OPRM1 and *COMT* Gene-Gene Interaction Effects on the Inter-individual Variability in Postoperative Pain and Response to Opioids

by

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OPRM1 and _COMT_ Gene-Gene Interaction Effects on the Inter-individual Variability in Postoperative Pain and Response to Opioids

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Background*:** Treatment of postoperative pain remains suboptimal. This is attributed, in part, to the individualized physiological and psychological perception of pain. Although the effect of genetic variation on pain is unequivocal, precise understating of the effect of multiple gene interaction is yet to be investigated. Catechol-O-methyltransferase (*COMT*) interacts with several neuroreceptors in the brain including the mu receptor (*OPRM1*). Hence, we sought to explore the gene-gene interaction effect of *OPRM1* and *COMT* on postoperative pain and opioid dose required for pain management. ***Methods*:** We used genotypes and clinical data for 153 postoperative orthopedic trauma patients. For the *COMT* gene four single nucleotide polymorphisms (rs6269, rs4633, rs4818 and rs4680), three haplotypes (ACCG, ATCA, and GCGG), and two diplotypes (low and high pain intensity) were considered for their interactions with A118G of *OPRM1* on postoperative pain and opioid consumption. Data were analyzed using descriptive statistics and multiple regression. Analyses were repeated including only the Caucasian subjects. ***Results: For postoperative opioid consumption; a significant interaction was found between *OPRM1* and *COMT* rs4680 (b=0.093, p=.037) and *OPRM1* and *COMT* rs4633 (b=0.097, p=.037). The interactions between *OPRM1* and *COMT* rs6269 and *OPRM1* and *COMT* diplotypes demonstrated trends toward significance (b=-0.075, p=.080 and b=0.071, p=.070, respectively). The results for *OPRM1*×*COMT* rs4680 and *OPRM1*×*COMT* rs4633 on opioid consumption were maintained even after restricting the analyses to only Caucasian

subjects. For postoperative pain scores, a significant interaction was found between *OPRM1* and the GCGG haplotype of *COMT* (*b*=-0.926, *p*=.017). The interaction between *OPRM1* and *COMT* rs4818 demonstrated a trend (*b*=-0.755, *p*=.060). When the sample was limited to only Caucasian subjects, only a trend was observed for the interaction effect of *OPRM1* and the GCGG haplotype of *COMT* on postoperative pain (*p*= .070). The interactions of *OPRM1*×ACCG haplotype and *OPRM1*×diplotypes of *COMT* also showed trends toward significance (*b*=1.110, *p*= .058 and *b*=0.831, *p*= .050, respectively). ***Conclusion*****:** *OPRM1*×*COMT* interactions may influence the variability in postoperative pain and response to opioids. Individualized pain management based on genetic variation can be an effective strategy to maximize the usefulness of pain management.

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PREFACE

First and foremost, thank you Allah for giving me the strength, determination and endurance to complete my PhD degree. Thank you Allah to have blessed me with the family I have. Mom and dad, what you have given me in this life is more valuable than I can express. Thank you for always supporting me and believing in me. Khalil, I could never thank you enough for all of the love, support and encouragement over the years. Yara, Maya and Furssan, you are my little angels. Thank you for the pure love and joy you have added to my life.

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1.0 INTRODUCTION

Pain is a subjective multidimensional human experience affecting millions of people annually in the United States (CDC, 2015). In spite of the development of new standards and guidelines for pain management and the recent evolution in the technology and pharmacology of postoperative analgesia, many patients still suffer pain after surgery; hence pain management protocols are currently insufficient. According to a national survey of 250 US adults who had undergone surgical procedures, approximately 80% of patients experienced acute postoperative pain, with most (86%) rating their pain as moderate, severe, or extreme (Apfelbaum, Chen, Mehta, & Gan, 2003). Experiencing postoperative pain was the most common concern (59%) of patients (Apfelbaum et al., 2003). Inadequate relief of postoperative pain may result in many harmful physiological, psychological and behavioral consequences that result in increased morbidity and mortality as well as health care costs (Caudill-Slosberg, Schwartz, & Woloshin, 2004; Feeney, 2004; Janssen, Spinhoven, & Arntz, 2004; Joshi & Ogunnaike, 2005).

A challenge of pain management results from the highly individualized effect of opioids which causes pain relief to vary among patients. Genetic variations have been suggested as a possible explanation for variation in pain intensity and in response to opioid treatment. Pain genetic studies have focused on single gene effects; however, pain treatment is suboptimal (Campa, Gioia, Tomei, Poli, & Barale, 2008; Chou, Wang, et al., 2006; Hayashida et al., 2008; Henker et al., 2013; Ross et al., 2008; Sia et al., 2008). Inconsistent results have been reported

from those studies investigating a single gene effect. One possible explanation for this would be ignoring other effects such as gene-gene interactions on pain and response to opioids. Recent evidence suggests that the interaction between two genes may have more impact on pain intensity and response to opioids than single gene (Kolesnikov, Gabovits, Levin, Voiko, & Veske, 2011; Reyes-Gibby et al., 2007).

Both *OPRM1* (mu opioid receptor) and *COMT* (Catechol-O-Methyltransferase; a catecholamine metabolizer) contribute to the neurotransmission pathways of pain within the brain and spinal cord. The A118G polymorphism of *OPRM1* has been shown to influence pain and opioid effect. The mutant G118 allele displays less mRNA expression and resultant protein level than A118 allele in brain tissues. It also has a structure alteration that affects its expression and translation into functional protein. This structural alteration translates into patients having G118 allele experiencing more pain and consuming higher opioid amount to achieve adequate pain control compared with those having the wild-type A118 allele (Chou, Yang, et al., 2006; Sia et al., 2008; Tan et al., 2009; Y. Zhang, Wang, Johnson, Papp, & Sadee, 2005). Val158Met is the most common SNP of *COMT*, in which the substitution of valine to methionine at codon 158 leads to a 3- to 4-fold decrease in *COMT* activity (Lotta et al., 1995). The decrease in the enzyme activity result in a reduction in pain threshold and an increase in pain score (Zubieta et al., 2003). Consistent with that, patients with homozygous Met were found to have more pain and consume less opioids compared with homozygous Val (Kolesnikov et al., 2011; Rakvag et al., 2005).

Physiologically, *COMT* interacts with several neuroreceptors, modifying function, in the brain including the mu opioid receptor (*OPRM1*). Cumulative evidence shows that *COMT* affects mu receptor (*OPRM1*) availability, 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). Thus, gene-gene interaction of *OPRM1* and *COMT* may influence the human experience of pain and explain greater variability in the individual differences compared to using either gene alone.

However, the interaction effect of *OPRM1* and *COMT* on postoperative pain and response to opioids is not well established. Findings of prior studies have not been replicated to confirm their results. Thus, gene-gene interaction of *OPRM1* and *COMT*, from both a statistical and biological perspective, need to be further investigated to establish the impact of this relationship on response to pain and pain management.

1.1 PURPOSE

The purpose of this study was to explore the effects of gene-gene interaction of *OPRM1* and *COMT* on the inter-individual variability in postoperative pain score and opioid consumption in postoperative orthopedic trauma patients.

1.2 SPECIFIC AIMS AND HYPOTHESES

1. Explore the gene-gene interaction effect of the *OPRM1* A118G SNP and *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680 or Val158Met), haplotypes and diplotypes on opioid dose required for pain management in postoperative orthopedic trauma patients.

Hypothesis 1a: Patients with the combination of G118 allele of *OPRM1* and Val158Val of *COMT* rs4680 will consume the largest opioid dose to control pain compared with other combinations.

Hypothesis 1b: Patients with the combination of A118 allele of *OPRM1* and Met158Met of *COMT* rs4680 will consume the lowest opioid dose to control pain compared with other combinations.

Hypothesis 1c: Patients with a combination of High Pain Sensitivity haplotype (HPS) or diplotype and G118 allele of *OPRM1* will consume the largest opioid dose to control pain compared with other combinations.

Hypothesis 1d: Patients with the combination of Low Pain Sensitivity haplotype (LPS) or diplotype and A118 allele of *OPRM1* will consume the lowest opioid dose to control that pain compared with other combinations.

2. Explore the gene-gene interaction effect of *OPRM1* A118G and *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680 or Val158Met), haplotypes and diplotypes on postoperative pain score in postoperative orthopedic trauma patients.

   Hypothesis 2a: Patients with the combination of the A118 allele of *OPRM1* and Val158Val genotype of *COMT* rs4680 will have the lowest pain score compared with other combinations.

   Hypothesis 2b: Patients with the combination of the G118 allele of *OPRM1* and Met158Met genotype of *COMT* rs4680 will have the highest pain score compared with other combinations.

Hypothesis 2c: Patients with combination of Low Pain Sensitivity haplotype (LPS) or diplotype and A118 allele of *OPRM1* will have the lowest pain score compared with other combinations.

Hypothesis 3d: Patients with the combination of High Pain Sensitivity haplotype (HPS) or diplotype and G118 allele of *OPRM1* will have the highest pain score compared with other combinations.

2.0 BACKGROUND AND SIGNIFICANCE

2.1 PAIN

Pain is one of the oldest medical problems. Tracking the history of pain over the ages revealed the debate and uncertainty about the nature of pain. The human definition of pain has evolved over time from being perceived as demons to a multidimensional neurological process that is the result of tissue damage (Bonica, 1991; Rey, 1993).

In ancient times when pain was considered as evil, demons or magic. Pain was treated with magical ceremonies, rattles gongs and other devices which were believed to frighten painful devils out of a person's body (Bonica, 1991; Sabatowski, Schafer, Kasper, Brunsch, & Radbruch, 2004). Later in Europe, religion influenced pain perception. Pain was considered a sacrificial offering that brought faithful believers closer to Christ. Therefore grieving pain and mourning were considered as practice to comfort and heal the pain (Sabatowski et al., 2004). The first scientific interpretation of pain was during the 5th century, when Hippocrates related the cause of pain to an imbalance of the four humors (blood, phlegm, yellow and black bile) (Bonica, 1991; Rey, 1993; Sabatowski et al., 2004). The tremendous scientific experiments and research that took place in the 20th century led to significant discoveries in the area of pain. Numerous pain concepts and theories have been developed to explain the complexity of pain. The most famous

pain theories are; specificity theory, pattern or intensity theory, and gate-control theory (Bonica, 1991; Dallenbach, 1939; Melzack & Wall, 1965; Rey, 1993; Sabatowski et al., 2004).

The “Gate-Control-Theory” combined for the first time the physiological as well as the psychological aspects of pain perception. The theory indicated a hypothetical gate within the spinal cord that controls our experience of pain. The hypothetical neurological gate opens and closes to control the traveling of pain signals to the brain. There are two type of signals passing through this gate; small nerve fibers (pain pathway fibers) and large fibers (sensory and touch neural pathways). The gate opens by activation of pain fiber and thus stimulating the sense of pain. However, the gate could be closed by stimulation of the sensory pathway with higher volume and intensity than the pain signals which prevent pain signals to transmit to the brain and consequently inhibit pain perception and vice a versa (Melzack & Wall, 1965). Accordingly, Melzack & Wall (1965) were the first to explain how the emotional and behavioral factors could increase or decrease the sensitivity of pain perception (Melzack & Wall, 1965).

Pain is now considered as a complex neurological process that could be categorized into different types (nociceptive, neuropathic, and psychogenic), each with distinct etiology, physiology, and remedy. Nociceptive Pain (acute pain) pathway involves the four processes of sensory neuronal transduction, transmission, perception and modulation; injured tissues release sensitizing chemicals (prostaglandins, bradykinin, substance P,..., etc.) that activate the peripheral nociceptors and lead to action potential generation. Action potentials transmit from site of injury to the spinal cord then continue to the brainstem, thalamus and finally to the cortex where pain processing and perception occurs. In the modulation phase the body releases substances such as endogenous opioids which bind to opioid receptors to inhibit nociceptive impulses (Brunton, 2011).

Opioids are one of the most potent drugs used to alleviate moderate to severe pain. All pharmacological effects of opioids are induced by activation of opioid receptors (mu, delta, and kappa). Opioid receptors are 7- transmembrane G-protein-coupled receptors (GPCRs) located synaptically and postsynaptically along pain transmission pathways (Brunton, 2011). By binding with those receptors, opioids cause hyperpolarization of nerve cells, inhibition of action potential transmission and inhibition of presynaptic neurotransmitter release, making the cellular pain signal less likely to reach upper the brain regions where pain perception occurs (Brunton, 2011). Most currently used opioid analgesics act mainly at mu opioid receptors so from the clinical perspective mu receptors are the most important (Matthes et al., 1996).

2.2 CONSEQUENCES OF UNTREATED POSTOPERATIVE PAIN

Up to 75% of surgical patients in the US complain of inadequate pain relief after surgery (Wu & Raja, 2011). Inadequate relief of postoperative pain may result in harmful physiologic consequences including; increased risk for myocardial ischemia and infarction, impaired immunity and increased postoperative pulmonary infection, reduced intestinal motility causing nausea and vomiting, urinary retention, increased deep vein thrombosis, and thromboembolism (Joshi & Ogunnaike, 2005). Inadequate relief of postoperative pain may also result in exacerbation of acute nociceptive pain, hyperalgesia and consequently the development of persistent chronic pain and disability after surgery (Joshi & Ogunnaike, 2005; Kehlet, Jensen, & Woolf, 2006). Moreover, evidence shows that inadequately treated pain also results in many psychological and behavioral consequences including; anxiety, anger, and exacerbation of physiologic and emotional responses (Feeney, 2004; Janssen et al., 2004).

2.3 FACTORS AFFECTING POSTOPERATIVE PAIN PERCEPTION AND RESPONSE TO OPIOIDS

Pain is a multidimensional experience. Age, gender and ethnicity are well established factors influencing pain perception and pain treatment (Aubrun, Salvi, Coriat, & Riou, 2005; Cepeda & Carr, 2003; Cepeda et al., 2001). Factors, such as emotion, cognition, behavior, and culture are known to influence pain response. Therefore, several studies have been focused on the different factors which contribute to the large inter-individual variability in pain perception and response to opioids and have attempted to predict such a response to ensure better pain management. (Ip, Abrishami, Peng, Wong, & Chung, 2009; Sommer et al., 2010).

Preoperative pain, expected pain, surgical fear, and pain catastrophizing by subjects were found to be the most important predictive factors of postoperative pain (Sommer et al., 2010). A recent systematic review reveled that preexisting pain, anxiety, age, and type of surgery are the four most significant predictive factors for the intensity of postoperative pain. Furthermore, the type of surgery, age, and psychological distress were identified as the three most important predictive factors for postoperative analgesic consumption (Ip et al., 2009).

Genetic variations have been suggested as a possible explanation for variation in pain intensity and response to opioids. According to the Pain Genes Database (PGD), 300 candidate genes were identified as pain genes based on animal research (Lacroix-Fralish, Ledoux, & Mogil, 2007). *OPRM1* and *COMT* are the best studied genes associated with pain perception and response to opioids. Both contribute to the neurotransmission pathway of pain within brain and spinal cord (Shi, Cleeland, Klepstad, Miaskowski, & Pedersen, 2010).

2.4 MU OPIOID RECEPTOR; *OPRM1*

The mu receptor, a G protein-coupled receptor, is the primary site of action for endogenous and exogenous opioids (Bond et al., 1998; Zadina, Hackler, Ge, & Kastin, 1997). Mu receptors are mainly distributed in the spinal cord, brain stem, thalamus, and cortex. Mu receptors play a major role in opioid-induced analgesia, depression of respiration, stimulation of nausea and vomiting, and opioid effects on mood and reward (Sora et al., 2001).

OPRM1 is the gene that encodes the mu opioid receptor. Over one hundred polymorphisms of the *OPRM1* gene have been identified (Pasternak, 2010); A118G polymorphism is the most common single nucleotide polymorphism (SNP) of *OPRM1*. A118G is located in exon 1 of the gene in the chromosome 6q24–25 region (Wang et al., 1994); in this SNP asparagine is changed to aspartic acid at position 40 of the resultant gene product. This results in less mRNA expression in brain tissues of G allele carriers compared to A carriers. The G allele also results in a 10-fold decrease in brain tissue protein levels compared with other *OPRM1* variants (Figure 1) (Y. Zhang et al., 2005).

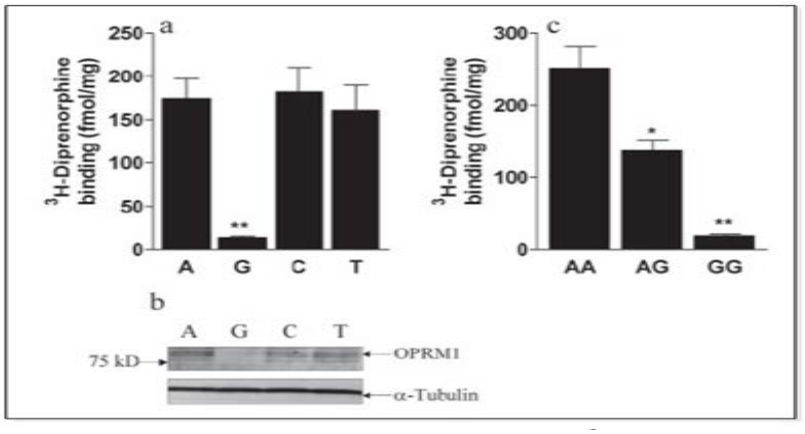


Figure 1*: OPRM1* expression in brain; tested with [3H]diprenorphine binding and Western blotting
Figure 1 was originally published in the Journal of Biological Chemistry. Zhang et al. Allelic Expression Imbalance of Human mu Opioid Receptor (*OPRM1*) Caused by Variant A118G. 2005; VOL.280, pp. 32618-32624© the American Society for Biochemistry and Molecular Biology.

The G118 has a secondary structure alteration. First, G118 indicated a well predicted helix in the mRNA that does not exist in the other variant structures. Second, mRNA structures for *OPRM1* variants commonly contain a loop motif that does not appear in G118 structure (Figure 2). This structural alteration in G118 mRNA affects its expression and translation into functional protein (Y. Zhang et al., 2005).

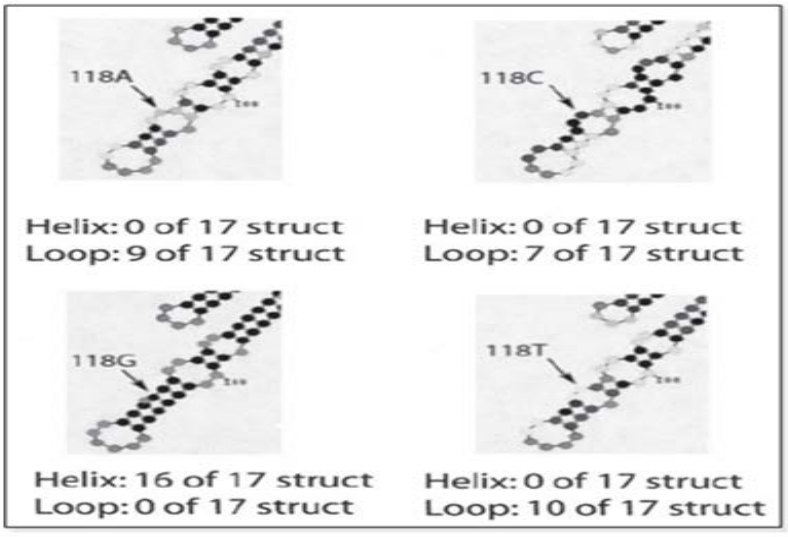


Figure 2: Secondary structures for 4 possible substitutions at position 118 in *OPRM1* mRNA
Figure 2 was originally published in the Journal of Biological Chemistry. Zhang et al. Allelic Expression Imbalance of Human mu Opioid Receptor (*OPRM1*) Caused by Variant A118G. 2005; VOL.280, pp. 32618-32624© the American Society for Biochemistry and Molecular Biology.

Given this, the functional effects of the A118G polymorphism have been demonstrated in the clinical setting in which the patients who are homozygous for the variant G experience more pain and need higher morphine doses to achieve adequate postoperative pain control compared with those who are homozygous for the wild-type A118 (Chou, Yang, et al., 2006; Sia et al., 2008; Tan et al., 2009).

2.5 CATECHOL-O-METHYLTRANSFERASE; *COMT*

Catechol-O-methyltransferase (*COMT*) is one of several enzymes responsible for metabolizing and degrading catecholamines, such as dopamine, epinephrine, and norepinephrine, which influence the opioids pathway. Catechol-O-methyltransferase is coded by the *COMT* gene which is located on chromosome 22 (22q11.21). *COMT* Val158Met is the most common SNP of *COMT*, in which the substitution of valine to methionine at position 158 lead to reduction in thermal stability of the *COMT* protein and a 3- to 4-fold decrease in the enzyme activity (Lotta et a l., 1995). Consequently, Met/Met genotype is associated with the lowest activity of *COMT* enzyme, followed by Met/Val and Val/Val genotype with the intermediate and the highest activities, respectively.

While it is well known that *COMT* influences pain sensitivity and perception, the exact underlying mechanism is still not well understood. It has been shown that the decrease in enzyme activity reduces the content of enkephalins in certain brain regions (Zubieta et al., 2003). Moreover, it affects opioid neurotransmitter pathways by increasing mu opioid receptors expression and density in the brain and decreasing their activation (Kowarik et al., 2012; Zubieta et al., 2003).

Another suggested mechanism is that the reduction in *COMT* activity leads to increased tonic dopamine (extrasynaptic) level. The high tonic dopamine level inhibits phasic dopamine (synaptic) which reduces postsynaptic D_2 receptors activation and descending inhibition, leading to a reduction in pain threshold and an increase in pain sensitivity (Zubieta et al., 2003). Others have suggested that the elevation in catecholamine levels caused by the inhibition in *COMT* enzyme activity leads to activation of β_2 and β_3 adrenergic receptors. Activation of these receptors also leads to increaseed pain sensitivity (Khasar, Green, Miao, & Levine, 2003; Nackley et al., 2006). In view of that, clinical studies have shown the patients with homozygous Met experience more pain compared with homozygous Val and heterozygous variant (Kolesnikov et al., 2011; Rakvag et al., 2005).

Other SNPs in the *COMT* gene were also found to be associated with pain sensitivity including rs6269, rs4633 and rs4818 (Lee, Delaney, Keogh, Sleeman, & Shorten, 2011; F. Zhang et al., 2014). rs6269 (A/G) is located in the promoter region of *COMT* gene, whereas rs4633 (C/T) and rs4818 (C/G) are located in the coding region at codons His62His and Leu136Leu, respectively. Although those SNPs are synonymous (not causing a change in the amino acid), they have a profound effect on maintaining mRNA secondary structure and mRNA stability (Nackley et al., 2006).

