Americans and are less frequently identified in Caucasians (Bond et al., 1998). We were not able to explore association with this SNP in the current study of only Caucasians because no individual had the TT genotype group and only 1 individual had the TC genotype. These findings further support that variants of C17T are infrequent in Caucasians.

The relationship of *COMT* genotypes with opioid use and variability in pain reports has been reported in an acute pain study, an experimental pain study in humans and in cancer patients (Rakvag et al.; Reyes-Gibb et al.). In those studies that evaluated *COMT* with pain in cancer patients there were significant differences in opioid requirements by rs4680 genotype. In addition, Rakvag and colleagues found that rs4818 genotype was also associated with variability in amounts of opioid use in Caucasian cancer patients with GG genotype carriers consuming more opioids. However, here we report the opposite trend as our GG homozygous group postoperative patients consumed less opioids than patients in the other two genotype groups.

Lotta et al. (Lotta et al., 1995) found that the individuals with the val (G)/val (G) genotype of rs4680 (Val158Met) tend to have the highest *COMT* enzyme activity (and lowest pain sensitivity), those with the met (A)/met (A) genotype have the lowest enzyme activity (and therefore the highest pain sensitivity), and heterozygous G/A individuals are intermediate. Our finding supports their findings by observing that patients in the A/A genotype group of rs4680 having the highest pain scores compared to scores in those patients in the heterozygous A/G group and homozygous G/G group.

We identified a common *COMT* haplotype ‘GCGG’ with a frequency of 12.1% and individuals carrying it have higher pain scores, higher opioid consumptions and higher heart rates than those not carrying it. This is in contrast to what was reported by Diatchenko and colleagues in which ‘GCGG’ (but with a higher frequency of 36.5%) was designated as the haplotype associated with ‘low pain sensitivity’. This inconsistency, however, is not without explanations. First, unlike the all female and mixed race population in the study of experimental pain sensitivity, the current study used only Caucasians and of both sexes. Second, there may be a critical difference between mechanisms of experimental pain and those of acute postoperative pain experienced after repair of orthopedic trauma in our study. Indeed, a previous study by Kim and colleagues also failed to associate the ‘GCGG’ haplotype with low pain sensitivity in patients following molar extraction. A recent finding from Tsao, Shabalina, Gauthier, Dokholyan, and Diatchenko (2011) may assist in clarifying these inconsistent findings given that the reduced enzyme activity of *COMT* that is driven by genetic variability of this gene may be compensated by increased mRNA translational efficiency.

There are some weaknesses that limit interpretation of results from this small pilot study. First, two of *COMT* SNPs were not in HWE, departure from which might suggest genotyping errors and population admixture. However, no problematic genotyping issues were identified in our systematic lab-review of raw genotype data. Caucasian patients were used in our study to alleviate the potential effects of the population admixture, although use of self-reported ancestry does not negate an admixture effect. Furthermore, it should be noted that although tests for HWE have been used to detect genotyping error and population admixture, those tests have relatively low power unless the sample size is large (Attia et al., 2010; Deng, Chen, & Recker, 2001). It is worth noting that deviation from HWE or HWD sometimes can actually indicate a valid gene-phenotype association in fine-scale mapping of mutations responsible for diseases like alcohol and drug dependence, and hereditary hemochromatosis (Feder et al., 1996; Jiang, Dong, Wang, & Sun, 2001; Lee, 2003). Therefore, it is highly possible that the uniqueness of our patient population (or “cases” in other fine-mapping studies) (Feder et al.; Jiang et al.) results in the HWD of these two *COMT* SNPs. In addition, our population may not represent the general population as patients that are involved in trauma often engage in risky behaviors whether they be athletics (Bovard, 2008), or while driving (Schermer et al., 2008). Schermer and colleagues identified non-alcohol related behaviors that are associated with traumatic injury such as tailgating, lack of seatbelt use, and absence of the use of a helmet when riding a motorcycle. Participation in high school and college sports has also been associated with risky behaviors that can lead to traumatic injury (Bovard).

Second, another major weakness results from the practical decision to include a number of different surgery, anesthesia drugs and administration techniques in this pilot study of small sample size. We tried to alleviate the problem by rigorously adjusting for effects of important clinical covariates on our dependent variable in the regression analysis. We fully acknowledge that a future larger and completely homogenous sample will allow for more detailed genotype-specific analyses and the requisite test of replicability of the current findings. Third, our study was limited to Caucasians, which might prevent generalization of our findings to other race populations. The fact that duration of patient follow-up was only 45 minutes limits our ability to investigate the effects of genetic markers on variability of OIS measures in postoperative patients beyond 45 minutes. Finally, several of our findings become non-significant after adjusting for multiple testings. However, the associations between ‘GCGG’ haplotype and patients’ pain scores ($P = 0.0013$) and opioid consumption ($P = 0.0024$) remain significant and represent a significant contribution to the understanding of heritable predisposition to postoperative pain and analgesia responses.