It has been found that postoperative patients who carried GG of rs6269 and GG of rs4818 experienced the lowest pain at rest and on movement after third molar (M3) extraction compared with patients homozygous for the common alleles (Lee et al., 2011). On the other hand, postoperative patients who carried AA of rs6269, TT of rs4633 and CC of rs4818 experienced lowest pain intensity before and one year after lumbar discectomy (Rut et al., 2014).

2.5.1 *COMT* haplotypes

Haplotype is a set of closely linked SNPs on the chromosome that are inherited as a unit. Diatchenko used the four *COMT* SNPs; rs6269, rs4633, rs4818, and rs4680 to define three major pain-sensitivity haplotypes in normal volunteers. The three *COMT* haplotypes were designated as low pain sensitivity (LPS; GCGG), average pain sensitivity (APS; ATCA) and high pain sensitivity (HPS; ACCG) (Diatchenko et al., 2005). *COMT* haplotypes vary with respect to mRNA secondary structures and subsequently the amount of translated protein. The HPS local stem-loop structure has a higher folding potential compared with LPS and APS haplotypes. Accordingly, HPS has longest, most stable structure associated with the lowest protein levels and enzymatic activity and thus the highest pain sensitivity (Nackley et al., 2006). Figure 4 (B) shows the local stem-loop structures associated with each of the three *COMT* haplotypes, (C and D) shows the enzymatic activity and protein levels in cells expressing *COMT*.

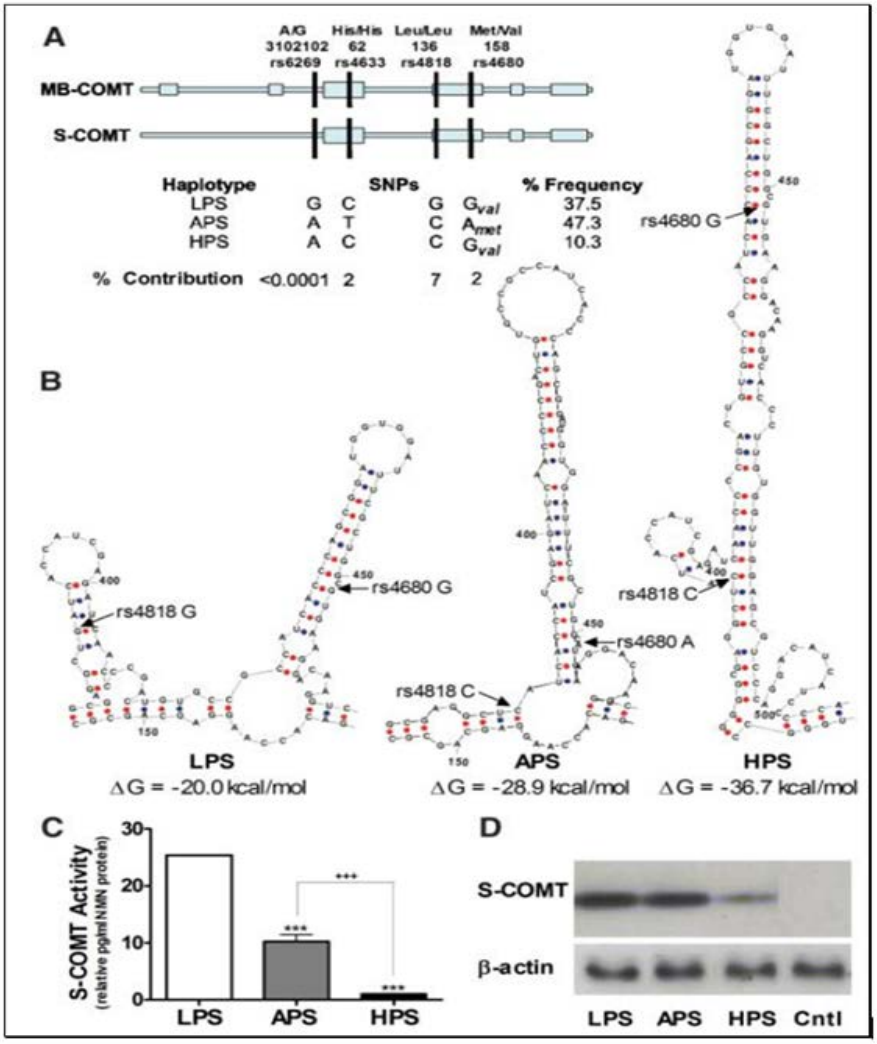


Figure 3: Common haplotypes of the human *COMT* gene (LPS, APS, and HPS) mRNA secondary structure and enzymatic activity

Figure 3 was originally published in the American Association for the Advancement of Science. AAAS. Nackley et al. Human Catechol-O-Methyltransferase Haplotypes Modulate Protein Expression by Altering mRNA Secondary Structure 2006; Vol. 314 no. 5807 pp. 1930-1933© AAAS.

Nackley and colleagues suggested that *COMT* haplotypes, rather than a single SNP, better account for the variability in pain sensitivity. The combinations of SNPs in haplotypes result in synergistic effects on protein function (Nackley et al., 2006). Therefore, the interaction of the Val158Met SNP with other known *COMT* SNPs have a greater effect on the mRNA structure, and subsequently the efficacy of protein translation and enzyme functions (Diatchenko et al., 2006; Diatchenko et al., 2005; Nackley et al., 2006). For instance, none of *COMT* SNPs

(rs6269, rs4633, rs4818 and rs4680) were associated with variable postoperative fentanyl consumption. However, *COMT* haplotypes constructed by those SNPs were significantly associated with fentanyl consumption at 24 and 48 hours after surgery (F. Zhang et al., 2014).

COMT diplotypes, combinations of haplotypes, were also found to be associated with pain sensitivity. Diatchenko et al. found that individuals with the HPS/APS diplotype were the most responsive to thermal painful stimuli and individuals with the LPS/LPS diplotype were the least responsive. However, *COMT* diplotypes were not associated with either mechanical or ischemic pain (Diatchenko et al., 2006). Moreover, George et al. found that *COMT* high pain sensitivity diplotype (APS/HPS, HPS/APS, HPS/HPS or APS/APS) and pain catastrophizing interactions were associated with higher pain intensity and greater risk of experiencing persistent pain following surgery compared with the low pain sensitivity diplotype (HPS/LPS, APS/LPS, LPS/LPS, or LPS/rare) (George et al., 2014).

2.6 *OPRM1* AND *COMT* MAIN EFFECTS; EVIDENCE AND GAPS IN PAIN LITERATURE

OPRM1 and *COMT* are considered key genes in predicting pain perception and opioid efficacy. Several genetic studies have been conducted to explore the association between the single gene effects of *OPRM1* or *COMT* on the variability in pain and response to opioids. However, overall contradictory results have been reported (Chou, Yang, et al., 2006; Fernández-de-las-Peñas et al., 2013; Janicki et al., 2006; Kambur et al., 2013; Rakvag et al., 2008; Sia et al., 2013).

Association studies investigating *OPRM1* A118G found that G118 allele patients have higher postoperative pain score than those having the A118 allele (Sia et al., 2008; Sia et al.,

2013; Tan et al., 2009; F. Zhang et al., 2013). However; other studies did not find any differences in the postoperative pain score among the A118 allele and G118 allele patients (Wang, et al., 2006; Chou, Yang, et al., 2006; Janicki et al., 2006; Kolesnikov et al., 2011; Liao et al., 2013). While some studies found that patients who are homozygous for the variant G need higher opioid doses to achieve adequate postoperative pain control compared with those who are homozygous for the wild-type A118 (Chou, Wang, et al., 2006; Hayashida, et al., 2008; Sia, et al., 2008; Tan, et al., 2009). Others did not find significant differences among *OPRM1* polymorphisms (Coulbault et al., 2006; Henker et al., 2013; Janicki et al., 2006; Kolesnikov et al., 2011). Table 1 summarizes studies investigating the association of *OPRM1* A118G polymorphisms with pain perception and response to opioids.

Table 1: Genetic association studies of *OPRM1* A118G polymorphism with pain and opioid consumption

Study	Country	n	Gender	Ethnic Group n	Medical condition	Phenotype	AA n(%)	AG n(%)	GG n(%)	Findings
Landau et al. (2004)	Mixed Genea, Switzerland. New York, USA.	181	Female=181	*Mixed* Caucasian 114 Hispanic 67 *Geneva* Caucasian 84(61%) Hispanic 53(39%) *New York* Caucasian 30(68%) Hispanic 14(32%)	Normal Birth Delivery	Genotype distribution	T=121 Geneva 89 (65%) New York 32 (72%) Caucasian 78 (68%) Hispanic 43 (64%)	T=52 Geneva 42 (31%) New York 10 (23%) Caucasian 31 (27%) Hispanic 21 (31%)	T=8 Geneva 6(4%) New York 2(5%) Caucasian 5(4%) Hispanic 3 (4%)	Describe genotype frequency among groups.
Klepstad et al. (2004)	Norway	99	Male=62 Female=37	Norwegian Caucasian	Cancer patients	Pain and morphine consumption	78(79%)	17(17%)	4(4%)	A118G patients have higher pain score compared with A118A or G118G. G118G patients consumed larger morphine dose compared with A118A or A118G.

Janicki et al. (2006)	USA	101	Male=23 Female=78	*Mixed* Caucasian 95(94%) Black 2 (2%) Hispanic 4 (4%)	laparoscopic abdominal surgery	Pain and morphine consumption	70 (69.2%)	30 (29.7%)	1 (1%)	No statistically significant differences among groups.
Coulbault et al. (2006)	France	74	Male =44 Female =30	Mixed Caucasian (70) Black (1) Biracial(black and Caucasian) (3)	abdominal surgery with a colorectal or coloanal anastomosis	Morphine consumption	57(77%)	15(20%)	2(3%)	No statistically significant differences among groups.
Chou et al. (2006)	Taiwan	120	Male=31 Female=89	Taiwanese	Total knee arthroplasty	Morphine consumption Pain	74(62%)	33(27%)	13(11%)	G118G patients consumed larger morphine dose compared with A118A or A118G. No significant differences in pain scores among groups.
Chou et al. (2006)	Taiwan	80	Female=80	Taiwanese	Total abdominal hysterectomy.	Morphine consumption	43(53.7%)	19(23.8%)	18(22.5%)	G118G patients consumed larger morphine dose

										compared with A118A.
Reyes-Gibby et al.(2007)	Norway	207	Male=116 Female=91	Caucasian	Cancer patients	Pain and morphine consumption	166(80%)	36(17%)	5(3%)	G118G patients consumed larger opioid dose compared with A118A. No significant differences in pain scores among groups.
Campa et al. (2008)	Italy	138	Male =62 Female =76	Italian from Caucasian origin	Cancer patients	Pain	106(77%)	22(16%)	10(7%)	G118 allele patients have higher pain score than A118 allele.
Sia et al. (2008)	Singapore	588	Female=588	Chinese Singaporean	Caesarian Section	Pain and morphine consumption	271(46%)	234(40%)	80(14%)	G118G patients have higher pain scores and consumed larger morphine doses compared with A118A.
Hayashida et al. (2008)	Japan	138	Male =79 Female =59	Japanese	Major open abdominal surgery	Morphine or Fentanyl consumption	41(29.7%)	70(50.7%)	27(19.6%)	G118G patients consumed larger opioid dose

										compared with A118A or A118G.
Tan et al. (2009)	Singapore	994	Female=994	*Mixed* Chinese 620(62.1%) Malays241 (24.1%) Indian137 (13.7%)	Caesarian Section	Pain and morphine consumption	389(39%)	435(44%)	170(17%)	G118G patients have higher pain scores and consumed larger morphine doses compared with A118A.
Kolesnikov et al. (2011)	Estonia	102	Male=45 Female=57	Estonian or Russian descent	Abdominal radical prostatectomy or hysterectomy	Pain and morphine consumption	80(80.3%)	17(16.8%)	3(2.9%)	No statistically significant differences among groups.
Henker et al. (2012)	United States	68	Male=57 Female=22	Caucasian	Surgical procedures for orthopedic trauma	Pain and morphine consumption	51(75%)	15(22%)	2(3%)	A118 allele patients have higher pain score than G118 allele. No statistically significant differences in morphine consumptiom among groups.
Landauet al. (2012)	United States	105	Female=105	*Mixed* Hispanic 39 (37%)	Labor	Analgesic success (Numerical	59 (60%)	34 (35%)	5 (5%)	No statistically significant

				Caucasian 33 (31%) Asian 26(25%) Other 7(7%)		Verbal Pain Scale score ≤ 10/100 15 minutes after the dose of fentanyl)				differences among groups.
Gong et al. (2013)	China	112	Male=75 Female=37	Chinese	Cancer patients	Opioid consumption	44(39%)	50(45%)	18(16%)	G118G and A118G patients consumed larger opioid dose compared with A118A
Liao et al. (2013)	China	97	Male=60 Female=37	Chinese	Radical gastrectomy	Pain and morphine consumption	42(43%)	41(42%)	14(15%)	No statistically significant differences among groups.
Zhang et al. (2013)	China	96	Female=96	Chinese	Caesarian Section	Pain and fentanyl consumption	35(36.5%)	45(46.9%)	16(16.7%)	G118G patients have higher pain score and consumed larger fentanyl doses compared with A118G or A118A.
Zhang et al. (2013)	China	128	Male=76 Female=52	Chinese	Radical gastrectomy	fentanyl consumption	54(42.2%)	53(41.4%)	21(16.4%)	No statistically significant differences

										among groups.
Sia et al. (2013)	Singapore	973	Female=973	*Mixed* Chinese 755(77.6%) Malays 136(14%) Asian Indian 82(8.4%)	Abdominal hysterectomy	Pain and morphine consumption	354(36.4%)	474(48.7%)	145(14.9%)	G118G patients have higher pain scores and consumed larger fentanyl doses compared with A118G or A118A.
Cajanus et al. (2014)	Finland	993	Female=993	Finns	Breast cancer surgery (breast resection or mastectomy with axillary surgery)	Pain and oxycodone consumption	631(63.5%)	327(32.9%)	35(3.5%)	G118 allele patients have higher pain score and consumed larger opioid dose than A118 allele.

n= Sample size included in the analysis , T= Total

Previous studies investigating the association between *COMT* Val158Met and postoperative pain and response to opioids have also had conflicting results (Ahlers et al., 2013; Henker et al., 2013; Ross et al., 2008; Rut et al., 2014). It has been found that patients with Met158Met genotype experience significantly more pain compared to those with Val158Val and Val158Met genotypes (Ahlers et al., 2013; Fernandez-de-las-Penas et al., 2012; Henker et al., 2013; Kolesnikov et al., 2011). However, others did not find this association (Kambur et al., 2013; Rakvag et al., 2005; Rakvag et al., 2008; Ross et al., 2008; Rut et al., 2014). In terms of opioid consumption, it has been reported that patients with Val158Val consume higher amounts of opioid compared to those with the Met158Met and Val158Met genotypes (Rakvag et al., 2008; Reyes-Gibby et al., 2007) However, others did not find significant differences among *COMT* polymorphisms (Kambur et al., 2013; Kolesnikov et al., 2011). Table 2 summarizes studies investigating the association of *COMT* Val158Met polymorphisms with pain perception and response to opioids.

In regard to *COMT* haplotypes, it has been found that patients having the ACCG haplotype reported higher postoperative pain and consumed more opioids than GCGG and ATCA (Rut et al., 2014; F. Zhang et al., 2014), whereas Henker et al. found that the common haplotype GCGG was associated with highest postoperative pain score and morphine consumption compared with other haplotypes in orthopedic trauma surgery patients (Henker et al., 2013). Kambur et al. did not find any association between the *COMT* haplotypes and pain sensitivity (Kambur et al., 2013)

Table 2: Genetic association studies of *COMT* Val158Met polymorphism with pain and opioid consumption

Study	Country	n	Gender	Ethnic Group n	Medical condition	Phenotype	Met/Met (AA) n(%)	Val/ Met(AG) n(%)	Val/Val GG n(%)	Findings
Rakvag et al. (2005)	Norway	207	Male=117 Female=90	Caucasians	Cancer patients	Pain and morphine consumption	67(32%)	96(47%)	44(21%)	Val158Val consumed larger morphine doses compared with Met158Val or Met158Met. No significant differences in the pain score amonge groups.
Kim et al. (2006)	United States	112	Male=52 Female=60	European Americans	Oral surgery	Pain	Met allele frequency (0.46) Val allele frequency (0.54)			No statistically significant differences among groups.
Lee et al. (2010)	Ireland	98	Male=39 Female=59	Caucasian of Irish ancestry.	Third Molar (M3) extraction	Pain	Not specified			No statistically significant differences among groups.
Kolesnikov et al. (2011)	Estonia	102	Male=45 Female=57	Estonian or Russian descent	Abdominal radical prostatectomy	Pain and morphine consumption	22(21.6%)	54(52.8%)	26(25.6%)	Met158Met patients have higher pain

					or hysterectomy					score compared with Val158Val. No significant differences in opioid consumptipon among groups.
Henker et al. (2012)	United States	73	Male=57 Female=22	Caucasian	Surgical procedures for orthopedic trauma	Pain and morphine consumption	22(30%)	27(37%)	24(33%)	Met158Met patients had higher pain scores and consumed larger morphine doses compared with Met158Val or Val158Val.
Fernandez-de-las-Penas et al. (2012)	Spain	128	Female=128	Caucasian	Cancer patients, post mastectomy	Post mastectomy pain	30(23%)	64(50%)	34(27%)	Met158Met patients had higher neck pain intensity compared with Val158Met or Val158Val.
Ahlers et al. (2013)	Netherlands	117	Male=85 Female=32	*Mixed* Caucasian=115 African American=1 Asian=1	Cardiac procedure	pain	21(18%)	66(56%)	30(26%)	Met158Met patients had higher pain score during cardiac

										procedure compared with Met158Val or Val158Val.
Kambur et al. (2013)	Finland	1000	Female=1000	Finns	Cancer patients, post mastectomy or breast conserving surgery	Pain oxycodone consumption	Met allele frequency (0.543) Val allele frequency (0.457)			No statistically significant differences among groups.
Fernandez-de-las-Penas et al. (2013)	Spain	109	Female=109	Caucasian	Carpal Tunnel Syndrome	Pain	23(21%)	46(42%)	40(37%)	Met158Met patients had higher pain scores compared with Val158Met or Val158Val.

The inconsistent findings from previous work on *OPRM1* and *COMT* have resulted in an insufficient understanding of the genetic basis of the individualized variability in pain perception and opioid responsiveness. Possible explanations for these inconsistencies include; first, heterogeneous samples. Although past research has revealed the role of ethnicity in pain perception and expression as well as response to pain treatment (Campbell & Edwards, 2012; Campbell, Edwards, & Fillingim, 2005; Cepeda et al., 2001), many studies included different racial/ethnic groups, e.g., subjects who were Caucasian, Black, Hispanic, and/or even biracial (Caucasian and Black) (Coulbault, et al., 2006; Janicki, et al., 2006). One study recruited a mixed sample of Chinese, Malays, and Indians (Tan, et al., 2009). Second, prior studies enrolled subjects with varied diagnoses and acuity. For instance, studies in cancer patients included lung, breast, and gastrointestinal cancer (Klepstad, et al., 2004; Rakvag, et al., 2005; Rakvag, et al., 2008; Ross, et al., 2008) or different surgical operations, e.g., abdominal radical prostatectomy or hysterectomy (Kolesnikov, et al., 2011), or varied procedures, e.g., gastric bypass, cholecystectomy, tubal ligation, diagnostic, and hernia repair (Janicki, et al., 2006). Third, the relatively small sample sizes in prior studies might not have the sufficient statistical power to adequately detect the differences among polymorphisms. In addition, the low frequency of the rare G118 allele in some of prior studies investigating *OPRM1* A118G might also reduce the statistical power. Finally, prior studies considered the effect of individual genes or haplotypes ignoring the possible effect of their interactions. Since single gene effects alone may not be responsible for all variations in pain and opioid responses, the interaction of various pain genes may ensure better explanation for wide variability in pain and response to opioid therapy (Kolesnikov et al., 2011; Reyes-Gibby et al., 2007).

2.7 GENE-GENE INTERACTIONS

The gene interaction definition takes into account both biological and statistical interactions. Although, there is no universally accepted definition of interaction in either biology or statistics, the frequently used biological definition of gene-gene interaction is the interaction between alleles at different loci. From a statistical standpoint, interactions represent the deviation from a statistical additive mode that describes how two or more variables predict a phenotypic outcome (X. Wang, Elston, & Zhu, 2010).

Gene-gene interactions play an important role in predicting complex pharmacologic treatment response and mechanisms (Lane, Tsai, & Lin, 2012). It has helped in predicting high risk individuals for drug under-treatment or over-treatment. Evidence has shown that gene-gene interaction has been significantly associated with treatment responsiveness of various drugs including; antidepressant drugs (Horstmann et al., 2010; Lin et al., 2009), antipsychotic drugs (Liou et al., 2012), IFNa and ribavirin for treating Chronic Hepatitis C (CHC) (Lin, Hwang, & Chen, 2007), Sibutramine for weight loss (Hsiao, Wu, Hwang, Huang, & Lin, 2010) , albuterol for asthma treatment (Choudhry et al., 2010; Corvol et al., 2009), and methotrexate for rheumatoid arthritis therapy (Sharma et al., 2008).

2.7.1 *OPRM1* and *COMT* gene-gene interaction

Both *OPRM1* and *COMT* contribute to the neurotransmission pathway of pain within the brain and spinal cord. Physiologically, *COMT* Val158Met interacts with several neuroreceptors, modifying function in the brain including the mu opioid receptor (*OPRM1*). Cumulative evidence shows that *COMT* Val158Met affects mu receptor (*OPRM1*) availability, expression

and density in brain tissue by affecting enkephalin levels, which inversely regulate mu receptor expression. Accordingly, the number of mu-opioid receptor binding sites is affected by *COMT* Val158 Met polymorphism such that *COMT* Met158 allele carriers that are significantly associated with the highest mu receptor expression and lowest enkephalin peptide levels compared with other *COMT* Val158 Met genotypes. (Berthele et al., 2005; Kowarik et al., 2012; Zubieta et al., 2003). The following sections briefly describe some of the evidences for the biological interaction between *COMT* and mu receptor (*OPRM1*);

COMT Val158Met has been shown to affect the number of binding sites of mu opioid receptors using Positron Emission Tomography (PET) and the radiotracer [11C] carfentanil, a mu receptor ligand. *COMT* Met158 homozygotes demonstrated the highest binding potential of [11C] carfentanil followed by heterozygotes then the Val158 homozygotes. The authors hypothesized that the high levels of dopamine cause by Met at position 158 in the *COMT* protein led to a reduction in enkephalin levels and consequently, mu receptor expression is upregulated (Zubieta et al., 2003).

Berthele et al. used another method, ligand binding autoradiography ([^{3}H] DAMGO receptor) in post-mortem tissue, to confirm the effect of *COMT* Val158Met polymorphism on mu receptor binding site expression in the human brain (Figure 4). It has been found that *COMT* Met158 allele was associated with a higher expression of mu binding sites in the caudate nucleus, the nucleus accumbens and the mediodorsal nucleus of the thalamus. Consistent with of Zubieta findings, the availability of mu receptor binding sites is affected by the Val158Met polymorphism of *COMT* gene (Berthele et al., 2005)

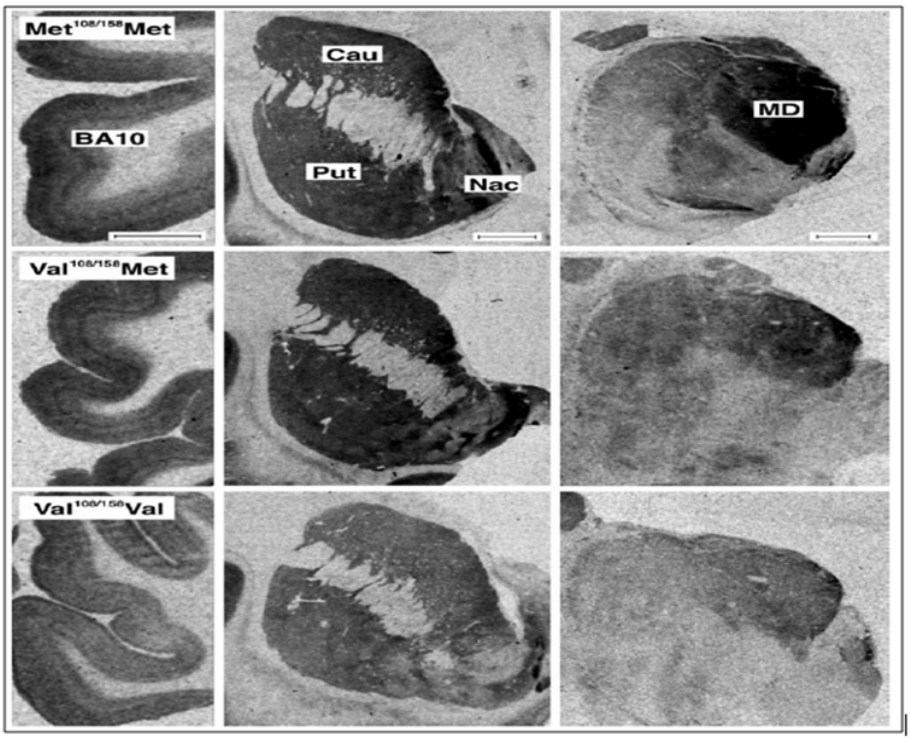


Figure 4: *COMT* Val158Met genotype associated differential expression of mu receptor binding sites
Figure shows autoradiographs depict [3H] DAMGO ligand-binding in brain tissues. Gray values correlate with increasing amounts of receptor ligand bound.

Recently, the effect of the *COMT* Val158Met polymorphism on mu receptor was investigated using semiquantitative immunostaining in human post-mortem brain tissue. It has been found that the Met158 homozygotes expressed significantly more mu receptor protein than Val158 homozygotes in human basal ganglia and thalamic tissues (Figure 5 "G and J" vs "I and L"). Moreover, the lowest levels of enkephalin peptide were found in Met158 homozygotes tissues, whereas Val158 homozygotes showed the highest peptide levels (Figure 5 "A and D" vs "C and F"). Accordingly, authors suggested that the *COMT* Val158Met polymorphism might influence the mu receptor expression through use-dependent down/up-regulation secondary to altered enkephalin levels (Kowarik et al, 2012).

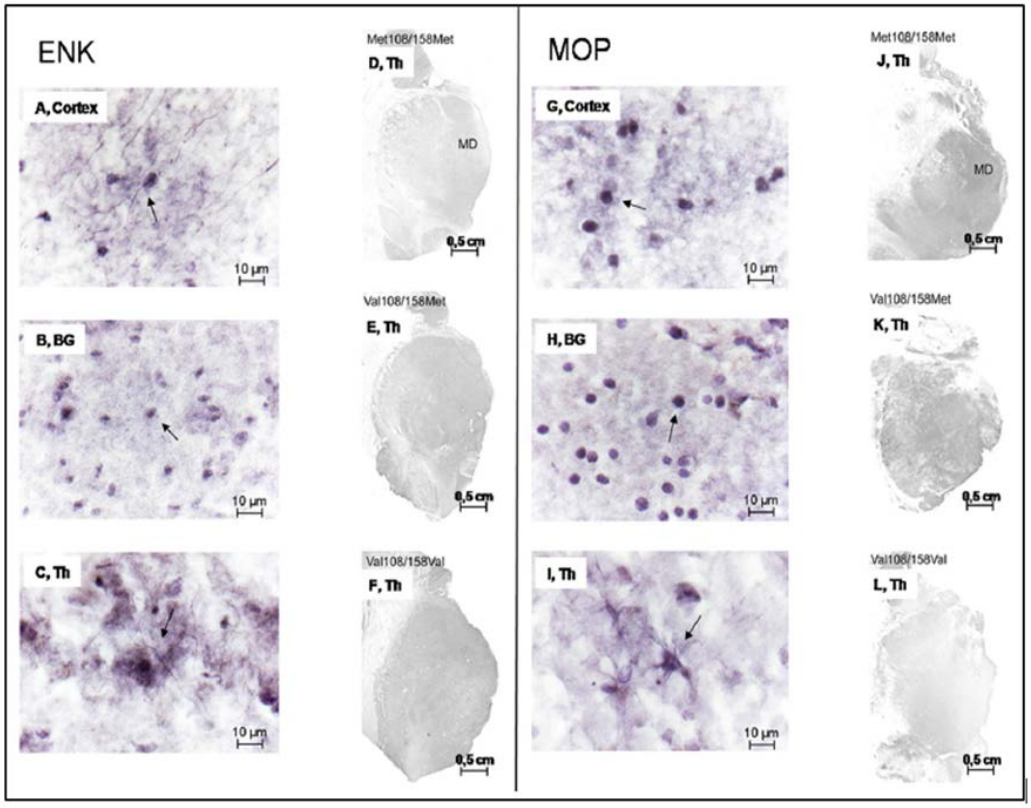


Figure 5: Met-enkephalin and mu receptor immunohistochemistry in brain tissues
Arrows highlight somata and neuropil immunoreactive for met-enkephalin or the mu opioid receptor in Cortex (A, G), basal ganglia (BG) (B, H), and mediodorsal nucleus of the thalamus (Th) (C, I). *COMT* Val158Met genotype related MOP receptor protein and met-enkephalin expression is shown in the macroscopic view of immunohistochemical stainings in the mediodorsal nucleus (MD) of the thalamus (Enkephalin D-F, MOP J-K). Increasing grey values correlate with increasing amounts of protein expression.

2.7.2 *OPRM1* and *COMT* interaction effect: evidence and gaps in pain literature

Few studies have investigated the association between *OPRM1* and *COMT* in relation to postoperative pain perception and response to opioids (Kolesnikov et al., 2011; Landau, Liu, Blouin, & Carvalho, 2013). Kolesnikov et al. investigated the combined effect of *OPRM1* A118G and *COMT* Val 158Met on pain perception and opioid response in a sample of 102 postoperative patients (Kolesnikov et al., 2011). The combined effect of *OPRM1* A118G and *COMT* Val158Met was associated with significant variability in morphine consumption during the first 48 hours post abdominal surgery (radical prostatectomy or hysterectomy), where the

patients with A118G and Val158Met combination needed significantly fewer morphine doses, approximately 18% less morphine, during the first 48 hours postoperatively compared with *OPRM1* A118A (Kolesnikov et al., 2011). Several limitations have been identified; first, the authors used different surgical models (prostatectomy or hysterectomy), which may influence the study results by causing different levels of pain for males and females and therefore increasing the variability in morphine consumption. Second, the continuous morphine infusion used in the study design may have masked a possible genetic effect on morphine efficacy and making it more difficult to detect any possible differences in the patient-administered analgesic bolus. Third, when authors analyzed combined effects of *OPRM1* and *COMT* on morphine consumption, they only compared morphine dose among the combined A118G/Val158Met carriers versus all *OPRM1* A118A carriers irrespective of *COMT* genotype. Lastly, the authors considered a single *COMT* SNP, not haplotypes, in their analyses though evidence suggests that *COMT* haplotypes rather than a single SNP better account for the variability in pain sensitivity (Diatchenko et al., 2006; Diatchenko et al., 2005; Landau, Ortner, & Carvalho, 2011; Nackley et al., 2006; Rakvag et al., 2008).

Recently, Landau et al investigated the effect of *COMT* and *OPRM1* on analgesic response to intravenous fentanyl during labor and delivery and found that women with the combination of *OPRM1* AA and *COMT* Met/Met have lower decrease in numerical verbal pain score (NVPS) at 15 minutes after IV fentanyl dose compared to other combinations. There was no significant differences in term of analgesic response to IV fentanyl between women with *OPRM1* AA and *COMT* Met/Met combination compared to women with any other allelic combination (Landau, Liu, Blouin, & Carvalho, 2013).

2.8 SIGNIFICANCE AND INNOVATION

The response to opioid treatment for acute or chronic pain is highly individualized and varies among patients. This variability interferes with optimal pain management and creates risk for undertreating or over-treating pain. Both the undertreatment and overtreatment of pain are associated with adverse outcomes, e.g., inadequate pain relief, respiratory depression, or even death (Webster et al., 2011).

By considering genetic factors that influence pain response and opioid effectiveness, high-risk individuals for opioid mismanagement can be identified. Consequently, opioid therapy can be individualized for better pain management, effective postoperative analgesia and preventing serious adverse events. Therefore, the results of this study are significant. They will improve our understanding of pain perception and response to opioids and they will improve strategies used for pain management of post-operative patients, providing a platform for safer, more efficacious opioid dosing. This study represents a first step towards individualized medicine in the post-operative setting.

The focus of the most recent genetic studies was on the effect of a single gene on pain sensitivity and response to opioid (Fernandez-de-las-Penas et al., 2012; Henker et al., 2013; Kambur et al., 2013). Though, the combination or interaction of multiple pain genes may be responsible for better understanding and a sturdier explanation of patient's variability in pain response. The gene–gene interaction effect of *OPRM1* and *COMT* on pain and opioid response is not well understood. Thus, more studies are needed to establish the impact of this relationship on response to pain management. We expanded *COMT* analyses from single SNP to haplotype analyses to warrant more detailed data and a better understanding of *COMT* variability in pain (Diatchenko et al., 2006; Diatchenko et al., 2005; Nackley et al., 2006; Rakvag et al., 2008), our

proposed study was innovative since it was the first to consider *COMT* haplotypes as well as *COMT* Val158Met SNP interaction with *OPRM1* A118G which provides a better understanding of the inter-individual variability of pain and responses to opioids rather than the effects of SNPs alone.

Our study had a relatively larger sample size and more uniform type of surgical operation as single orthopedic procedures, addressing certain limitations of previous work (Kolesnikov et al., 2011). Evidence shows that patients undergoing orthopedic surgical procedures experience moderate to severe pain (Chung, Ritchie, & Su, 1997; Morin et al., 2005); supported by the fact that bone injury is more painful than soft tissue injury (Chung et al., 1997). Thus, our population was ideal for investigating postoperative pain and patient response to opioids.

3.0 METHODS

3.1 RESEARCH DESIGN

This descriptive observational study used previously obtained genotyping data of a parent study that prospectively enrolled a convenience sample of 153 patients between 2005 and 2009. The previous study examined the independent effects of *OPRM1* and *COMT* genotypes on postoperative pain, opioid use, and opioid-induced sedation (Henker et al., 2013). The parent study had 79 patients while the proposed study included an additional 74 patients. Recruitment and data on demographic and other clinical characteristics from these additional patients were collected using the same procedures as the initial 79 patients.

3.2 CLINICAL SETTING OF THE PARENT STUDY

The University of Pittsburgh Medical Center (UPMC)-Presbyterian University Hospital (PUH) is an 816 bed hospital designated as a Level I Regional Trauma Center fully accredited by the Pennsylvania Trauma Systems Foundation. UPMC-PUH has 43 operating rooms and 2 post anesthesia care units (PACU).

3.3 SAMPLE

Saliva samples were available for analysis from 153 postoperative orthopedic trauma patients. Inclusion criteria of the parent study (that will also be used for the proposed study) were 1) 18 to 80 years of age; 2) opioid-naïve; 3) received general or general and regional anesthesia; 4) single orthopedic procedure; and 5) planned surgical time of 1 to 4 hours in length.

Exclusion criteria were 1) second orthopedic trauma site; 2) abdominal or thoracic trauma; 3) history of mental illness (e.g., depression, bipolar disorder, schizophrenia); 4) currently taking phenothiazines; 5) history of hepatic disease (e.g., history of hepatitis C); 6) history of renal disease; 7) American Society of Anesthesiologist Physical Status >3; 8) previous history or neurologic conditions such as stroke, head injury, spinal cord injury, intracerebral hemorrhage; and 9) previous history of arthritis or bone disease.

3.3.1 Justification of the Sample Size

Testing interaction effects generally requires a much larger sample size than testing for main effect of single SNP or haplotype. As such, our study is an exploratory, rather than a confirmatory study of the putative interaction effects of *COMT* and *OPRM1* using data collected from 153 patients enrolled to date. A formal sample size calculation was not performed; however, the effect sizes estimated from our study will be informative for estimating the sample size for a large scale genomic study in the same patient population.

3.4 RECRUITMENT

Subjects were recruited as part of the parent study. Once patient eligibility was confirmed, the study was explained in detail including the study purpose, risks, benefits, and data collection procedure by the study personnel. If the patient was willing to participate, informed consent was obtained.

3.5 DATA COLLECTION

Investigators with clinical privileges at UPMC-PUH identified patients scheduled to receive general anesthesia for a single orthopedic surgical procedure with an anticipated duration of 1 to 4 hours. Once eligibility was confirmed, the patient was approached and the study explained in detail. If the patient was willing to participate, informed consent was obtained in the preoperative holding area or the patient's hospital room. Once enrolled, subjects were taught how to use the Numerical Pain Scale (NPS); the NPS was recorded prior to entering the Operation Room (OR) and then at 45 minutes after PACU admission by study personnel. The total amount of opioids used during PACU stay was recorded by study personnel. 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 extracted from the medical record. Saliva was collected the first day after surgery (subjects have difficulty providing saliva prior to surgery due to being NPO for 8 hours prior to surgery) and stored at room temperature and evaluated for genotype**.**

3.6 STUDY VARIABLES

Pain score and opioid use in the immediate postoperative period were treated as dependent variables. *OPRM1* A118G (rs1799971) SNP and *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680) haplotypes and diplotypes were treated as the main independent variables of interest, with selected patient demographics and baseline clinical characteristics as covariates.

3.6.1 Opioid consumption

Opioid consumption was the amount of opioids per kg received by subjects in the OR and PACU. Time and amount of opioids administered during PACU were abstracted from the PACU record. The amount of opioid administered was converted to morphine equivalents as follows: 100 µg fentanyl was converted to 10 mg of morphine, 1.5 mg hydromorphone was converted to 10 mg of morphine, and 75 mg of meperidine was converted to 10mg of morphine. Opioid dose was analyzed as a continuous dependent variable for the first aim.

3.6.2 Pain response

Pain was assessed using the numeric pain scale (NPS), an 11-point verbal pain response scale from 0 (“no pain”) to 10 (“pain as bad as I can imagine”). Pain scores were collected in the preoperative holding area and within 45 minutes after arrival in the PACU. Pain was analyzed as a continuous dependent variable for the second aim.

3.6.3 Genetic data

Oragene DNA self-collection kit from DNA Genotek Incorporated (Ottawa, Ontario, Canada) was used to collect saliva samples from subjects. DNA was extracted from saliva samples applying the protocol and reagents for extraction supplied with the Oragene kit. The extracted DNA was stored and then used to evaluate genotypes.

3.6.3.1 *OPRM1* A118G

OPRM1 A118G (rs1799971) was genotyped using sequencing. The forward primer (50-TCAGTACCATGGACAGCAG-30) and reverse primer (50-GGAGTAGAGGGCCATGAT-30) were used in a polymerase chain reaction (PCR) with an annealing temperature of 59 °C. The PCR products were cleaned with ExoSAP reagents (USBiochemicals, Cleveland, OH). Then, the sequencing was performed using the reverse primer and Big Dye Cycle Sequencing reagents (Applied Biosystems, ABI, Foster City, CA). The sequencing products were electrophoresed using ABI377 automated sequencer (ABI). Finally, the data were viewed and genotypes were assigned using Sequencer software (Gene Codes Corporation, Ann Arbor, MI). Only two patients (1.3%) were homozygous for the G variant. Thus, we combined heterozygous and homozygous for the G variant into one group. *OPRM1* A118G was coded as having two levels (‘no minor allele G’ and ‘1 or 2 minor allele G’) and was treated as a nominal independent variable for both aims.

3.6.3.2 *COMT*

The four *COMT* SNPs including rs6269, rs4633, rs4818, and rs4680 were genotyped using 5' exonuclease Assay-on-Demand TaqMan assays (ABI). Amplification and genotype assignments

were next conducted using the ABI7000 and SDS 2.0 software (ABI). *COMT* SNPs were treated as independent variable for both aims. *COMT* haplotypes, constructed by four *COMT* SNPs; rs6269, rs4633, rs4818 and rs4680, were designated as low pain sensitivity (LPS; GCGG), average pain sensitivity (APS; ATCA) and high pain sensitivity (HPS; ACCG) (Diatchenko et al., 2005). Using combinations of the three major haplotypes, we created two major diplotypes; low pain sensitivity diplotype (two copies of LPS haplotype or one copy each of LPS/APS haplotypes) and high pain sensitivity diplotype (two copies of APS haplotype, two copies of HPS haplotype, one copy each of LPS/HPS haplotypes, one copy each of APS/HPS haplotypes). Each of *COMT* haplotypes has three levels; having no copies, having one copy and having two copies of the haplotype. Since we did not have enough patients in those three levels, we combined patients having one copy of the haplotype and patients having two copies of the haplotype into one group as "having at least one copy of the haplotype". Thus, *COMT* haplotypes were coded into two levels ('having no copies' and 'having at least one copy'). *COMT* haplotypes and diplotypes were also treated as independent variables for both aims.

3.6.4 Demographic and clinical characteristics

Gender (2 levels; male and female), race (2 levels; Caucasian and other), smoking status (2 levels; smoker and non-smoker), fracture types (5 levels; ankle, tibia/fibula, tibial plateau, femur and other), and preoperative pain (2 levels; no/mild pain and moderate/severe pain) were treated as nominal variables. Age (measured in years) and OR opioid (measured in mg/kg/hr) were treated as continuous variables. All data were collected from the patient and medical records, and their effects were adjusted as possible covariates in our association analyses.

3.7 ANALYSIS PLAN

3.7.1 Genotype quality control

To ensure accuracy of association results, we assessed the quality of the genotyping data using filters on call rate (CR) (>0.95), repeatability of calls (>0.99), and checks comparing expected homozygosity to observed homozygosity at each marker. We assessed Hardy–Weinberg equilibrium (HWE) for all genotyped SNPs using Fisher's exact test implemented in the software PLINK (Purcell et al., 2007), which was also used to estimate the allele frequencies of SNPs as well as the frequencies of *COMT* haplotypes and diplotypes. The Haploview software (http://www.broad.mit.edu/mpg/haploview) was used to visualize the pairwise linkage disequilibrium (LD) between the selected four *COMT* SNPs measured by Lewontin's D' (Barrett, Fry, Maller, & Daly, 2005).

3.7.2 Data screening procedures

Data analyses were performed using IBM® SPSS® Statistics (Version 22, IBM Corp., Armonk, NY). Assessment of data accuracy was performed prior to the main data analysis using descriptive statistics and graphical plots to ensure the valid analysis of data. The amounts and patterns of missing data were reviewed. For each variable in our model, a variable missing value indicator (missing=1, observed=0) was created, then t-tests or chi-square tests were conducted between each of these indicator variables and the observed values of the other variables in the model. Missing observations were assumed to be missing completely at random. Most missing data were genetic information for *OPRM1* or *COMT* or both. Cases with missing genetic data

(n=28, 18%) or missing postoperative pain score (n=2, 1.3%) or opioid dose (n=2, 1.3%) were excluded from the analysis.

Univariate outliers were reviewed carefully and verified against the research records. Score alteration was applied for outliers of opioid consumption during PACU and opioid consumption in the OR, setting outliers or extreme values closer to the remaining cases while keeping the relative ordering (Tabachnick, 2013). Multivariate outliers and potentially influential observations (based on studentized deleted residuals and leverage values) were further evaluated using, Cook's D, |DFFITS| and |DFBETAS| during model assessment.

3.7.3 Data analysis for specific aims

All statistical analyses to address study aims were preceded by detailed descriptive analyses of the data, using standard descriptive summaries (e.g., means, standard deviations, median, minimum, maximum, percentiles, ranges) and graphical techniques (e.g., histograms and scatterplots) for all continuous variables including pain scores, opioid doses, and age. Frequencies and percentages were examined for categorical variables including *OPRM1*, *COMT*, gender, race, smoking, preoperative pain and fracture types. No adjustment to significance level was made for multiple testing, since the purpose of this study was more exploratory than confirmatory of the putative interaction effects of *COMT* and *OPRM1*.

Since haplotype structure may vary by race, we conducted a subgroup analysis limited to only Caucasian subjects (n=121; 79%). Though our sample size decreased, we were interested in exploring the magnitude and direction of the estimated regression coefficients for a homogeneous sample of Caucasians. Sample sizes for other ethnicities were too small to conduct subgroup analyses.

3.7.3.1 Specific aim #1

To explore the gene-gene interaction effect of *OPRM1* and *COMT* on opioid dose required for pain management during PACU stay.

First, descriptive statistics as well as graphical plots were created to describe the distribution of postoperative opioid dose in PACU stay among *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680), haplotypes and diplotypes and among their combined groups with *OPRM1* A118G. Opioid distribution patterns among *OPRM1* levels were also described. Hierarchical multiple regression analysis modeling was applied considering each of *COMT* SNPs (including the additive, dominant and recessive effects), haplotypes and diplotypes with A118G SNP to investigate their individual and interactive effects on PACU opioid consumption after adjustment identified covariates including gender, race, age, smoking, fracture type and OR opioid consumption. The significance of the interaction terms and their regression coefficients with confidence intervals were reported as well as the proportion of the variance accounted by each interaction term.

Any violations of assumptions underlying the planned methods of analysis were assessed including non-normality of model errors and heterogeneity of error variance and serious multicollinearity. Histogram of the studentized deleted residuals and P-P plot of studentized deleted residuals against the predicted values were examined for any deviation from normality. The assumption of normality was met for postoperative opioid consumption in the PACU. The Levene's test was also used to examine the homogeneity of group variances. The variance inflation factors (VIF) and tolerance values were examined to screen for multicollinearity. The assumption of homogeneity of the variance was also met and no serious multicollinearity was found.