Significance and Nursing Practice

Opioids continue to be the primary treatment for pain in postoperative patients despite a wide range of responses among patients and multiple and even serious side effects of OIS and opioid induced respiratory depression. While the current results generally agree with previous studies of association between *OPRM1* polymorphisms and pain and analgesia, the results indicate that association with opioid-induced side-effects merit further investigation in postoperative patient populations. Although the current results of association of *COMT* genotype with postoperative pain, analgesia requirements and physiological responses require validation in additional populations, it suggests a role for this gene in the immediate postoperative period, and that different relationships with specific genotypes may exist across different types of patient or subject cohorts. This information is critical for the development of individualized treatment strategies and risk assessments.

Acknowledgments

Funding

This publication was made possible by the American Association of Nurse Anesthetist Foundation and Grant Number UL1 RR024153 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research, and a special grant from the Office of the Senior Vice Chancellor for the Health Sciences, University of Pittsburgh. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NCRR or NIH.

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Figure 1. Barplot of raw numerical pain scores and sedation scores for each genotype of *OPRM1* and *COMT* SNPs. A, minor allele; B, major allele. See table 2 for specific alleles for each SNP. *: $P < 0.05$

1	2	3	4	Frequency
A	T	C	G	27.9%
G	C	G	A	24.7%
A	T	C	A	14.3%
G	C	G	G	12.1%
A	C	C	G	6.5%
A	C	C	A	6.0%
G	T	G	G	2.7%
A	C	G	A	1.2%
x	x	x	x	4.6%

Figure 2.

Graphical representation of candidate SNPs and linkage disequilibrium (LD) structure of *COMT* gene. The numbers inside the squares are $100 \times |D'|$ varies between 0 (no disequilibrium) and 1 (maximum disequilibrium), represented by shades of white to light grey to dark grey. Color scheme: dark grey = strong evidence of LD; light grey = uninformative; white = strong evidence of recombination or weak LD. 'xxxx' indicates that no explicit alleles are depicted as the rare haplotype is pooled from several haplotypes with a frequency below 1%. Significantly associated haplotype 'GCGG' is highlighted BOLD and italic.

Table 1

Demographics and clinical characteristics of 79 study participants. Data are expressed as mean (SD) for continuous variables and n (percentage) for categorical variables.

Variable	Summary
Age (years)	39 (13)
Height (cm)	174 (17)
Weight (kg)	87 (17)
Surgical time (minutes)	124 (58)
Time in PACU (minutes)	129 (66)
Anesthesia plan	
General	66 (83.5%)
General & Regional	13 (16.5%)
Fracture type (frequency)	
Ankle	29 (36.7%)
Femur	11 (13.9%)
Tibial plateau	15 (19.0%)
Tibia-fibula	19 (24.1%)
Others (acetabular, humerus, radius, ulna, hip)	5 (6.3%)
Gender (frequency)	
Male	57 (72.2%)
Female	22 (27.8%)
Heart Rate after 15 minutes in the PACU (beats/minute)	87 (18)
Heart Rate after 45 minutes in PACU (beats/minute)	79 (15)
Respiratory Rate after 15 minutes in the PACU (breaths /minute)	16 (4)
Respiratory Rate after 45 minutes in the PACU (breaths / minute)	16 (4)
Numerical Pain Scale score after 15 minutes in the PACU	7 (3)
Numerical Pain Scale score after 45 minutes in the PACU	6 (3)
Amount of Opioids provided during the first 45 minutes in the PACU (mg)	6 (6)
Sedation score after 15 minutes in the PACU (n=60)	1 (1)
Sedation score after 45 minutes in the PACU (n=60)	1 (1)

Table 2

Details of six genotyped SNPs in the study

Gene	SNP	Chromosome	Position	Alleles		MAF ^a	Genotypes				HWE ^b
				A1	A2		A1/A1	A1/A2	A2/A2		
<i>OPRM1</i>	rs1799971	6	154402490	G	A	0.14	2	15	51	0.6	
	rs1799972	6	154402389	T	C	0.007	0	1	67	1	
<i>COMT</i>	rs6269	22	18329952	G	A	0.42	17	29	29	0.1	
	rs4633	22	18330235	C	T	0.48	22	23	25	0.004	
	rs4818	22	18331207	G	C	0.44	17	29	26	0.15	
	rs4680	22	18331271	A	G	0.49	22	27	24	0.03	

Note: A1: minor allele, A2: major allele.

^aMAF: minor allele frequency.

^bHardy-Weinberg equilibrium (HWE) test p-value.