3.7.3.2 Specific aim #2

To explore the gene-gene interaction effect of *OPRM1* and *COMT* on postoperative pain score at 45 minutes in PACU.

Descriptive statistics as well as graphical plots were used to characterize the distribution of postoperative pain among *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680), haplotypes (LPS, APS, and HPS), and diplotypes (low pain intensity and high pain intensity) and among their combinations with *OPRM1* A118G. Pain distribution patterns among *OPRM1* levels was also described. Hierarchical multiple linear regression was used, where each of the *COMT* SNPs (including the additive, dominant and recessive effects), haplotypes and diplotypes was included in the regression model with A118G SNP to investigate their individual and interactive effects on pain score at 45 minutes in the PACU after adjustment for the associated covariates including gender, race, age, smoking, fracture type, preoperative pain, OR opioid consumption and opioid consumption during the first 45 minutes in PACU. The significance of the interaction terms and their regression coefficients with confidence intervals were reported as well as the proportion of the variance accounted by each interaction term.

Any violations of assumptions underlying the planned methods of analysis were checked, including non-normality of model errors and heterogeneity of error variance and serious multicollinearity. Histogram of the studentized deleted residuals and P-P plot of studentized deleted residuals against the predicted values were examined for any deviation from normality. The assumption of normality of model errors was not met for postoperative pain at 45 minutes in PACU (severely negatively skewed residuals). Data transformations (square root transformation of reflected values of postoperative pain at 45 minutes in the PACU) were used to remediate the violation of normality assumption. The Levene's test was used to examine the homogeneity of

group variances. The variance inflation factors (VIF) and tolerance values were examined to screen for multicollinearity. The assumption of homogeneity of the error variance was satisfied and no severe multicollinearity was found.

3.8 LIMITATIONS

While our proposed sample size is larger than what is reported in the literature in the area of pain (Kolesnikov et al., 2011), it is still relatively small for a general genetic study of a common disease or phenotype. Because of the small sample size, the homozygous and heterozygous G variants for A118G were combined into one group as 'having 1 or 2 G alleles'. We also combined subjects having one copy and two copies of each of *COMT* haplotype into one group as "having at least one copy of the haplotype".

There are also limitations of using data that were collected previously as we were not able to consider other pain-related factors that may contribute to the variability in pain and response to opioids such as expected pain, surgical fear, and pain catastrophizing. Moreover, mixing different types of opioids in the analysis may affect our final findings; different types of opioid display different pharmacological properties as they bind with different affinity to opioid receptors.

3.9 HUMAN SUBJECTS

This secondary analysis will use data collected for the parent study (NIH 1 UL1 RR024153) that was approved by the Institutional Review Board (IRB) at the University of Pittsburgh. The risk in the proposed study is minimal and is limited to potential loss of confidentiality related to disclosure of genetic information. The risk of confidentiality was minimized by assignment of a unique identification number rather than personal identifiers for all the subjects. All data files were maintained in a locked office in a locked department in the School of Nursing. Electronic data were stored on password protected computers. No exclusion of potential participants was made based on gender, ethnicity or race.

4.0 SUMMARY OF STUDY

4.1 RESULTS

4.1.1 Demographic and clinical characteristics

One hundred and fifty-three postoperative patients with average (±SD) age of 38.48 ±13.1 years that underwent surgical orthopedic trauma repair were included in the study. Most (68%) of the sample was male (n=104), 80% (n=121) were non-Hispanic Caucasian, and 57 % (n=86) were nonsmokers.

Thirty-nine percent (n=56) of the patients were admitted with ankle fractures, 24% (n=35) with tibia fibula fractures, 21% (n=30) with tibial plateau fractures, 11% (n=16) with femur fractures, and 5% (n=7) with other types of fractures including; radial, ulnar, humerus, acetabular and hip. The average (±SD) of preoperative pain score was 4.53 ± 2.92. The average (±SD) surgical time was 119.96 ± 59.51 minutes. OR opioid consumption ranged from 0 to 1.35 mg/kg with an average (±SD) of 0.42 ± 0.22. The average postoperative pain scores at 15 and 45 minutes of PACU admission were 6.59 ± 3.2 and 6.42 ± 2.79, respectively. Total opioid consumption during PACU stay ranged from 0 to 0.36 mg/kg with an average (±SD) of 0.11 ± 0.09 mg/kg. The average (±SD) of PACU stay was 128.99 ± 56.40 minutes. Descriptive statistics by *OPRM1* A118G and *COMT* SNPs, haplotypes and diplotypes are presented in Table 3.

Table 3. Descriptive statistics by *OPRM1* A118G and *COMT* SNPs, haplotypes and diplotypes

	n	Gender (Male)	Race (Caucasian)	Age (Years)	Preoperative Pain	Postoperative Pain in PACU (at 45 min)	OR Opioid (mg/kg/hr)	PACU Opioid (mg/kg)
A118G(Dominant)								
AA	95	70 (71)	77 (79)	39.8±13.4	4.6±2.9	6.4±2.9	.24±.14	.10±.08
AG,GG	27	16 (57)	22 (79)	36.5±14.3	4.2±3.0	6.5±2.7	.26±.14	.16±.11
RS4680(Recessive)								
AA	32	20 (63)	30(94)	37.4±13.0	4.5±2.8	7.1±2.6	.25±.13	.13±.08
AG,GG	90	66 (70)	69(73)	39.7±13.8	4.5±2.9	6.2±2.9	.24±.14	.11±.09
RS6269(Recessive)								
AA	38	25(66)	34(90)	37.4±12.9	4.3±2.7	7.1±2.6	.25±.13	.12±.08
GA,GG	79	56(68)	61(74)	40.4±13.9	4.6±2.9	6.0±2.9	.24±.14	.11±.10
RS4633(Recessive)								
TT	30	20(67)	28(93)	38.3±13.2	4.3±2.8	7.3±2.7	.24±.13	.13±.09
CT,CC	89	64(69)	68(73)	39.9±13.7	4.5±3.0	6.2±2.8	.24±.14	.11±.09
RS4818(Dominant)								
CC	42	29(69)	33(79)	36.3±11.6	4.3±3.1	7.3±2.6	.27±.14	.12±.08
GG,GC	76	55(69)	63(79)	40.8±14.2	4.5±2.8	6.1±2.9	.23±.14	.12±.10
LPS Haplotype								
0 copy	48	30(63)	38(79)	37.5±13.2	4.6±3.1	7.0±2.6	.26±.14	.12±.08
1 or 2 copies	74	56(72)	61(78)	40.1±13.9	4.4±2.8	6.1±2.9	.23±.14	.11±.10
APS Haplotype								
0 copy	54	38(66)	42(72)	41.1±14.0	5.3±3.0	6.2±3.0	.25±.15	.11±.09
1 or 2 copies	68	48(71)	57(84)	37.5±13.1	3.8±2.6	6.6±2.7	.24±.13	.12±.09
HPS Haplotype								
0 copy	99	73(71)	86(84)	39.9±13.7	4.2±2.7	6.4±2.8	.24±.14	.12±.10
1 or 2 copies	23	13(57)	13(57)	35.7±12.7	5.6±3.4	6.4±3.0	.27±.15	.10±.07
Diplotype								
Low	69	53(73)	58(80)	41.5±14.1	4.2±2.7	6.1±2.8	.23±.14	.11±.10
High	53	33(62)	41(77)	35.9±12.4	4.8±3.1	6.9±2.8	.26±.14	.12±.08

Descriptive statistics reported in cell are expressed as mean±SD for continuous variables and n (%) for categorical variables.
LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity; PACU = postanesthesia care unit; OR= Operation Room.

4.1.2 Genetic characteristics

One SNP from *OPRM1* (rs1799971) and four SNPs from *COMT* (rs4680, rs4633, rs4818, and rs6269) were included in the analysis. Genotype and allele frequencies of *OPRM1* and *COMT* SNPs are presented in Table 2. For *OPRM1* A118G; there were 106 (78%) patients that had no minor allele G (AA) and 30 (22%) that had 1 or 2 minor alleles G (AG and GG). For *COMT* rs4680; 37 (27%) had Met158Met (AA), 46 (34%) had Met158Val (AG), and 53 (39%) had Val158Val (GG). For *COMT* rs6269; 40 (31%) had AA, 49 (37%) had GA, and 42 (32%) had GG. For *COMT* rs4633; 52 (39%) had CC, 48 (36%) had CT, and 33 (25%) had TT. For *COMT* rs4818; 30 (23%) had GG, 56 (43%) GC, and 45 (34%) CC.

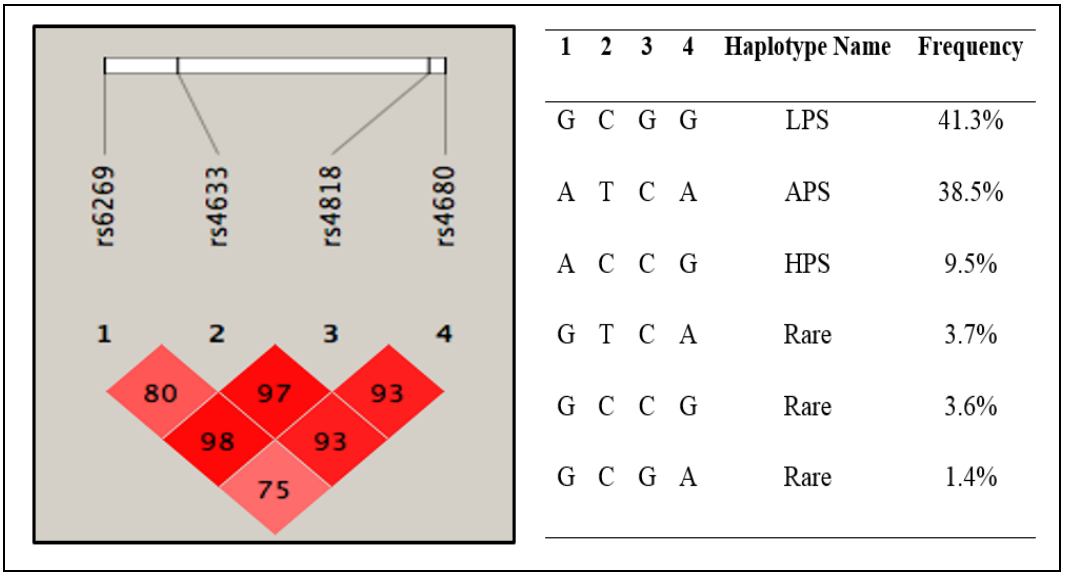
Three of the four *COMT* SNPs (rs4680, rs4633, and rs6269) displayed significant deviation from HWE and corresponding p-values are shown in Table 4. *COMT* SNPs were out of equilibrium even after restricting our analyses to Caucasian subjects. Systematic lab review of raw genotype data was performed to exclude that deviations from HWE were due to genotyping error. It is possible that deviations from HWE in *COMT* SNPs as a result of the small size of our sample. Another possible explanation is that deviation from HWE is due to the distinctive characteristics of our sample. Thus, our sample may not be completely representative of the general population. Despite violations of HWE, *COMT* SNPs are genetically related; they collectively influence the biological process of pain. Furthermore, pairwise linkage disequilibrium values computed by Haploview software showed that all *COMT* SNPs rs4680, rs4633, rs4818 and rs6269 were in strong LD ($D' > 0.75$) (Figure 6).

Table 4. Genotype and allele frequency of *OPRM1* and *COMT* SNPs

Gene	SNP	Chromosome	Position	Alleles[a]		MAF[b]	Genotypes			HWE[c]
				A1	A2		A1/A1	A1/A2	A2/A2	
OPRM1	rs1799971	6	154360797	G	A	0.1176	2	28	106	1
COMT	rs4680	22	19951271	A	G	0.4412	37	46	53	0.00025
	rs4633	22	19950235	T	C	0.4286	33	48	52	0.00258
	rs4818	22	19951207	G	C	0.4427	30	56	45	0.15560
	rs6269	22	19949952	A	G	0.4924	40	49	42	0.00494

[a]A1: Minor allele; A2: Major allele; [b]MAF: Minor Allelic Frequency; [c](HWE) : Hardy-Weinberg Equilibrium test p-value.

The distribution of *COMT* haplotypes and diplotypes were as follow; 52(39%) patients had no copies of LPS haplotype and 82(61%) had at least one copy; 63(47%) patients had no copies of APS haplotype and 71(53%) had at least one copy; 110(82%) patients had no copies of HPS haplotype and 24(18%) had at least one copy. Regarding *COMT* diplotypes; 77(57%) patients had low pain sensitivity diplotype and 57(43%) had high pain sensitivity diplotype (Figure 6).



1	2	3	4	Haplotype Name	Frequency
G	C	G	G	LPS	41.3%
A	T	C	A	APS	38.5%
A	C	C	G	HPS	9.5%
G	T	C	A	Rare	3.7%
G	C	C	G	Rare	3.6%
G	C	G	A	Rare	1.4%

Figure 6. Linkage disequilibrium (LD) graph of *COMT* SNPs

Linkage disequilibrium (LD) graph of COMT SNPs "left" and Haplotype frequency "right". LD was calculated using D' (0= no disequilibrium; 1= maximum disequilibrium). The numbers inside the squares are 100 × D'. Graph was created using Haploview software. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

4.1.3 *OPRM1* × *COMT* effect on postoperative pain and opioid consumption

One hundred and fifty-three patients were available for our data analysis. A hierarchical multiple regression was performed using postoperative pain score at 45 minutes in PACU and opioids consumption during PACU stay as the dependent variables. *OPRM1* A118G (under dominant genetic model), the four *COMT* SNPs including rs6269, rs4633, rs4818 and rs4680 (under additive, dominant, and recessive genetic models), the three *COMT* haplotypes (low, average, and high pain sensitivity), and *COMT* diplotypes (low and high pain sensitivity) as independent variables. Main and interaction effects of the independent variables were investigated after adjustment for the associated covariates. Correlations between *OPRM1* and *COMT* and variables of interest are presented in Table 5. Correlations between pain and opioid variables and other variables of interest are presented in Table 6.

Table 5. Spearman's rank correlation coefficient (r_s) between *OPRM1*, *COMT*, dependent variables and covariates

	A118G	rs4680	rs4633	rs4818	rs6269	LPS	APS	HPS	Diplotype
Male Gender	.128	-.083	-.054	.066	.015	-.10	-.05	.119	.110
Caucasian Race	<.001	.159	-.063	.242**	.207*	.011	-.14	.254**	.025
Age (years)	-.098	-.014	-.081	-.079	-.179*	.082	-.11	-.113	-.189*
None smoker	-.146	-.083	-.106	-.108	.001	.006	-.02	-.070	-.010
Ankle Fracture Type	-.049	-.016	.017	-.120	-.048	-.05	.058	-.104	-.016
Surgical Time (minutes)	.096	.014	.103	-.061	-.043	.065	.058	-.146	-.115
Preoperative Pain Score	-.069	-.091	-.195*	.008	-.166	-.02	-.3**	.170	.084
Pain 45 minutes in PACU	.015	-.102	-.012	-.009	.054	-.16	.082	.002	.143
OR Opioid (mg/kg)	.217*	-.082	-.033	-.148	-.041	-.04	-.00	-.092	-.023
PACU Opioid (mg/kg)	.258**	-.028	.114	.105	.090	-.06	.116	-.056	.044
PACU Time (minutes)	.113	.060	.122	.166	.207*	-.07	.088	.101	.030

* p<.05, ** p<.001, LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity; PACU = Post Anesthesia Care Unit; OR= Operation Room.

Table 6. Spearman's rank correlation coefficient (r_s) between pain, opioid, and covariates

	Preoperative Pain Score	Postoperative Pain Score in PACU	OR Opioid	PACU Opioid
Preoperative Pain Score	--	.137	.139	.085
Pain 15 minutes in PACU	-.023	.433**	.328**	.386**
Pain 45 minutes in PACU	.137	--	.173	.400**
OR Opioid (mg/kg)	.139	.173	--	.203**
PACU Opioid (mg/kg)	.085	.400**	.203*	--
Male Gender	.050	-.030	.037	.065
Caucasian Race	.056	.130	.081	-.099
Age (years)	.009	-.104	-.210*	-.123
None smoker	.179*	.079	.130	-.069
Ankle Fracture Type	.105	.260**	.129	.137
Surgical Time (minutes)	.018	-.067	.259**	-.069
PACU Time (min)	.176	.106	.010	.015

* p<.05, ** p<.001; PACU = postanesthesia care unit; OR= Operation Room.

4.1.4 *OPRM1* × *COMT* effect on opioid consumption

Regression analysis results for predicting PACU opioid consumption by *OPRM1* × *COMT* are summarized in Table 7. In addition to the main effects of both *OPRM1* and *COMT*, the interaction analysis controlled for gender, race, age, smoking, fracture type and OR opioid consumption. Figure 7 displays the interaction effects of *OPRM1* × *COMT* on PACU opioid consumption for the typical subject in our sample; Caucasian, male, with ankle fracture, a mean age of 38.9 years, and a mean OR opioid consumption of 0.246 mg/kg/hr.

Table 7. Regression analysis summary for predicting PACU opioid (mg/kg) by *OPRM1*× *COMT* (Total sample)

Interaction Variable	n	b	95%CI	Beta	p-value	sr^2
OPRM1 A118G×*COMT* rs4680 (Recessive)	123	0.093	(0.006,0.179)	0.235	.037	.031
OPRM1 A118G×*COMT* rs4633 (Recessive)	120	0.097	(0.006,0.189)	0.231	.037	.033
OPRM1 A118G×*COMT* rs4818 (Dominant)	119	-0.044	(-0.126,0.037)	-0.170	.284	.009
OPRM1 A118G×*COMT* rs6269 (Recessive)	118	-0.075	(-0.160,0.010)	-0.305	.082	.023
OPRM1 A118G×*COMT* LPS Haplotype	123	-0.047	(-0.125,0.030)	0.174	.230	.011
OPRM1 A118G×*COMT* APS Haplotype	123	0.009	(-0.068,0.086)	0.034	.818	<.001
OPRM1 A118G×*COMT* HPS Haplotype	123	0.001	(-0.095,0.098)	0.003	.982	. <.001
OPRM1 A118G×*COMT* Diplotypes	123	0.071	(-0.006,0.148)	0.231	.070	.024

Interaction analysis controlled for the main effects of both *OPRM1* and *COMT*, gender, race, age, smoking, fracture type and OR opioid consumption. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

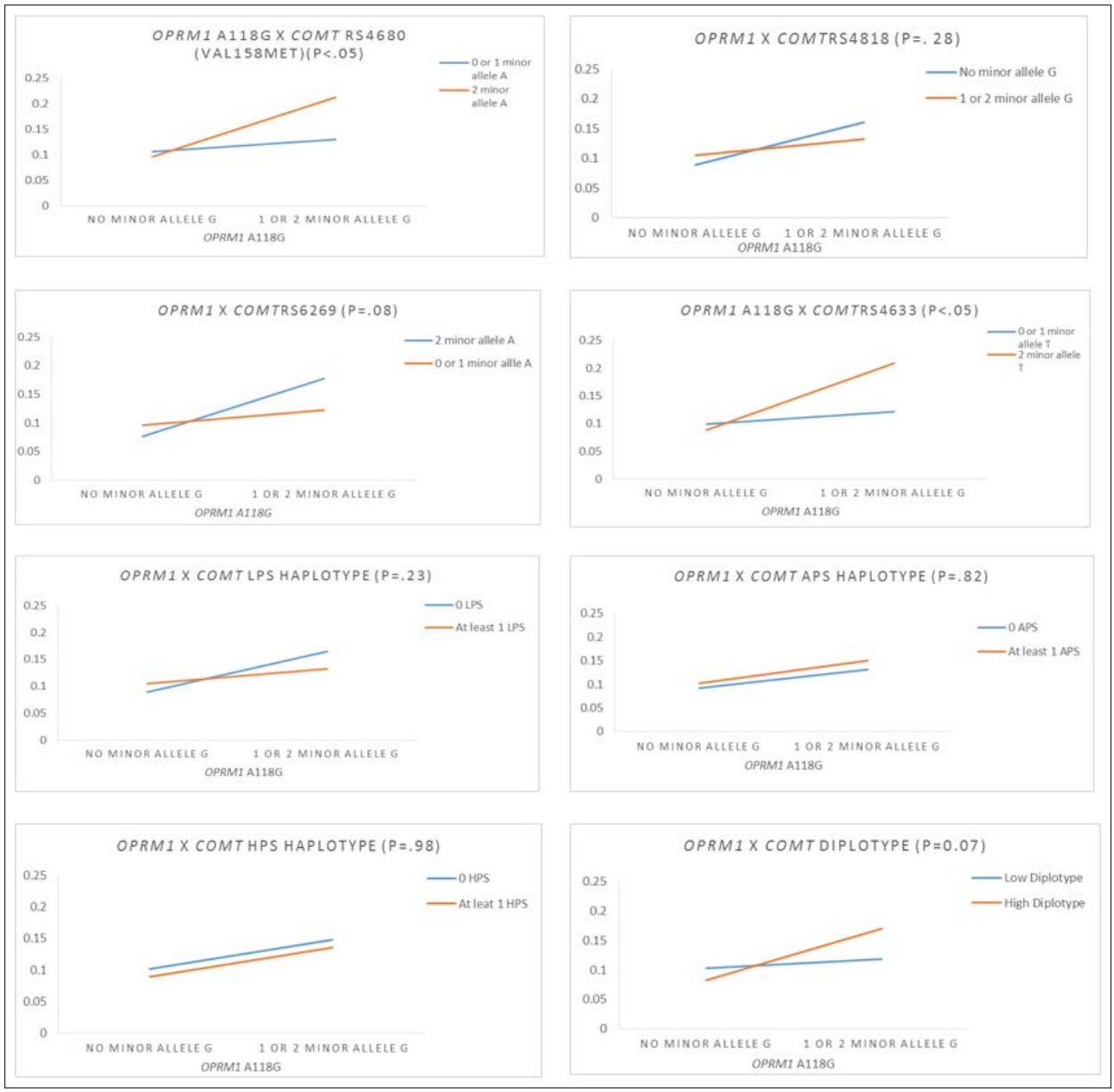


Figure 7. The interaction effects of *OPRM1*× *COMT* (SNPs, haplotypes, and diplotypes) on PACU opioid for the total sample

The displayed graphs are for the typical subject in our sample in terms of the covariates; Caucasian, male, with ankle fracture, a mean age of 38.9 years, and a mean OR opioid consumption of 0.246mg/kg/hr. , LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity.

One multivariate outlier and possibly influential point was identified in PACU opioid consumption analyses. Case 25 has high values of studentized deleted residuals, leverage, Cook's D, |DFFITS| and |DFBETAS|. This case represents a 23 year old Caucasian male subject with radial fracture. This subject had the largest total opioid consumption during PACU stay (total PACU opioid dose = 40 mg; .36mg/kg). Of a maximum verbal pain rating of 10, his pain score before surgery was 9. His postoperative pain ratings at 15 and 45 minutes in PACU were 10. The genetic characteristics of this subject were AG for *OPRM1* A118G, GA for *COMT*rs6269, CT for *COMT*rs4633, GC for *COMT*rs4818, AG for *COMT*rs4680, one copy of each of LPS and APS, and low pain sensitivity diplotype. A sensitivity analysis was conducted to investigate the influence of this outlier on regression model results. Sensitivity analysis showed the findings (in terms estimated regression coefficients and standard errors) were robust to the exclusion of case 25 as findings were not changed when case 25 was excluded (Table 8).

Table 8. Regression results for the *OPRM1* × *COMT* interaction for PACU opioid (mg/kg) with inclusion (*) and exclusion (**) of case #25

Interaction Term	b^{*}	b^{**}	$\%\Delta_b$	SE^{*}	SE^{**}	$\%\ \Delta_{SE}$	p^{*}	p^{**}
OPRM1 A118G×*COMT* rs4680	.093	.098	5.4	.044	.043	2.3	.037	.024
OPRM1 A118G×*COMT* rs4633	.097	.103	6.2	.046	.045	2.2	.037	.023
OPRM1 A118G×*COMT* rs4818	-.044	-.051	16.0	.041	.040	2.4	.284	.208
OPRM1 A118G×*COMT* rs6269	-.075	-.081	8.0	.043	.042	2.3	.082	.055
OPRM1 A118G×*COMT* LPS Haplotype	-.047	-.056	19.1	.039	.038	2.3	.230	.150
OPRM1 A118G×*COMT* APS Haplotype	. 009	-.006	166.67	.039	.039	0	.818	.878
OPRM1 A118G×*COMT* HPS Haplotype	.001	.013	1200	.049	.048	2.3	.982	.786
OPRM1 A118G×*COMT* Diplotype	.071	.083	16.9	.039	.038	2.3	.070	.032

LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity.

A significant interaction was found between *COMT* Val158Met (rs4680) assuming a recessive genetic model (Met158Met vs Val158Met/Val158Val) and *OPRM1* A118G assuming a dominant genetic model (A118G/G118G vs A118A), b = 0.093, p < .05. The effect of *OPRM1* on opioid consumption is varied for the different genotypes of *COMT* Val158Met. Considering Met158Met of *COMT*, there was a significant increase in opioid consumption as we move from *OPRM1* wild type (AA) to variants (AG/GG). Patients having Met158Met (AA) of *COMT*rs4680 and (AG/GG) of *OPRM1*A118G consumed more opioids compared to patients having Met158Met (AA) of *COMT*rs4680 and AA of *OPRM1*A118G, 95% CI = [0.006, 0.179]. *OPRM1*A118G × *COMT*rs4680 accounted uniquely for 3.1% of the total PACU opioid consumption variance.

A significant interaction was also found between *COMT*rs4633 assuming a recessive genetic model (TT vs. CT/CC) and *OPRM1* A118G assuming a dominant genetic model (AG/GG vs. AA), b = 0.097, p < .05. Considering TT of *COMT* rs4633, there was a significant increase in opioid consumption as we move from *OPRM1* wild type (AA) to variants (AG/GG). Patients having TT of *COMT*rs4633 and (AG/GG) of *OPRM1*A118G consumed more opioids compared to patients having TT of *COMT*rs4633 and AA of *OPRM1*A118G, 95% CI = [0.006, 0.189]. *OPRM1*A118G × *COMT*rs4633 accounted uniquely for 3.3% of the total PACU opioid consumption variance.

No significant interactions were found between *OPRM1* and the *COMT* SNPs of rs6269 and rs4818. However, the interaction of *COMT* rs6269 assuming a recessive genetic model and *OPRM1* assuming dominant genetic model demonstrated a trend (p = .08). Patients having AA of *COMT*rs6269 and AG/GG of *OPRM1*A118G consumed more opioids compared to patients having AA of *COMT*rs6269 and AA of *OPRM1*A118G, b=-0.075, 95% CI = [-0.16, 0.01].

No significant interaction was found between *OPRM1* and *COMT* haplotypes or diplotypes. However, the interaction of *COMT* diplotype and *OPRM1* assuming a dominant genetic model demonstrated a trend towards significance (p = .07). Patients having high pain sensitivity diplotype and (AG/GG) of *OPRM1*A118G consumed more opioids compared to patients having high pain sensitivity diplotype and AA of *OPRM1*A118G, b = 0.071 95% CI = [-0.006, 0.148].

OPRM1 A118G assuming a dominant genetic model was significantly associated with opioid consumption during PACU stay, b = 0.046, p < .05, sr^2 = .04. Patients having one or two minor alleles G (AG and GG) consumed significantly 0.046 mg/Kg more opioids during PACU stay compared to patients having AA, 95% CI = [0.008, 0.085].

Restricting our analysis to Caucasian subjects did not change the statistical significance for the interaction effects of both *OPRM1*A118G × *COMT* rs4680 and *OPRM1*A118G × *COMT* rs4633 on PACU opioid consumption (Table 9). Figure 8 shows the interaction effect of *OPRM1* × *COMT* on PACU opioid consumption for Caucasian subjects who were male, with ankle fracture, with a mean age of 39.9 years, and a mean OR opioid consumption of 0.233 mg/kg/hr. Restricting our analysis to Caucasian subjects changed the direction of the regression coefficient for the interaction effect of *OPRM1*A118G × APS and *OPRM1*A118G × HPS haplotypes. However, changes were small and not statistically significant. Table 10 represents the changes in regression statistics for PACU opioid (mg/kg) when the analysis is restricted to Caucasian subjects.

Table 9. Regression analysis summary for predicting PACU opioid (mg/kg) by *OPRM1*× *COMT* (Caucasian only sample)

Interaction Variable	n	b	95%CI	Beta	p-value	sr^2
OPRM1 A118G×*COMT* rs4680 (Recessive)	96	0.108	(0.017,0.200)	0.300	.021	.047
OPRM1 A118G×*COMT* rs4633 (Recessive)	93	0.102	(0.006,0.199)	0.264	.038	.039
OPRM1 A118G×*COMT* rs4818 (Dominant)	93	-0.058	(-0.153,0.036)	-0.217	.223	.014
OPRM1 A118G×*COMT* rs6269 (Recessive)	92	-0.070	(-0.163,0.023)	-0.264	.136	.021
OPRM1 A118G×*COMT* LPS Haplotype	96	-0.059	(-0.149,0.031)	-0.208	. 194	.015
OPRM1 A118G×*COMT* APS Haplotype	96	-0.015	(-0.1060,.077)	-0.057	. 750	.<.001
OPRM1 A118G×*COMT* HPS Haplotype	96	-0.073	(-0.191,0.045)	-0.155	.222	.013
OPRM1 A118G×*COMT* Diplotypes	96	0.047	(-0.040,0.135)	0.154	.285	.010

Interaction analysis controlled for the main effects of both *OPRM1* and *COMT*, gender, age, smoking, fracture type and OR opioid consumption. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

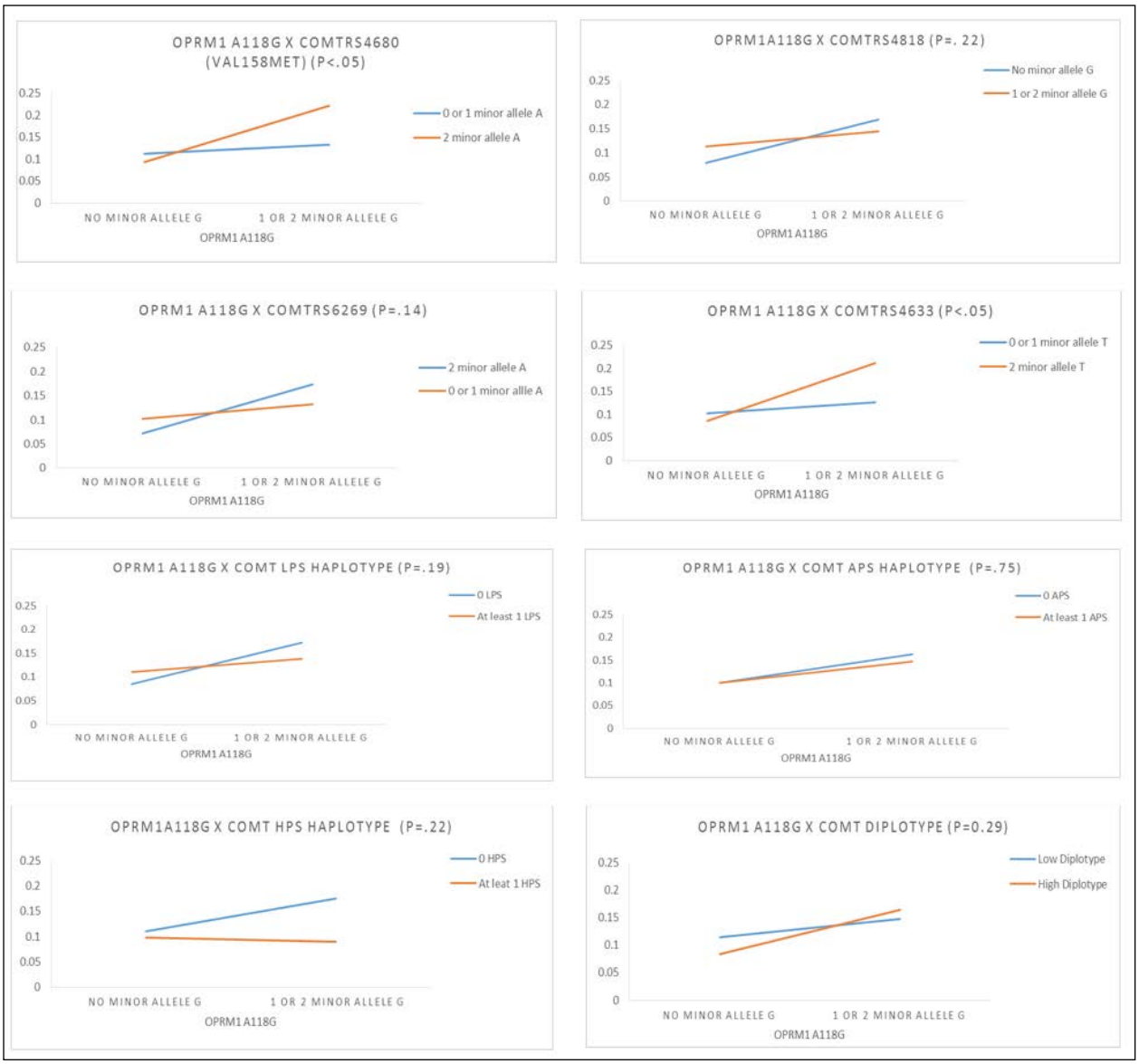


Figure 8. The interaction effect of *OPRM1× COMT* (SNPs, haplotypes, and diplotypes) on PACU opioid using Caucasian sample

Graphs display the *OPRM1 × COMT* interaction effects for the typical Caucasian subject in our sample; male, with ankle fracture, a mean age of 39.9 years, and a mean OR opioid consumption of 0.233mg/kg/hr. , LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity.

Table 10. Changes and percentage changes in PACU opioid regression statistics when the sample is restricted to Caucasians

Interaction Variable	b_T*	b_C**	%***	SE_T	SE_C	%	p_T	p_C
OPRM1 A118G×*COMT* rs4680(Recessive)	.093	.108	16.1	.044	.046	4.5	.037	.021
OPRM1 A118G×*COMT* rs4633(Recessive)	.097	.102	5.2	.046	.049	6.5	.037	.038
OPRM1 A118G×*COMT* rs4818(Dominant)	-.044	-.058	31.8	.041	.048	17.1	.284	.223
OPRM1 A118G×*COMT* rs6269(Recessive)	-.075	-.070	6.7	.043	.047	9.3	.082	.136
OPRM1 A118G×*COMT* LPS Haplotype	-.047	-.059	25.5	.039	.045	15.4	.230	.194
OPRM1 A118G×*COMT* APS Haplotype	. 009	-.015	266.7	.039	.046	17.9	.818	.750
OPRM1 A118G×*COMT* HPS Haplotype	.001	-.073	7400	.059	.059	20.4	.982	.222
OPRM1 A118G×*COMT* Diplotypes	.071	.047	33.8	.039	.044	12.8	.070	.285

T*: Total sample, C**: Caucasian sample, %***: Percentage change. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

4.1.5 *OPRM1* × *COMT* effect on postoperative pain

The results of the regression analysis for predicting postoperative pain at 45 minutes in PACU by *OPRM1* × *COMT* are summarized in Table 11. In addition to the main effects of both *OPRM1* and *COMT*, the interaction analysis controlled for gender, race, age, smoking, fracture type, preoperative pain, OR opioid consumption and opioid consumption during the first 45 minutes in PACU. Figure 9 shows the interaction effect of *OPRM1* × *COMT* on postoperative pain where the pain variable was reflected and transformed using square root to meet the assumption of normality of model errors. Interaction graph represents the typical subjects in our sample; Caucasian, male, with ankle fracture, a mean age of 38.9, a mean OR opioid consumption of

0.246 mg/kg/hr, and a mean opioid consumption during the first 45 minutes in PACU of.08 mg/kg.

Table 11. Regression analysis summary for predicting postoperative pain by *OPRM1* × *COMT* (Total sample)

Interaction Variable	n	b	95%CI	β	p-value	sr^2
OPRM1 A118G×*COMT* rs4680 (Recessive)	121	0.444	(-0.436,1.324)	0.110	.319	.007
OPRM1 A118G×*COMT* rs4633 (Recessive)	118	0.359	(-0.559,1.276)	0.083	.440	.004
OPRM1 A118G×*COMT* rs4818 (Dominant)	117	-0.755	(-1.543,0.033)	-0.276	.060	.024
OPRM1 A118G×*COMT* rs6269 (Recessive)	116	-0.500	(-1.297,0.296)	-0.199	.216	.010
OPRM1 A118G×*COMT* LPS Haplotype	121	-0.926	(-1.686, -0.166)	-0.324	.017	.037
OPRM1 A118G×*COMT* APS Haplotype	121	0.288	(-0.510,1.085)	0.106	.476	.003
OPRM1 A118G×*COMT* HPS Haplotype	121	0.596	(-0.372,1.563)	0.137	.225	.010
OPRM1 A118G×*COMT* Diplotypes	121	0.637	(-0.132,1.407)	0.203	.103	.017

Interaction analysis controlled for the main effects of both *OPRM1* and *COMT* gender, race, age, smoking, fracture type, preoperative pain, OR opioid consumption and opioid consumption during the first 45 minutes in PACU.
LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

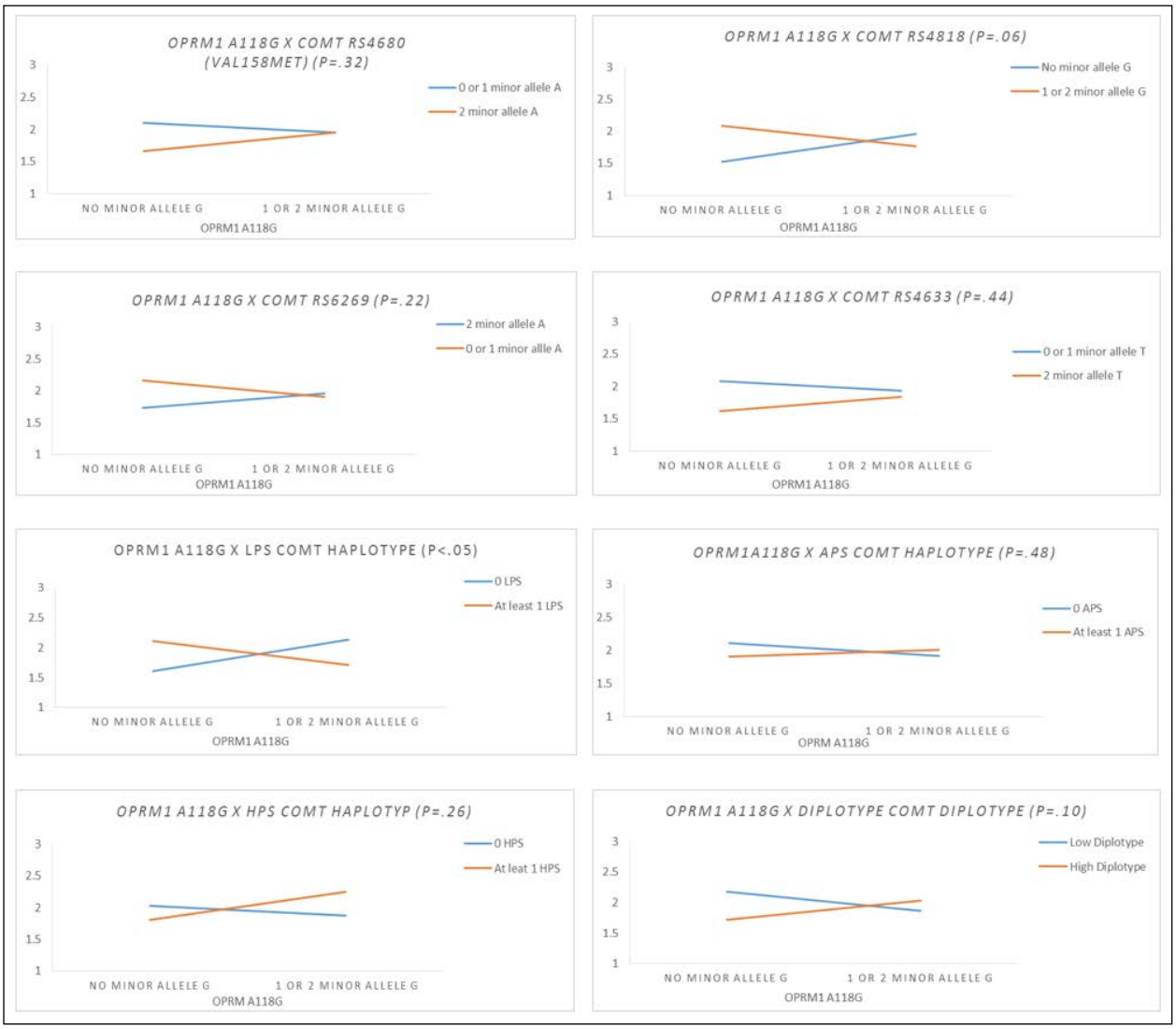


Figure 9. The interaction effect of *OPRM1* × *COMT* (SNPs, haplotypes, and diplotypes) on postoperative pain at 45 minutes in PACU using total sample

Graphs display the *OPRM1* × *COMT* interaction effects for the typical subject in our sample; Caucasian, male, with ankle fracture, a mean age of 38.9, a mean OR opioid consumption of .246mg/kg/hr and a mean opioid consumption during the first 45 minutes in PACU of.08 mg/kg. Note: Pain variable was reflected and transformed using square root to meet the assumption of normality. , LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity.

A significant interaction was found between *COMT* LPS haplotype and *OPRM1* A118G assuming a dominant genetic model (A118G/G118G vs. A118A), b = -0.93, p < .05. The effect of *OPRM1* on postoperative pain varies across levels of *COMT* LPS haplotype. Considering having at least of one copy of LPS haplotype, there was a significant increase in pain score as we move from the wild type of *OPRM1* (AA) to variants (AG/GG). Patients having at least one copy of *COMT* LPS haplotype and minor allele variants (AG/GG) of *OPRM1*A118G experienced more pain compared with patients having at least one copy of *COMT* LPS haplotype and AA of *OPRM1*A118G. However, not having the LPS haplotype was associated with the opposite direction, that is, there was a significant decrease in pain score as we move from the wild type of *OPRM1* (AA) to variants (AG/GG). *OPRM1*A118G × *COMT* LPS haplotype accounted uniquely for 3.7% of the total postoperative pain variance. No significant interaction was found between *OPRM1* and other *COMT* haplotypes and diplotypes. However, *COMT* diplotypes were significantly associated with postoperative pain in PACU, b = -0.321, p < .05, sr^2 = .03. Patients having the high pain sensitivity diplotype experienced significantly more postoperative pain compared to patients having the low pain sensitivity diplotype, b=-0.321, 95%CI = [-0.636, -0.005].

No significant interaction was found between *OPRM1* and *COMT* SNPs. However, the interaction of *COMT* rs4818 and *OPRM1* assuming dominant genetic models was leaning towards significance (p = .06). The *COMT* SNP rs4633, assuming a recessive genetic model, was significantly associated with postoperative pain in PACU, b = -0.389, p<.05, sr^2 = .03. Patients with two minor alleles T (TT) significantly experienced more postoperative pain at 45 minutes in PACU compared to patients having CT or CC, b=-0.389, 95%CI = [-0.759, -0.019]. Moreover, *COMT* rs4818 assuming a dominant genetic model was also significantly associated with

postoperative pain in PACU, b = 0.387, $p < .05$, $sr^2 = .04$. Patients having one or two minor alleles G (GC and GG) significantly experienced more postoperative pain compared to patients having CC, b = 0.387, 95%CI = [0.051, 0.723]. The main effects of *COMT* rs4680 and rs6269 assuming recessive genetic models were leaning towards significance with p-values of .063 and .055, respectively.

None of *OPRM1*A118G × *COMT* SNPs, haplotypes and diplotypes was significantly associated with postoperative pain when the analysis was limited to Caucasian subjects (Table 12). Figure 10 shows the interaction effect of *OPRM1* × *COMT* on postoperative pain for the average/typical subject who was Caucasian and male, with ankle fracture, with a mean age of 39.9 years, a mean OR opioid consumption of 0.233mg/kg/hr and a mean opioid consumption during the first 45 minutes in PACU of 0.08 mg/kg. The interaction effects for *OPRM1*A118G × LPS, *OPRM1*A118G × HPS, and *OPRM1*A118G × diplotypes were trending towards significance ($p = .07$, $p = .058$, $p = .05$, respectively). Table 13 represents the changes in regression statistics for postoperative pain data restricted to Caucasian subjects.

Table 12. Regression analysis summary for predicting postoperative pain in PACU by *OPRM1*× *COMT* (Caucasian only sample)

Interaction Variable	n	b	95%CI	Beta	p-value	sr^2
OPRM1 A118G×*COMT* rs4680 (Recessive)	95	0.421	(-0.501,1.342)	0.121	.366	.007
OPRM1 A118G×*COMT* rs4633 (Recessive)	92	0.419	(-0.526,1.364)	0.113	.380	.007
OPRM1 A118G×*COMT* rs4818 (Dominant)	92	-0.486	(-1.390,0.420)	-0.186	.289	.010
OPRM1 A118G×*COMT* rs6269 (Recessive)	91	-0.640	(-1.470,0.188)	-0.261	.128	.020
OPRM1 A118G×*COMT* LPS Haplotype	95	-0.794	(-1.660,0.067)	-0.291	.070	.023
OPRM1 A118G×*COMT* APS Haplotype	95	0.080	(-0.811,0.971)	0.032	.858	<.001
OPRM1 A118G×*COMT* HPS Haplotype	95	1.110	(-0.04,2.261)	0.246	.058	.031
OPRM1 A118G×*COMT* Diplotypes	95	0.831	(-0.001,1.662)	0.281	.050	.033

Interaction analysis controlled for the main effects of both *OPRM1* and *COMT* gender, age, smoking, fracture type, preoperative pain, OR opioid consumption and opioid consumption during the first 45 minutes in PACU. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity.

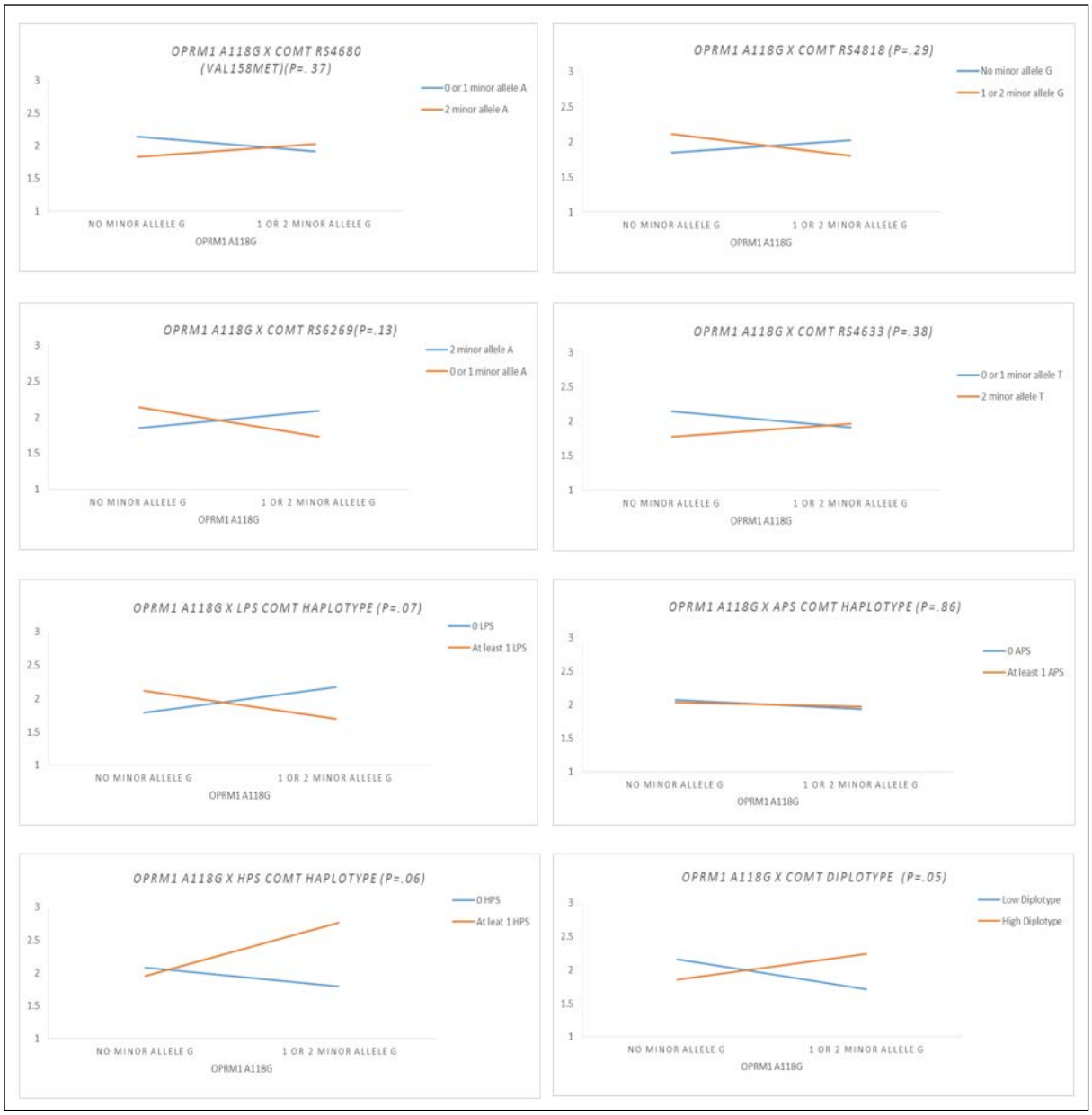


Figure 10. The interaction effect of *OPRM1* × *COMT* (SNPs, haplotypes, and diplotypes) on pain at 45 minutes in PACU for Caucasian subjects

Graphs display the *OPRM1* × CPOMT interaction effects for the typical Caucasian subject who is male, with ankle fracture, a mean age of 39.9, a mean OR opioid consumption of .233 mg/kg/hr and a mean opioid consumption during the first 45 minutes in PACU of.08 mg/kg. Note: Pain variable was reflected and transformed using square root to meet the assumption of normality. , LPS= Low Pain Sensitivity; APS= Average Pain Sensitivity; HPS= High Pain Sensitivity.

Table 13. Changes and percentage changes in regression statistics of postoperative pain when the sample is restricted to Caucasians

Interaction Variable	b_T*	b_C**	%***	SE_T	SE_C	%	p_T	p_C
OPRM1 A118G×*COMT* rs4680(Recessive)	.444	.421	5.2	.444	.463	4.3	.319	.366
OPRM1 A118G×*COMT* rs4633(Recessive)	.359	.419	16.7	.463	.475	2.6	.440	.380
OPRM1 A118G×*COMT* rs4818(Dominant)	-.755	-.486	35.6	.398	.455	14.3	.060	.289
OPRM1 A118G×*COMT* rs6269(Recessive)	-.500	-.640	28.0	.401	.416	3.7	.216	.128
OPRM1 A118G×*COMT* LPS Haplotype	-.926	-.794	14.3	.383	.433	13.1	.017	.070
OPRM1 A118G×*COMT* APS Haplotype	.288	.080	72.2	.402	.448	11.4	.476	.858
OPRM1 A118G×*COMT* HPS Haplotype	.596	1.11	86.4	.488	.578	18.4	.225	.058
OPRM1 A118G×*COMT* Diplotypes	.637	.831	30.5	.388	.418	7.7	.103	.050

T*: Total sample, W**: Caucasian sample, %***: Percentage change. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity.

4.2 DISCUSSION

The purpose of this study was to explore *OPRM1* and *COMT* gene-gene interaction effects on postoperative pain score and opioid consumption. Our findings showed that the interaction between *OPRM1* and *COMT* partially explained the inter-individual variability in postoperative pain and response to opioids. To our knowledge, this is the first study to investigate the interaction of four *COMT* SNPs (rs6269, rs4633, rs4818 and rs4680) as well as *COMT* haplotypes and diplotypes with *OPRM1* A118G SNPs to explain the variability in the pain phenotype. We found that combining the minor allele variants of both *OPRM1* A118G and *COMT* Val158Met there was a poor response to opioid analgesia. Patients with the Met158Met

of *COMT* Val158Met and (AG/GG) of *OPRM1* A118G combination consumed more opioid compared to patients with other combinations. Yao and colleagues found that cancer patients with this combination experienced higher preoperative pain sensitivity compared to other genotype combinations (Yao et al., 2015), in which, they had a lower pain threshold and pain tolerance threshold. Consistent with previous work of Reyes-Gibby et al, we found that carriers of the *OPRM1* AA and *COMT* Met/Met genotype required the lowest opioid dose compared to other combinations (Reyes-Gibby et al., 2007). However, Landau et al. found that women with *OPRM1* AA and *COMT* Met/Met genotype were associated with lower decrease in NVPS at 15 minutes after single fentanyl dose during labor and delivery (Landau et al., 2013).

This study is the first to include the interaction between *OPRM1* A118G and silent SNPs of *COMT* (rs4633, rs4818 and rs6269). We found that patients having TT of *COMT* rs4633 and (AG/GG) of *OPRM1* A118G consumed the largest amount of opioids compared to other combinations. Recent genetic studies focused on investigating haplotype reconstruction suggesting that combinations/ interactions of SNPs within haplotypes resulted in synergistic effects on the resultant protein and it might result in functional consequences that are different from the independent effects of those SNPs (Diatchenko et al., 2005; Duan et al., 2003). Thus, we expanded our analysis by including *COMT* haplotypes and diplotypes. We found that the Low Pain Sensitivity (LPS) haplotype has a "protective" effect on pain when combined with the wild type of *OPRM1*. Patients with the combination of *OPRM1* A118A and LPS haplotype reported the lowest pain score compared to all other combinations. Moreover, patients with the high pain sensitivity diplotype reported higher pain compared to those with the low pain sensitivity diplotype.

It has been found that the synonymous" silent" SNPs of *COMT* gene could affect mRNA stability and consequently *COMT* protein expression and enzymatic activity (Nackley et al., 2006). Previous pain genetic studies showed that silent SNPs of the *COMT* gene (rs4633, rs4818 and rs6269) influence pain sensitivity and opioid efficacy (Lee et al., 2011; Rut et al., 2014). Consistent with that, we found that the two synonymous SNPs of *COMT*, rs4633 and rs4818, were significantly associated with postoperative pain in the PACU. The TT of rs4633 and AG/GG of rs4818 interaction was associated with higher pain sensitivity compared to other combinations for these SNPs.

Some of our findings on postoperative pain were changed after restricting the analyses to Caucasians. For example, the significant interaction of *OPRM1* and LPS haplotype using the total sample was not significant in Caucasians. Both OPRM1×ACCG and *OPRM1*×diplotypes showed a trend toward significance in Caucasians that was not observed when using the total sample. The changes in our findings might be partly due to the variation in the allele and haplotype frequencies among the different racial populations (Beuten, Payne, Ma, & Li, 2006; DeMille et al., 2002; Gabriel et al., 2002; Hastie et al., 2012; Palmatier, Kang, & Kidd, 1999). In conclusion, our findings suggest the *OPRM1*×*COMT* gene-gene interaction may contribute to the variability of postoperative pain and response to opioids. Future studies with larger sample sizes and more diversity are needed to confirm these effects.

5.0 MANUSCRIPT #1: GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS PREDICT PAIN PHENOTYPES: A REVIEW OF THE LITERATURE

5.1 ABSTRACT

Purpose: The aim of this paper was to review the existing literature in the area of gene-gene and gene-environment interactions related to pain phenotypes.

Organizing Construct: This review examined previous literature in the area of gene-gene and gene-environment interactions related to pain. The review focuses on the gene-gene interaction of *OPRM1* and *COMT* and its effect on predicting pain and opioid efficacy, and finally it reviews the effects of other gene-gene and gene-environment interactions related to phenotypes of pain.

Findings: Gene-gene and gene-environment interactions are significant factors affecting the various disease susceptibility and predicting complex pharmacologic responses including pain sensitivity and opioid efficacy. A single gene alone is not likely to be responsible for all variation in pain sensitivity or opioid efficacy. But more of the variation in responses to pain may be able to be explained by evaluating the interaction of various genes and environmental factors associated with pain pathways.

Conclusion: Gene-gene and gene-environment interactions may be a way to explain the complex mechanisms associated with the various phenotypes including pain and opioid efficacy.

Clinical Relevance: Exploring the gene-gene and gene-environment interactions may lead to more precise risk prediction of pain phenotype. Subsequently, it could improve current strategies to personalized pain management. Personalized pain management can be an effective strategy to maximize the usefulness of pain treatment and minimize complications and adverse effects associated with opioids.

Key Words: gene interaction, epistasis, gene-environment interactions, pain, analgesics, opioid.

5.2 INTRODUCTION

The complex relationship between genotype and phenotype is partially explained by the interactions among genes. At the cellular level, gene-gene interaction is the interaction between alleles at different loci to express a single trait. However, from a statistical standpoint, gene-gene interaction represents the deviation from a statistical additive mode that describes how two or more variables predict a phenotype (Fisher, 1918). The integration of the statistical interaction models into the biological mechanisms is still challenging.

Gene-gene interaction is a significant factor that affects various human traits, disorders, diseases, and drug responsiveness (Dean, 2003; Dean et al., 1996; Rivolta, Sharon, DeAngelis, & Dryja, 2002; Shin et al., 2000; Smith et al., 1997). In this review, we provided a brief introduction of gene-gene interaction role in predicting human complex diseases and pharmacologic treatment response. Then we focus on the effect of *OPRM1* and *COMT* gene-gene interaction on the phenotypes of pain and opioid efficacy. We discussed evidence to support the importance of the interaction of these genes in predicting pain and opioid efficacy.

Finally, we highlighted evidence for other gene-gene and gene-environment interactions related to pain phenotype.

5.3 GENE INTERACTIONS PREDICT VARIOUS HUMAN COMPLEX DISEASES AND PHARMACOLOGIC TREATMENT RESPONSE

Complex human traits involve various biological and biochemical pathways, each is influenced by multiple genes. Thus, examining the gene interactions instead of a single gene might explicate the disease underlying biological mechanisms and pathways, which in turn, improve the understanding of complex disease etiology. Various genetics studies have described the effect of gene-gene interaction on human complex diseases such as asthma, cancers, diabetes, inflammatory bowel disease, psychiatric disorders, systemic lupus erythematosus and others (Chan et al., 2008; Chen et al., 2009; Hughes et al., 2012; Lin, Hong, et al., 2009; Manso et al., 2012; Polgar et al., 2012; Ryu et al., 2011; Turnbull et al., 2012; Xiao et al., 2011).

Gene-gene interaction has also played an important role in predicting complex pharmacologic treatment responses and mechanisms (Lane, Tsai, & Lin, 2012). It has helped in predicting high risk individual for drug under-treatment or over-treatment. Evidence has shown that gene-gene interaction has been significantly associated with treatment responsiveness of various drugs including; IFNa and ribavirin for treating Chronic Hepatitis C (CHC) (Lin, Hwang, & Chen, 2007), Sibutramine for weight loss (Hsiao, Wu, Hwang, Huang, & Lin, 2010) , albuterol for asthma treatment (Choudhry et al., 2010; Corvol et al., 2009), and methotrexate for rheumatoid arthritis therapy (Sharma et al., 2008)..Etc.

5.4 THE INTERACTIONS BETWEEN LINKED GENES IS A VALUED STRATEGY TO BE CONSIDERED TO BETTER EXPLAIN HUMAN TRAITS

Genetic studies often consider the effect of individual genes or haplotypes on phenotypes ignoring the possible effect of their interactions. However, evidence shows that gene-gene interaction can significantly predict various phenotypes in the absence of an independent main effect of a single gene (Manso et al., 2012; Moore & Williams, 2002; Nelson, Kardia, Ferrell, & Sing, 2001). Nelson and colleagues identified gene-gene interaction between multiple loci from six cardiovascular disease susceptibility genes, in which gene-gene interactions explained the inter-individual variability in plasma triglycerides whereas evaluating only SNPs failed to predict such variability (Nelson et al., 2001). Manso and colleagues investigated the contribution of three growth factor genes; BDNF, FGF2, and VEGFA on recovery after stroke, while none of those genes was independently associated with stroke outcome, two significant gene–gene interactions were identified (Manso et al., 2012). A pharmacogenomics study of major depressive disorder evaluated four serotonin-related genes (GNB3, HTR1A, HTR2A, and SLC6A4) on antidepressant treatment outcomes. Only one SNP of four (GNB3 rs5443) was found to be associated with antidepressant treatment outcome whereas the interaction analyses revealed that there were 2 significant locus gene-gene interactions between GNB3 and HTR2A, as well as a significant 3rd locus gene-gene interaction among GNB3, HTR2A, and SLC6A4. Those finding suggesting that serotonin-related genes contribute to the short-term antidepressant treatment outcome in an interactive manner (Lin, Chen, et al., 2009). Taken together, complex human traits might not explained only by the independent main effect of genes. Exploring the gene-gene interaction may lead to more precise risk prediction of human traits.

5.5 GENE-GENE INTERACTIONS IN PAIN GENETICS STUDIES

According to the Pain Genes Database, 300 candidate genes have been identified as being associated with pain (Lacroix-Fralish, Ledoux, & Mogil, 2007). Genetic variants in those genes are known to affect pain sensitivity, analgesic efficacy, and the incidence of analgesia adverse effects such as, nausea, vomiting and sedation (Stamer & Stuber, 2007). Pain is a complex phenomenon that might be better explained with gene-gene interactions rather than with a single SNP. Therefore, gene-gene interaction may influence the human experience of pain and explain the inter-individual differences. However, gene interaction studies of pain and opioid efficacy are limited. Few studies investigated the gene–gene interaction of pain related gene; *OPRM1*, *COMT*, CYP3A4 and ABCB1/MDR1 on pain sensitivity and opioid efficacy (Campa, Gioia, Tomei, Poli, & Barale, 2008; De Gregori et al., 2013; Kolesnikov, Gabovits, Levin, Voiko, & Veske, 2011; Landau, Liu, Blouin, & Carvalho, 2013; Liao et al., 2013; Matic et al., 2014; Reyes-Gibby et al., 2007; Yao et al., 2015). *OPRM1* and *COMT* are by far the most extensively studied pain genes. Thus, the focus of this paper is the interaction between those genes. The following sections highlight some of evidence to support the importance of *OPRM1* and *COMT* and their interaction effects on predicting pain sensitivity and opioid efficacy.

5.5.1 *OPRM1* and *COMT* are considered the genes most associated with pain sensitivity and opioid efficacy.

The main effect of each of *OPRM1* and *COMT* genes on pain sensitivity and opioid efficacy are well established in the pain literature. *OPRM1* is the gene that encodes for the mu receptor, the primary site of action for endogenous and exogenous opioids (Bond et al., 1998; Zadina,

Hackler, Ge, & Kastin, 1997). The A118G polymorphism is the most common single nucleotide polymorphism (SNP) of *OPRM1*. In this mutation asparagine is changed to aspartic acid at position 40 of the resultant gene product. This substitution results in less mRNA expression in brain tissues of G allele carriers (Y. Zhang et al., 2005). Moreover, the G118 has a secondary structure alteration that affects its expression and translation into functional protein; mRNA of G118 showed a well predicted helix that does not exist in the other variant structures. Furthermore, the G118 mRNA structures does not contain a loop motif that all *OPRM1* variants commonly contain (Zhang, Wang, Johnson, Papp, & Sadee, 2005). Given this finding, carriers of the G allele were found to experience more pain and need higher opioid dose to achieve adequate pain control compared with those with the wild-type A118 (Chou, Yang, et al., 2006; Sia et al., 2008; Tan et al., 2009).

Catechol-O-methyltransferase (*COMT*) is one of several enzymes responsible for metabolizing and degrading catecholamines. Genetic variants in the gene coding for *COMT* have been found to influence opioid pathways. *COMT* Val158Met is the most common SNP of *COMT* in which the substitution of valine to methionine at codon 158 leads to a 3- to 4-fold decrease in *COMT* activity (Lotta et al., 1995). The Met/Met genotype is associated with the lowest activity of *COMT* enzyme, followed by Met/Val and Val/Val genotypes with the intermediate and highest activity respectively. It has been shown that the decrease in enzyme activity reduces the content of enkephalins in certain brain regions and consequently increase pain sensitivity (Zubieta et al., 2003). In view of this finding, clinical studies have shown the patients with homozygous Met experience more pain compared with Val/Val and Val/Met (Kolesnikov et al., 2011; Rakvag et al., 2005).

5.5.2 Both genes interact on the neurotransmission pathways of pain

Cumulative evidence shows that *COMT* Val158Met affects mu receptor (*OPRM1*) availability, expression and density in brain tissue by affecting enkephalin levels, which inversely regulate mu receptor expression. Accordingly, the number of mu-opioid receptor binding sites is affected by *COMT* Val158 Met polymorphism such that *COMT* Met158 allele carriers are significantly associated with the highest mu receptor expression and lowest enkephalin peptide levels compared with other *COMT* Val158 Met genotypes. (Berthele et al., 2005; Kowarik et al., 2012; Zubieta et al., 2003).

COMT Val158Met has been shown to affect the number of binding sites of mu opioid receptors using Positron Emission Tomography (PET) and the radiotracer [11C] carfentanil, a mu receptor ligand. Met158 allele of *COMT* Val158Met demonstrated highest binding potential of [11C] carfentanil compared with the Val158 homozygotes. The high levels of dopamine are caused by Met at position 158 in the *COMT* protein led to a reduction in enkephalin levels and consequently, mu receptor expression is upregulated (Zubieta et al., 2003). Ligand binding autoradiography ([3H] DAMGO receptor) in post-mortem tissue has also been used to confirm the effect of *COMT* Val158Met polymorphism on mu receptor binding site expression in the human brain. Met158 allele of *COMT* was associated with a higher expression of mu binding sites in various brain regions including the caudate nucleus, the nucleus accumbens and the mediodorsal nucleus of the thalamus (Berthele et al., 2005). Recently, Kowarik and colleagues examined the effect of the *COMT* Val158Met polymorphism on mu receptor using semiquantitative immunostaining on post-mortem human brain tissues. The Met158 homozygotes expressed significantly more mu receptor protein than Val158 homozygotes in human basal ganglia and thalamic tissues. Moreover, the lowest levels of enkephalin peptide

were found in Met158 homozygote tissues, whereas Val158 homozygotes showed the highest peptide levels. Accordingly, the *COMT* Val158Met polymorphism might influence the mu receptor expression through use-dependent down/up-regulation secondary to altered enkephalin levels (Kowarik et al, 2012). Since Interactions between the dopaminergic and opioidergic system are recognized as explained above, gene-gene interaction of *COMT* and *OPRM1* may influence the human experience of pain and explain the inter-individual differences.

5.5.3 Findings of previous studies investigating the main effects of *OPRM1* or *COMT* failed to be replicated

Several studies have investigated the association between the single gene effects of *OPRM1* or *COMT* and the variability in acute or chronic pain and opioid efficacy. However, overall contradictory results have been obtained (Campa et al., 2008; Chou, Wang, et al., 2006; Chou, Yang, et al., 2006; Coulbault et al., 2006; Hayashida et al., 2008; R. A. Henker et al., 2012; Janicki et al., 2006; Kim, Clark, & Dionne, 2009; Kim et al., 2004; Klepstad et al., 2004; Kolesnikov et al., 2011; Rakvag et al., 2005; Rakvag et al., 2008; Reyes-Gibby et al., 2007; Ross et al., 2008; Tan et al., 2009). For instance, examining the genetic effect of A118G SNP of *OPRM1* on pain and response to opioids has shown that patients with the *OPRM1* A118G polymorphism who were homozygous for the variant G allele were reported to need more morphine to achieve adequate post-operative pain control compared with the those homozygous for the wild-type A118 allele (Chou, Wang, et al., 2006; Chou, Yang, et al., 2006; Hayashida et al., 2008; Sia et al., 2008; Tan et al., 2009). However, other studies did not find a statistically significant association between presence of the A118G polymorphism and the dose of opioid

required for pain relief (Coulbault et al., 2006; Richard A. Henker; Janicki et al., 2006; Kolesnikov et al., 2011).

Conflicting results were also found among studies investigating the impact of the *COMT* gene on pain sensitivity and opioid efficacy. Some genetic studies found that patients with the Val/Val genotype consumed a significantly higher opioid dose to manage their pain, compared to those with the Met/Met and Val/Met genotypes (Henker et al., 2013; Rakvag et al., 2005). However, other studies did not find the same association between *COMT* Val158Met presence and opioid dose requirement (Kolesnikov et al., 2011; Ross et al., 2008).

The inability to replicate prior genetics studies investigating the main effect of *OPRM1* or *COMT* has several explanations. First, previous studies included heterogeneous samples using different racial/ethnic groups, different diagnoses and different surgical operations. Second, the relatively small sample sizes in prior studies might not have the sufficient statistical power to adequately detect the differences among polymorphisms. Third, the low frequency of the rare G118 allele in some of prior studies investigating *OPRM1* A118G might also reduce the statistical power. Finally, prior studies considered the effect of individual genes or haplotypes ignoring the possible effects of their interactions. The failure of single locus studies' findings to be replicated may indicate that the impact of gene-gene interaction is more crucial than the single SNPs effect. Since gene effects alone may not be responsible for all variation in pain and opioids efficacy. The interaction of various pain genes may ensure better explanation for wide variability in pain sensitivity and opioid efficacy (Kolesnikov et al., 2011; Reyes-Gibby et al., 2007).

5.5.4 Gene-gene interaction of *OPRM1* and *COMT* is one of the most extensively studied gene interactions in relation to the pain phenotype

The interaction effect of *COMT* Val158Met and *OPRM1* A118G has been found to explain variability in acute postoperative or chronic cancer pain sensitivity and opioid efficacy (De Gregori et al., 2013; Kolesnikov et al., 2011; Landau et al., 2013; Matic et al., 2014; Reyes-Gibby et al., 2007; Yao et al., 2015). Regarding chronic cancer pain; Reyes-Gibby et al found that cancer patients who carry *OPRM1* A118A and *COMT* Met158Met genotype required the lowest morphine dose to achieve adequate pain control. On the other hand, the patients who did not carry Met158Met or A118A genotype at all requested the highest morphine dose. Moreover, the morphine requirement in the joint effect of both *OPRM1* and *COMT* genotype was lower than in the single effect for these SNPs alone (Reyes-Gibby et al., 2007). Yao and colleagues found that cancer patients undergoing elective surgery with the combination of G118G of *OPRM1* and Met158Met of *COMT* had a more significant decrease in pain threshold and pain tolerance (Yao et al., 2015).

Four studies were found to investigate the gene-gene interaction between *OPRM1* A118G and *COMT* Val158Met in relation to acute postoperative pain. Kolesnikov et al investigated the combined effect of *OPRM1* A118G and *COMT* Val 158Met on postoperative pain and opioid efficacy after abdominal surgery (Kolesnikov et al., 2011). Patients with A118G and Val158Met combination needed significantly fewer morphine doses, approximately 18% less morphine, during the first 48 hours postoperatively compared with *OPRM1* A118A (Kolesnikov et al., 2011). De Gregori et al examined the interaction effect of these genes on opioid consumption after major abdominal and urological surgery. However, no significant differences in opioid consumption during the first 24 postoperative hours was found (De Gregori et al., 2013).

Recently, Landau et al investigated the effect of *COMT* and *OPRM1* on analgesic response to intravenous fentanyl during labor and delivery. It has been found that women with the combination of *OPRM1* A118A and *COMT* Met158Met have lower decrease in numerical verbal pain score (NVPS) at 15 minutes after receiving an IV fentanyl dose compared to other genotype combinations. However, there was no significant differences in term of analgesic response to IV fentanyl between women with the *OPRM1* A118A and *COMT* Met158Met combination compared to women with other allelic combination (Landau et al., 2013). Lastly, Matic et al. investigated the effect of *COMT* and *OPRM1* on rescue morphine requirement (yes/no) and total morphine consumption in newborns under mechanical ventilation (Matic et al., 2014). The combination of *OPRM1* 118G allele and *COMT* Val158Val was significantly associated with the need for morphine rescue. However, this combination was not associated with total morphine consumption.

The findings of previous genetic studies investigating the interaction effects between *OPRM1* and *COMT* have not been replicated. Moreover, findings from those studies are troublesome to compare because of the major differences among studies. Previous studies investigating the interaction between *OPRM1* and *COMT* used different types and causes of pain; (acute postoperative versus chronic cancer pain), (preoperative versus postoperative pain), and (experimentally induced pain versus clinical pain). Furthermore, the definition and assessment of the final pain outcome varies among those studies; (pain sensitivity versus pain threshold and pain tolerance) and (pain at specific time point versus the decrease in pain from baseline). Finally, various opioid regimens were used to examine pain response including; long-term opioid therapy, short-term postoperative therapy, single opioid dose and rescue morphine

requirement. These differences make it difficult to compare findings from those studies and draw general conclusions about the interaction effect of *OPRM1* and *COMT* genes on pain.

5.5.5 Other evidence of gene-gene interactions in the area of pain genetics

Genetic pain studies have investigated the interactions of other genes involved in pain sensitivity and opioid efficacy. Two studies were conducted to investigate the interaction effect of *OPRM1* A118G and *ABCB1*/*MDR1*on pain-related phenotypes. The *ABCB1*/*MDR1* gene codes for a P-glycoprotein efflux transporter, which transports various substrates including drugs across the cell membrane and blood–brain barrier (Mizutani et al., 2008; Wandel, Kim, Wood, & Wood, 2002). The first study investigated the interaction effect of *OPRM1* A118G and *ABCB1* C3435T in term of pain relief after morphine administration for cancer patients (Campa et al., 2008). No significant interaction between of *OPRM1* A118G and *ABCB1*/*MDR1* C3435T was found. However, a simple additive effect of single allele combination has been shown. For instance, cancer patients with the combined *ABCB1* TT and *OPRM1* AA showed significantly greater pain relief after morphine administration than patients with either *ABCB1* TT or *OPRM1* AA. The second study investigated the interaction of *OPRM1* A118G and *ABCB1* haplotypes on methadone dose and trough plasma (R)-methadone concentrations (C_{trough}) for opioid-dependent subjects (Barratt et al., 2012). Among *OPRM1* A118A subjects, there was a significant difference in methadone dose and C_{trough} between *ABCB1* haplotype groups, with the AGCTT variant haplotype (61A; 1199G; 1236C; 2677T; 3435T) associated with a significantly lower dose and C_{trough} than the wild-type *ABCB1* subjects. Among subjects with the *ABCB1* AGCGC/AGTTT haplotype combination, the *OPRM1* A118G genotype was associated with significantly higher C_{trough} than the A118A.

Liao and colleagues investigated the interaction between *OPRM1* and *CYP3A4* on postoperative fentanyl analgesia in Chinese Han patients undergoing radical gastrectomy (Liao et al., 2013). *CYP3A4* encodes the cytochrome P450 enzymes which catalyze many reactions involved in drug metabolism (Labroo, Paine, Thummel, & Kharasch, 1997). A significant interaction between *OPRM1* A118G and *CYP3A4**18B polymorphisms was found, in which patient with combined AG of *OPRM1* A118G and *1*1 of *CYP3A4* received larger fentanyl doses compared with those with AA and *1*18B or those with AG and *1*18B. However, the interaction of *OPRM1* and *CYP3A4* on postoperative nausea, vomiting and dizziness was not significant.

5.6 GENE-ENVIRONMENT INTERACTIONS IN PAIN GENETICS STUDIES

Several studies have investigated the interaction between pain genes and environmental factors to explain the variability in pain sensitivity and opioid efficacy. Hastie and colleagues investigated the ethnic differences in *OPRM1* allelic association with experimental pain responses (Hastie et al., 2012). Three different ethnic groups including African Americans, non-White Hispanics and non-Hispanic whites were included. Across the three ethnic groups, *OPRM1* genotype did not significantly affect pain sensitivity. However, when each ethnic group was analyzed separately there was a significant effect of *OPRM1* for most pain modalities only in the non-Hispanic White group. Specifically, the G allele was associated with decreased pain sensitivity in this group. The author suggested that the ethnicity-dependent association of *OPRM1* with pain sensitivity could be due to linkage with other functional polymorphisms or

gene–gene interactions with other variants with different frequency among ethnic groups (Hastie et al., 2012).

Gene-sex interactions have also been shown to explain the inter-individual variability in pain. Rhodin and colleagues investigated the sex- *ABCB1* C3435T interaction on plasma β-endorphin level in chronic low back pain patients (Rhodin et al., 2013). It has been found that men with the minor TT allele of *ABCB1* had higher β-endorphin levels than men with the major CC allele but the reverse was true for women (Rhodin et al., 2013). Two recent studies have investigated sex -*OPRM1* A118G interaction on pain sensitivity. Olsen et al found that *OPRM1* G allele increases the pain intensity in women, but has a protective effect in men the first year after disc herniation. Moreover, the G allele women had 2.3 times as much pain as the G allele men (Olsen et al., 2012). Hasvik et al found that all patients, with lumbar radicular pain and disc herniation, except female carriers of the *OPRM1* G-allele reported a decrease in pain from baseline to 1 year (Hasvik et al., 2014). Moreover, female carriers of the G-allele reported a significantly higher subjective health complaint score than male carriers of the G-allele when controlling for pain and pain duration (Hasvik et al., 2014). The differences in the type and level of glycosylation site of the mu receptor between men and women is a possible explanation for the sex-specific differences of *OPRM1* (Ding et al., 2011). Belfer and colleagues investigated the sex differences in the effect of *COMT* variants on capsaicin-induced pain in mice and humans (Belfer et al., 2013). The *COMT* High Pain Sensitivity haplotype (ATCA; HPS) was significantly associated with higher pain in females compared to males in both species. Sex differences in *COMT* HPS haplotype effect on pain could possibly be explained by that fact that females have lower *COMT* levels compared to men. Furthermore, males have additional receptor pathways

stimulated by catecholamines that are not as functional in females. Thus, compared with males, females tend to be more sensitive to pain (Belfer et al., 2013).

Interactions between genetic and psychological factors were also found to be predictors of pain**.** George and colleagues found significant interactions between the *COMT* diplotype and pain catastrophizing on postoperative pain for patients receiving operative treatment of shoulder pain (George, Dover, et al., 2008). Patients with high pain catastrophizing and low *COMT* activity (APS/HPS group) were found to be more likely to have post-operative pain ratings of 4.0/10 or higher (George, Wallace, et al., 2008). In another study the same author investigated the interactions between *COMT* diplotype and pain catastrophizing using experimental pain model. Delayed Onset Muscle Soreness (DOMS) was induced at the shoulder (George, Dover, et al., 2008). Findings from this experimental pain model converge with those from their previous clinical pain model; as participants with high pain catastrophizing and low *COMT* enzyme activity (APS/HPS group) were more likely to have elevated pain intensity ratings (40/100 or higher).

5.7 CONCLUSION

Pain is a subjective multidimensional human experience. Thus, interaction of various pain genes and environmental factors may provide better explanation for the wide variety in pain sensitivity and opioid efficacy. Exploring the gene-gene and gene-environment interactions may help establish personalized pain management to achieve effective pain control and minimize risk of opioid adverse effects.

6.0 MANUSCRIPT #2: *OPRM1* AND *COMT* GENE–GENE INTERACTION IS ASSOCIATED WITH POSTOPERATIVE PAIN AND OPIOID CONSUMPTION AFTER ORTHOPEDIC TRAUMA SURGERY

6.1 ABSTRACT

Background: *OPRM1* (mu-opioid receptor) and *COMT* (Catechol-O-Methyltransferase) contribute to the neurotransmission pathway of pain. Evidence shows that *COMT* affects mu receptor availability, expression and density in brain tissue. Thus, investigating the interaction of *COMT* and *OPRM1* on pain and pain management is warranted. The aim of this study was to explore the *OPRM1* and *COMT* interaction on postoperative pain and opioid consumption.

Method: This cross-sectional study used genotype and clinical data from 153 postoperative orthopedic trauma 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*on postoperative pain and opioid consumption. All analyses were performed in the total sample and in Caucasian-only patients.

Results: For postoperative opioid consumption, a significant interaction was found between *OPRM1* A118G and *COMT* rs4680 (p=.037). Patients having Met158Met of *COMT* rs4680 and (AG/GG) of *OPRM1* consumed the largest opioid compared to other combinations. A significant interaction was also found between *OPRM1* and *COMT* rs4633 (p=.037). Patients having TT of

COMT rs4633 and (AG/GG) of *OPRM1* consumed the largest amount of opioid compared to other combinations. The results for *OPRM1*×*COMT* rs4680 and *OPRM1*×*COMT* rs4633 on opioid consumption were maintained even after restricting the analyses to Caucasian subjects. 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 LPS haplotype. When the sample was limited to Caucasian, only a trend was observed for the interaction of *OPRM1* and LPS haplotype on postoperative pain (*p*=.070).

Conclusions and Clinical Relevance: *OPRM1*×*COMT* interaction appears to be important to postoperative pain and response to opioids. Individualized pain management based on genetic variations might improve future strategies for pain management.

6.2 INTRODUCTION

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 (Caudill-Slosberg et al., 2004; Feeney, 2004; Janssen et al., 2004; Joshi & Ogunnaike, 2005). The highly individualized effect of opioids on patients makes optimal pain management challenging. Genetic variations have been suggested as a possible explanation for variation in pain intensity and response to opioids.

The single nucleotide polymorphism (SNP) effects of *OPRM1* and *COMT* gene on the variability in pain and response to opioids are well established. The variant alleles of both

OPRM1 A118G (AG and GG) and *COMT* Val158Met (Met/Met) SNPs have been reported to be independently associated with higher postoperative pain compared to their wild types (Henker et al., 2013; Klepstad et al., 2004; Kolesnikov et al., 2011; Sia et al., 2013; S. Zhang, Li, & Tan, 2013). The G variant of *OPRM1* A118G was also associated with larger opioid consumption (Chou, Yang, et al., 2006; Klepstad et al., 2004; Sia et al., 2013; S. Zhang et al., 2013). However, evidence suggests that the relationships between these genes and pain are more complex and might involve interaction of multiple genes (Landau et al., 2013; Reyes-Gibby et al., 2007). *COMT* genetic variants have been found to affect mu receptor (*OPRM1*) availability, 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 *OPRM1*×*COMT* interaction may significantly impact pain perception and response to opioids.

The interaction effect of *OPRM1* A118G and *COMT* Val158Met was first evaluated on opioid dose needed to control cancer pain. 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 combination had the least pain relief after intravenous fentanyl dose during labor and delivery (Landau et al., 2013). De Gregori et al did not find significant interactions between these genes on postoperative opioid consumption (De Gregori et al., 2013). Lastly, cancer patients undergoing elective surgery with *OPRM1* GG and *COMT* Met/Met genotype have lowest preoperative pain threshold and tolerance (Yao et al., 2015). As shown a bove, the findings of *OPRM1* and *COMT* interaction studies have not been replicated. The definition of pain outcomes and opioid regimens vary among those studies make it difficult to compare their findings and draw general conclusions.

Haplotype is a set of closely linked SNPs on the chromosome that are inherited as a unit. Diatchenko used the four *COMT* SNPs; rs6269, rs4633, rs4818, and rs4680 to define three major pain-sensitivity haplotypes; low pain sensitivity (LPS; GCGG), average pain sensitivity (APS; ATCA) and high pain sensitivity (HPS; ACCG) (Diatchenko et al., 2006). No published studies have investigated the interaction of *COMT* haplotypes or diplotypes (i.e., combination of haplotypes) with *OPRM1* on pain and opioid consumption.

The purpose of this study was to explore the gene-gene interaction of *COMT* and *OPRM1* on postoperative pain and opioid consumption in orthopedic trauma patients. This is the first study to investigate the interaction 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.

6.3 MATERIALS AND METHODS

6.3.1 Subjects

This cross-sectional descriptive study used previously obtained phenotype, demographic, clinical and genotyping data from a parent study (Henker et al., 2013). Incluion criteria for that study were: patients from 18 to 80 years of age, who received general, and had a single orthopedic surgery with planned surgical time of 1 to 4 hours in length. Patients were excluded if they had a second trauma site, history of mental illness, neurologic conditions, hepatic or renal disease. Subjects were prospectively enrolled in the parent study after written informed consent was obtained. **Human subjects approval was obtained from the University of Pittsburgh**

Institutional Review Board. Demographic and clinical data were collected from the patients and their medical records.

6.3.2 Postoperative pain and opioids

Pain was assessed using the Numeric Rating Scale (NRS), an 11-point verbal pain response scale from 0 (“no pain”) to 10 (“pain as bad as I can imagine”). Pain scores were collected in the preoperative holding area and then at 45 minutes after arrival in the PACU. Opioids administered during PACU stay included fentanyl, hydromorphone, morphine, and meperidine. The amount of opioid administered was converted to morphine equivalents, where 100 mg fentanyl, 1.5 mg hydromorphone, or 75 mg of meperidine were equivalent to 10 mg of morphine (Berry & C., 2006).

6.3.3 Genotyping data

Oragene DNA self-collection kit from DNA Genotek Incorporated (Ottawa, Ontario, Canada) was used to collect saliva samples from patients and DNA was extracted using the manufacture’s protocol. *OPRM1* A118G (rs1799971) was genotyped using sequencing and coded into two levels; no minor allele G “wild” and 1 or 2 minor allele G “variant”.

The four *COMT* SNPs including rs6269, rs4633, rs4818, and rs4680 were genotyped using 5' exonuclease Assay-on-Demand TaqMan assays. The Haploview software (http://www.broad.mit.edu/mpg/haploview) was used to visualize the pairwise linkage disequilibrium (LD) between the selected four *COMT* SNPs measured by Lewontin’s D' (Barrett, Fry, Maller, & Daly, 2005) (Figure 11). *COMT* haplotypes, constructed from the four *COMT*

SNPs, were designated as low pain sensitivity (LPS), average pain sensitivity (APS) and high pain sensitivity (HPS) (Diatchenko et al., 2006). *COMT* haplotypes were coded into two levels as 'having no copies' and 'having at least one copy'. Using combinations of these three major haplotypes, we created two diplotypes; low pain sensitivity (LPS/LPS or LPS/APS) and high pain sensitivity (APS/APS, HPS/HPS, LPS/HPS or APS/HPS). Details for sample collection and genotyping procedure were previously described by Henker et al. (Henker et al., 2013).

6.3.4 Statistical analysis

All statistical analyses were preceded by detailed descriptive and exploratory analyses of the data. Hardy–Weinberg equilibrium (HWE) was assessed for all genotyped SNPs using the exact test implemented in the software PLINK (Purcell et al., 2007). Hierarchical multiple regression analyses were performed with postoperative pain scores at 45 minutes in PACU and opioid consumption during PACU stay as dependent variables. Square root transformation of reflected values of postoperative pain score were used for regression analyses to remediate the violation of normality assumption for model residuals. Independent variables were: *OPRM1* A118G (under dominant genetic model), the four *COMT* SNPs including rs6269, rs4633, rs4818 and rs4680 (under additive, dominant, and recessive genetic models), the three *COMT* haplotypes (low, average, and high pain sensitivity), and *COMT* diplotypes (low and high pain sensitivity). The main and interaction effects of the independent variables were investigated after adjustment for covariates identified from the literature. Gender, race/ethnicity, age, smoking, fracture type and OR opioids consumption (mg/kg/hr) were covariates for opioid consumption analyses. Same covariates were included in pain analyses in addition to preoperative pain and opioid consumption during the first 45 minutes in the PACU (mg/kg). Since the frequencies of alleles

and haplotypes may vary among the different ancestries, we also conducted a subgroup analysis limited to Caucasian subjects (n=121). All analyses were carried out using IBM® SPSS® Statistics version 22 (IBM Corporation, Armonk, NY) and a two-sided p-value of less than 0.05 was considered to be statistically significant. Due to the exploratory nature of current data analyses, no multiple comparisons adjustment was made.

6.3.5 Source of funding

The parent study was funded by the American Association of Nurse Anesthetist Foundation, as well as a grant (UL1 RR024153) from the National Institutes of Health (NIH). This study was funded by the Margaret E. Wilkes scholarship fund award from University of Pittsburgh School of Nursing.

6.4 RESULTS

6.4.1 Demographic characteristics

The sample of postoperative patients (N=153) who underwent surgical orthopedic trauma repair was mostly male (n=104, 68%), non-Hispanic Caucasian (n=121, 80%), and nonsmokers (n=86, 57%) and on average (±SD) 38.48 ±13.1 years of age. Further description of the entire sample is presented in Table 14. Caucasian sample description is provided in Table 15.

6.4.2 Genetic characteristics

Genotype and allele frequencies of *OPRM1* and *COMT* SNPs are presented in Table 16. Three of the four *COMT* SNPs (rs4680, rs4633, and rs6269) displayed significant deviation from HWE as shown in Table 16. *COMT* SNPs were out of equilibrium even after restricting our analyses to Caucasian patients. Systematic lab review of raw genotype data was performed to examine if deviations from HWE were due to genotyping error. Pairwise linkage disequilibrium values computed by Haploview software showed that all *COMT* SNPs (rs4680, rs4633, rs4818and rs6269) were in strong LD (D' > 0.75) (Figure 11). The distribution of *COMT* haplotypes and diplotypes were as follow; 52 (39%) patients had no copy of LPS haplotype and 82 (61%) had at least one copy; 63 (47%) patients had no copy of APS haplotype and 71 (53%) had at least one copy; 110 (82%) patients had no copy of HPS haplotype and 24 (18%) had at least one copy. Regarding the *COMT* diplotypes; 77 (57%) patients had the low pain sensitivity diplotype and 57 (43%) had the high pain sensitivity diplotype.

6.4.3 OPRM1 × COMT interaction on PACU opioid consumption

Regression analysis results for predicting PACU opioid (mg/kg) consumption by *OPRM1*× *COMT* are reported in the Table 17. A significant interaction was found between *COMT* Val158Met (rs4680) when assuming a recessive genetic model (Met158Met vs. Val158Met/Val158Val) and *OPRM1* A118G assuming a dominant genetic model (A118G/G118G vs. A118A), beta = 0.093 (95%CI [0.006, 0.179]), $p < .05$. We found that patients having Met158Met (AA) of *COMT* rs4680 and (AG/GG) of *OPRM1* A118G consumed the largest amount of opioids compared to other combinations (Figure 12).

A significant interaction was also found between *COMT* rs4633 assuming a recessive genetic model (TT vs. CT/CC) and *OPRM1* A118G, b = 0.097 (95% CI [0.006, 0.189]), $p < .05$. We found that patients having TT of *COMT* rs4633 and (AG/GG) of *OPRM1* A118G consumed the largest opioids compared to other combinations (Figure 13). *OPRM1* A118G×*COMT* rs4680 and *OPRM1* A118G×*COMT* rs4633 accounted uniquely for 3.1% and 3.3% of the variance in total PACU opioid consumption, respectively. No significant interactions were found between *OPRM1* A118G and the other two SNPs of *COMT* rs6269 and rs4818. However, the interaction of *COMT* rs6269 and *OPRM1* demonstrated a trend ($p = .08$). Patients who have AA of *COMT* rs6269 and AG/GG of *OPRM1* A118G consumed more opioids compared to other combination, b = -0.075, 95%CI [-0.16, 0.01].

No significant interaction was found between *OPRM1* and *COMT* haplotypes or diplotypes. However, the interaction of *COMT* diplotype and *OPRM1* demonstrated a trend towards significance ($p = .07$). Patients having high pain sensitivity diplotype and (AG/GG) of *OPRM1* A118G seemed to consume more opioids compared to other combinations, b = 0.071, 95%CI [-0.006, 0.148]. Restricting our analysis to Caucasian subjects did not change the direction or the statistical significance for the interaction effects of both *OPRM1* A118G×*COMT* rs4680 and *OPRM1* A118G×*COMT* rs4633 on PACU opioid consumption (data not shown).

6.4.4 OPRM1 × COMT interaction on postoperative pain in PACU

Regression analysis results for predicting postoperative pain ratings (square root transformed) by *OPRM1* × *COMT* are summarized in Table 18. A significant interaction was found between *COMT* LPS (GCGG) haplotype and *OPRM1* A118G, beta = -0.93 (95%CI [-1.686, -0.166]), $p < .05$. The effect of *OPRM1* on postoperative pain ratings varied across levels of *COMT* LPS

haplotype. For patients with no copies of 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 have at least one copy of LPS haplotype, (AA) of *OPRM1* A118G was significantly associated with lower pain score compared with those having the variant (AG/GG) (Figure 14). *OPRM1*A118G×*COMT* LPS haplotype accounted uniquely for 3.7% of the variance in total postoperative pain ratings. No significant interaction was found among *OPRM1* and other *COMT* haplotypes and dipolotypes. No significant interaction was found between *OPRM1* and *COMT* SNPs; however, the interaction of *COMT* rs4818 and *OPRM1* assuming dominant genetic models demonstrated a trend near significance ($p = .06$).

Analyses of the main effect of *COMT* showed that patients with high pain sensitivity *COMT* diplotype reported significantly higher postoperative pain scores compared to patients with low pain sensitivity diplotype, beta = -0.32 (95%CI [-0.636, -0.005]), $p < .05$. Moreover, the main effect of *COMT* rs4633, assuming a recessive genetic model, was significantly associated with postoperative pain in the PACU, beta = -0.389 (95%CI [-0.759, -0.019), $p<0.05$. Patients with two minor alleles T (TT) reported significantly higher postoperative pain scores at 45 minutes in PACU compared to patients with CT or CC. The main effect of *COMT* rs4818, assuming a dominant genetic model, was also significantly associated with postoperative pain, beta = 0.38 (95%CI [0.051, 0.723]), $p < .05$. Patients with one or two minor alleles G (GC and GG) reported significantly higher postoperative pain scores compared to patients with CC, Assuming recessive genetic models, the main effects of *COMT* rs4680 and rs6269 showed a trend toward significance with *p*-values of .063 and .055, respectively.

Restricting the analyses to Caucasian subjects, none of *OPRM1* A118G×*COMT* SNPs, haplotypes and diplotypes were significantly associated with postoperative pain. However**,** the

interaction effects for *OPRM1* A118G×*COMT* LPS, *OPRM1*A118G×*COMT* HPS, and *OPRM1* A118G×*COMT* diplotypes were near significant (p= .070, p= .058, p= .050, respectively).

6.5 DISCUSSION

Our findings provide the first evidence that *OPRM1* A118G × *COMT* (four SNPs as well as their haplotypes and diplotypes) interactions may explain the variability in postoperative pain and response to opioids. Several important conclusions can be drawn. First, combining the mutant variant of both *OPRM1* A118G and *COMT* Val158Met is associated with poor response to opioid analgesia. We found that patients with combined Met158Met of *COMT* Val158Met and (AG/GG) of *OPRM1* A118G consumed more opioid compared to patients with other combinations. These findings suggest that combining both variants results in synergistic effect associated with poor response to opioid therapy. Consistent with previous work of Reyes-Gibby et al, we also found that carriers of the *OPRM1* AA and *COMT* Met/Met genotype required the lowest opioid dose compared to other combinations.

Second, synonymous SNPs of the *COMT* gene might influence pain sensitivity. It has been found that synonymous" silent" SNPs of *COMT* gene could affect mRNA stability and consequently *COMT* protein expression and enzymatic activity (Nackley et al., 2006). Consistent with that, we found that the two synonymous SNPs of *COMT*, rs4633 and rs4818, were significantly associated with postoperative pain in PACU. TT of rs4633 and AG/GG of rs4818 were associated with high pain sensitivity.

Third, *COMT* haplotypes/ diplotypes have greater impact on pain than single SNPs. Recent genetic studies focused on investigating haplotype reconstruction suggesting that

combinations/ interactions of SNPs within haplotypes lead to synergistic effects on the resultant protein and it might result in functional consequences that is different from the independent effect of those SNPs (Diatchenko et al., 2005; Duan et al., 2003). For instance, LPS and HPS haplotypes of *COMT* are identical for Val158Met, both of which have the Val allele. However, they significantly differ in the resultant protein and enzyme activity. LPS shown to catabolize catecholamine 11.4 times higher than HPS and decrease pain sensitivity (Diatchenko et al., 2006). This suggests that the interactions of multiple SNPs within *COMT* haplotype determines the functional outcomes of *COMT* enzyme rather than only single SNP of Val158met. Our main effect analysis findings suggested that the single SNP Val158Met was not associated with postoperative pain; however, *COMT* diplotypes were significantly associated with postoperative pain.

Fourth, the LPS haplotype has a "protective" effect on pain when combined with the wild type of *OPRM1*. We have shown that having at least of one copy of LPS haplotype and wild type of *OPRM1* A118G was associated with lower postoperative pain scores compared to variant (AG/GG), yet not carrying the LPS haplotype was associated with the opposite direction. The reasons for this dichotomy are unclear. Thus, additional research is required to establish the exact mechanisms influencing these relationships.

Finally, racial differences in allele and haplotype frequencies may influence postoperative pain sensitivity. Our findings on postoperative pain were changed after restricting the analyses to Caucasians. The interaction of *OPRM1* and LPS haplotype was not significant in Caucasians; however, the interaction effectwas similar to that for total sample. *OPRM1*×ACCG and *OPRM1*×diplotypes showed a trend toward significance that was not observed using the total sample. None of the significant main effect of *COMT* rs4818, *COMT* rs4633, or *COMT*

diplotypes were significant in the Caucasian-only sample. The changes in our findings might be partly due to the variation in the allele and haplotype frequencies among the different racial populations. The G allele frequencies of *OPRM1* A118G vary across racial and ethnic groups, it ranges from 12% to 20% in Caucasians, 1% to 4% in African Americans, and 19% to 24% in Hispanics (Hastie et al., 2012). *COMT* allele frequencies and *COMT* haplotype structures and frequencies also vary among the different racial groups (Beuten et al., 2006; DeMille et al., 2002; Gabriel et al., 2002; Palmatier et al., 1999). Moreover, one study found that *COMT* enzyme activity was significantly higher in African American compared to Caucasian (McLeod, Fang, Luo, Scott, & Evans, 1994).

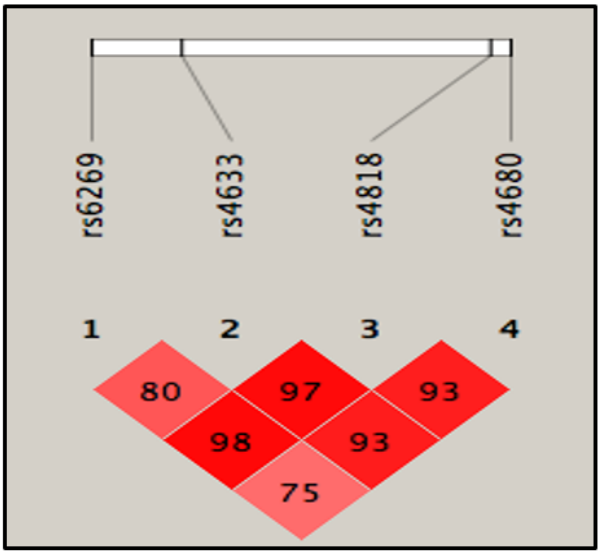
Our study has several limitations. While our sample size is larger than what was reported in the literature in the pain area (Kolesnikov et al., 2011; Landau et al., 2013), it is still relatively small for general genetic study of a common phenotype. However, even with this small sample size, it appears that significant associations exist. Three of the *COMT* SNPs were out of HWE; however, it is possible that deviation from HWE is due to the distinctive characteristics of our sample. Our sample may not be completely representative of the general population. Despite violations of HWE, *COMT* SNPs are genetically related (i.e., in strong LD) and they collectively influence the biological process of pain.

6.6 CONCLUSION

Our findings suggest the *OPRM1*×*COMT* may contribute to variability of postoperative pain and response to opioids. Incorporation of diagnostic markers such as *OPRM1* and *COMT* will potentially facilitate the identification of high-risk individuals for opioid mismanagement.

Consequently, opioid therapy can be individualized for better pain management. Future studies with larger sample sizes and more diversity are needed to confirm these effects.

6.7 FIGURES AND TABLES



1	2	3	4	Haplotype Name	Frequency
G	C	G	G	LPS	41.3%
A	T	C	A	APS	38.5%
A	C	C	G	HPS	9.5%
G	T	C	A	Rare	3.7%
G	C	C	G	Rare	3.6%
G	C	G	A	Rare	1.4%

Figure 11. Linkage disequilibrium (LD) graph of COMT SNPs

Linkage disequilibrium (LD) graph of COMT SNPs "left" and Haplotype frequency "right". LD was calculated using D' (0= no disequilibrium; 1= maximum disequilibrium). The numbers inside the squares are 100 × D'. Graph was created using Haploview software. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity

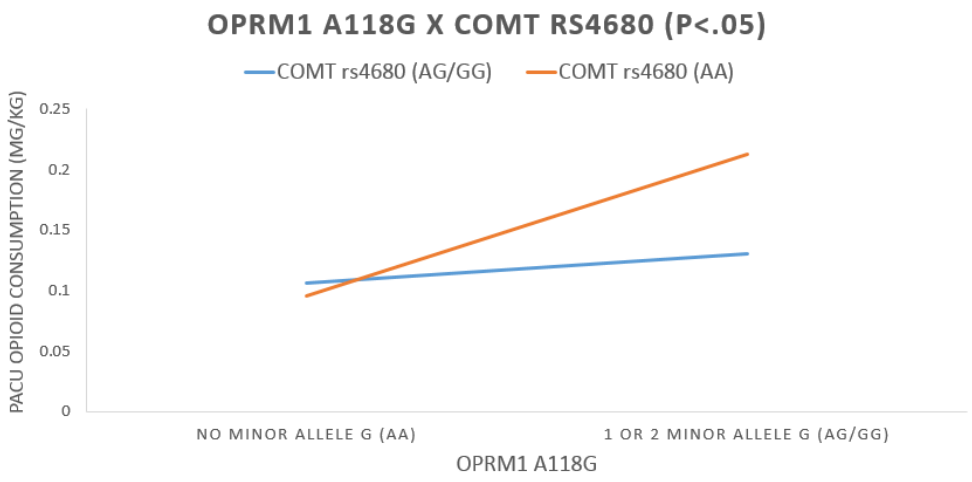


Figure 12. The interaction of OPRM1 A118G × COMT rs4680 on PACU opioid consumption for the total sample

Graphs display the OPRM1 × COMT interaction for the typical participant in our sample; Caucasian, male, with ankle fracture, age of 39.9 years, and OR opioid consumption of 0.233mg/kg/hr.

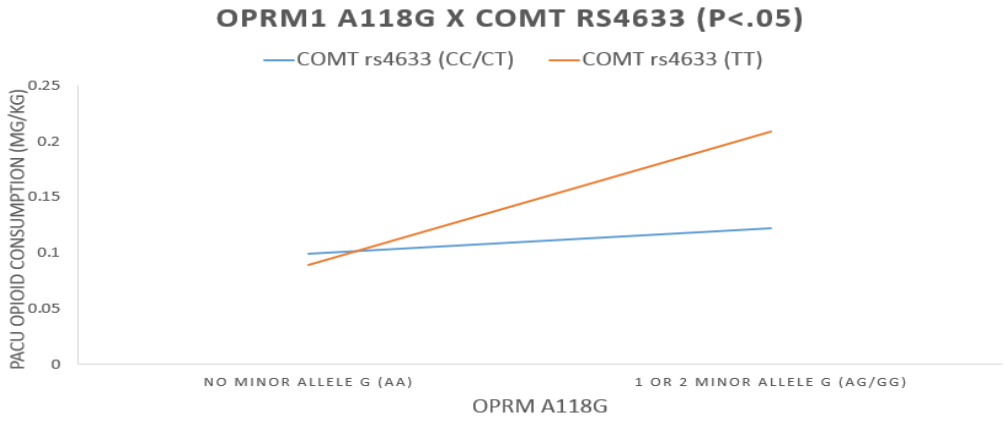


Figure 13. The interaction of OPRM1 A118G × COMT rs4633 on PACU opioid consumption for the total sample

Graphs display the OPRM1 × COMT interaction for the typical participant in our sample; Caucasian, male, with ankle fracture, age of 39.9 years, and OR opioid consumption of 0.233mg/kg/hr.

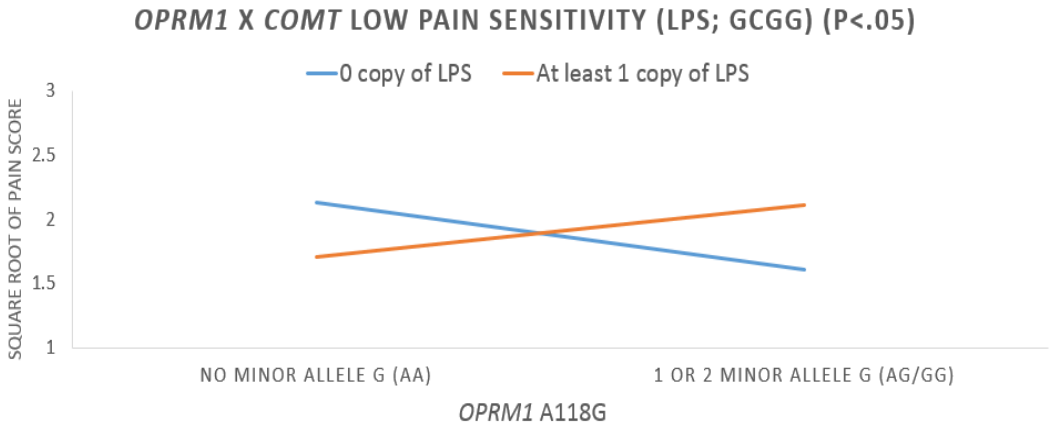


Figure 14. The interaction of OPRM1 × COMT Low Pain Sensitivity (LPS; GCGG) haplotype on postoperative pain ratings at 45 minutes in PACU for the total sample

Graphs display the OPRM1 × COMT interaction for the typical participant in our sample; Caucasian, male, with ankle fracture, age of 39.9 years, and OR opioid consumption of 0.233mg/kg/hr.

Table 14. Descriptive statistics for the total sample (n=153)

Variable	Descriptive statistics
Age (years)	38.48 ± 13.13
Gender (Male)	104 (68)
Ethnicity (Caucasian)	121 (80)
Smoking (non-smoker)	86 (57)
Body Mass Index (kg/m^2)	29.09 ± 7.43
Fracture type	
Ankle	56 (39)
Femur	16 (11)
Tibial plateau	30 (21)
Tibia-fibula	35 (24)
Other	7 (5)
Surgical time (min)	120.00 ± 59.51
PACU time (min)	125.30 ± 48.00
Preoperative Numerical Pain scale score	4.56 ± 2.95
Postoperative Numerical Pain scale score at 45 min in PACU	6.48 ± 2.70
Opioid consumption during OR (mg)	35.86 ± 18.17
Opioid consumption during the first 45 min in PACU (mg)	6.80 ± 5.41
Opioid consumption during PACU stay (mg)	9.60 ± 7.74

Descriptive statistics reported in cell are expressed as M ± SD for continuous variables and n (%) for categorical variables. OR = Operation Room; PACU = Post Anesthesia Care Unit.

Table 15. Descriptive statistics for the Caucasian sample (n=121)

Variable	Descriptive statistics
Age (years)	39.85 ± 13.16
Gender (Male)	88 (73)
Smoking (non-smoker)	69 (58)
Body Mass Index (kg/m^2)	29.60 ± 6.83
Fracture type	
Ankle	44 (38)
Femur	14 (12)
Tibial plateau	25 (22)
Tibia-fibula	25 (25)
Other	7 (6)
Surgical time (min)	120.00 ± 59.51
PACU time (min)	126.11 ± 61.73
Preoperative Numerical Pain scale score	4.44 ± 2.73
Postoperative Numerical Pain scale score at 45 min in PACU	6.23 ± 2.76
Opioid consumption during OR (mg)	35.08 ± 17.04
Opioid consumption during the first 45 min in PACU (mg)	6.87 ± 5.62
Opioid consumption during PACU stay (mg)	10.11 ± 7.74

Descriptive statistics reported in cell are expressed as M ± SD for continuous variables and n (%) for categorical variables. OR = Operation Room; PACU = Post Anesthesia Care Unit.

Table 16. Genotype and allele frequency of OPRM1 and COMT SNPs

				Alleles[a]			Genotypes			
Gene	SNP	Chromosome	Position	A1	A2	MAF[b]	A1/A1	A1/A2	A2/A2	HWE[c]
OPRM1	rs1799971	6	154360797	G	A	0.1176	2	28	106	1
COMT	rs4680	22	19951271	A	G	0.4412	37	46	53	0.00025
	rs4633	22	19950235	T	C	0.4286	33	48	52	0.00258
	rs4818	22	19951207	G	C	0.4427	30	56	45	0.15560
	rs6269	22	19949952	A	G	0.4924	40	49	42	0.00494

[a]A1: Minor allele; A2: Major allele; [b]MAF: Minor Allelic Frequency; [c]Hardy-Weinberg Equilibrium (HWE) test p-value.

Table 17. Regression analysis results for predicting PACU opioid (mg/kg) consumption by OPRM1×COMT

Interaction Variable	Sample	n	beta	95%CI	*p*-value	sr^2
OPRM1 A118G×*COMT* rs4680	Total	123	0.093	(0.006,0.179)	.037	.031
	Caucasians	96	0.108	(0.017,0.200)	.021	.047
OPRM1 A118G×*COMT* rs4633	Total	120	0.097	(0.006,0.189)	.037	.033
	Caucasians	93	0.102	(0.006,0.199)	.038	.039
OPRM1 A118G×*COMT* rs4818	Total	119	-0.044	(-0.126,0.037)	.284	.009
	Caucasians	93	-0.058	(-0.153,0.036)	.223	.014
OPRM1 A118G×*COMT* rs6269	Total	118	-0.075	(-0.160,0.010)	.082	.023
	Caucasians	92	-0.070	(-0.163,0.023)	.136	.021
OPRM1 A118G×*COMT* LPS haplotype (GCGG)	Total	123	-0.047	(-0.125,0.030)	.230	.011
	Caucasians	96	-0.059	(-0.149,0.031)	.194	.015
OPRM1 A118G×*COMT* APS haplotype (ATCA)	Total	123	0.009	(-0.068,0.086)	.818	<.001
	Caucasians	96	-0.015	(-0.1060,.077)	.750	<.001
OPRM1 A118G×*COMT* HPS haplotype (ACCG)	Total	123	0.001	(-0.095,0.098)	.982	<.001
	Caucasians	96	-0.073	(-0.191,0.045)	.222	.013
OPRM1 A118G×*COMT* Diplotypes	Total	123	0.071	(-0.006,0.148)	.070	.024
	Caucasians	96	0.047	(-0.040,0.135)	.285	.010

In addition to the *OPRM1*×*COMT* interaction, models included the main effects of both *OPRM1* and *COMT*, gender, race, age, smoking, fracture type and OR opioid consumption. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity.

Table 18. Regression analysis results for predicting postoperative pain ratings by *OPRM1×COMT*

Interaction Variable	Sample	n	beta	95%CI	*p*-value	sr^2
OPRM1 A118G×*COMT* rs4680	Total	192	0.444	(-0.436,1.324)	.319	.007
	Caucasians	95	0.421	(-0.501,1.342)	.366	.007
OPRM1 A118G×*COMT* rs4633	Total	118	0.359	(-0.559,1.276)	.440	.004
	Caucasians	92	0.419	(-0.526,1.364)	.380	.007
OPRM1 A118G×*COMT* rs4818	Total	117	-0.755	(-1.543,0.033)	.060	.024
	Caucasians	92	-0.486	(-1.390,0.420)	.128	.020
OPRM1 A118G×*COMT* rs6269	Total	116	-0.500	(-1.297,0.296)	.216	.010
	Caucasians	91	-0.640	(-1.470,0.188)	.128	.020
OPRM1 A118G×*COMT* LPS haplotype (GCGG)	Total	121	-0.926	(-1.686,-0.166)	.017	.037
	Caucasians	95	-0.794	(-1.66,0.067)	.070	.023
OPRM1 A118G×*COMT* APS haplotype (ATCA)	Total	121	0.288	(-0.510,1.085)	.476	.003
	Caucasians	95	0.080	(-0.811,0.971)	.858	<.001
OPRM1 A118G×*COMT* HPS haplotype (ACCG)	Total	121	0.596	(-0.372,1.563)	.225	.010
	Caucasians	95	1.110	(-0.04,2.261)	.058	.031
OPRM1 A118G× *COMT* Diplotypes	Total	121	0.637	(-0.132,1.407)	.103	.017
	Caucasians	95	0.831	(-0.001,1.662)	.050	.033

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, OR opioid consumption and opioid consumption during the first 45 minutes in PACU. LPS= Low pain Sensitivity; APS= Average pain Sensitivity; HPS= High pain Sensitivity.

APPENDIX A

PARENT STUDY IRB APPROVAL

University of Pittsburgh
Institutional Review Board

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(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Richard Henker PHD CNRA
From: IRB Office
Date: 5/8/2015
IRB#: REN14050278 / PRO07070196
Subject: The association between mu-receptor genotypes and postoperative pain response

---

Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:
45 CFR 46.110.(9)

Please note the following information:

Approval Date: 5/8/2015
Expiration Date: 5/13/2016

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

APPENDIX B

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