RISK FACTORS FOR RESPIRATORY DEPRESSION IN POSTOPERATIVE ORTHOPEDIC TRAUMA PATIENTS

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Respiratory depression is a serious and potentially lethal adverse effect of opioid medications that must be considered to achieve safe opioid pain management in clinical practice. The patient demographics; age, sex, height, weight, body mass index, smoking status, race, amount of opioid received in the operating room, amount of opioid received in the post anesthesia care unit (PACU), sedation score postoperatively, American Society of Anesthesiologists (ASA) Physical Status, and genetic variants from identified candidate genes; *OPRM1*, *ABCB1*, *COMT*, and *CYP2D6* were potential risk factors that were analyzed for association with opioid induced respiratory depression after surgery for orthopedic trauma.

A sample of 152 subjects aged 18-70 that received surgeries for orthopedic trauma were included in this study. Subject characteristics data were collected from both subject reports and medical records. Data regarding respiratory rate postoperatively was collected in the post anesthesia care unit at 15 and 45 minutes postoperatively. DNA extraction was performed with saliva samples from the subjects. Sequencing was performed using Big Dye Cycle Sequencing reagents to determine *OPRM1* genotype. Taqman allele discrimination was used to genotype *ABCB1*, *COMT*, and *CYP2D6* genes.

The patient characteristics ASA physical status and sedation score were associated with differences in respiratory rate at 15 and 45 minutes postoperatively. Differences in subject genotype on the *ABCB1* gene single nucleotide polymorphism (SNP) (rs1045642) were

associated with differences in respiratory rate 45 minutes postoperatively. *CYP2D6* metabolizer group differences were associated with difference in respiratory rate at 15 minutes postoperatively.

This study provided evidence in support of the use of ASA Physical Status, sedation score, *ABCB1* SNP (rs1045642), and *CYP2D6* metabolizer groups to identify individuals at increased risk for opioid induced respiratory depression postoperatively.

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PREFACE

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1.0 CHAPTER 1: INTRODUCTION

Effective pain management is crucial to safe and effective patient care. A sentinel event alert was issued by the Joint Commission in August 2012 that emphasized the importance of safe pain management with opioids, as many adverse events have been linked with the use of opioid medications (The Joint Commission, 2009). Respiratory depression is a serious and potentially lethal adverse effect of opioid administration that must be considered to achieve safe opioid pain management in clinical practice.

Data collected from 152 adult patients that received surgery for orthopedic trauma were used for analysis of respiratory depression with associated risk factors. Orthopedic trauma patients receiving surgery are an optimal patient population for studying pain management, as orthopedic surgical procedures are rated as being moderate to severely painful (Sinatra, Torres, & Bustos, 2002). Potential risk factors for opioid induced respiratory depression that were analyzed included age, sex, height, weight, body mass index, smoking status, race, amount of opioid received in the operating room, amount of opioid received in the post anesthesia care unit (PACU), sedation score postoperatively, American Society of Anesthesiologists (ASA) Physical Status, and genotype data including: *CYP2D6* metabolizer group, and single nucleotide polymorphism (SNPs) from the candidate genes of interest *OPRM1*, *COMT*, and *ABCB1*. This study sought to answer the question; what are the risk factors for respiratory depression in orthopedic trauma patients receiving opioids for pain management postoperatively?

Identification of risk factors for opioid induced respiratory depression will allow healthcare providers to manage delivery, develop monitoring strategies, and increase vigilance in the delivery of opioids in patients with known risks. An awareness of an individual patient's risk factors for opioid induced respiratory depression will improve patient safety with the use of opioids.

A sample of 152 adult subjects that received surgery for orthopedic trauma was recruited from Presbyterian Hospital of UPMC. Age, sex, height, weight, body mass index, smoking status, race, amount of opioid received in the operating room, amount of opioid received in the post anesthesia care unit (PACU), sedation score postoperatively, and American Society of Anesthesiologists (ASA) Physical Status, were determined by patient report or from information retrieved from the medical record. Saliva samples were collected from consented and enrolled subjects for DNA extraction. Genotyping for *COMT*, *ABCB1*, and *CYP2D6* SNPs was determined through the use of Taqman Allelic Discrimination Assays (Applied Biosystems, Foster City, CA). Genotypes for *OPRM1* SNPs were identified through the use of Big Dye Cycle Sequencing reagents (Applied Biosystems, Foster City, CA).

The subject characteristics that were found to have a significant effect on respiratory rate postoperatively at both 15 and 45 minutes were American Society of Anesthesiologists (ASA) Physical Status, sedation score at 15 minutes postoperatively and sedation score at 45 minutes postoperatively. *CYP2D6* metabolizer group classification was found to be significantly associated with respiratory rate at 15 minutes postoperatively. The *ABCB1* SNP (rs1045642) was found to be significantly associated with respiratory rate at 45 minutes postoperatively. These results support the use of ASA physical status, sedation score at 15 minutes and 45 minutes,

CYP2D6 metabolizer group, and *ABCB1* SNP (rs1045642) in the identification of individuals at risk for postoperative opioid induced respiratory depression.

1.1 PURPOSE

Currently there is limited evidence to determine risk factors for opioid induced respiratory depression (Jarzyna et al., 2011; Stoelting & Weinger, 2009). Further evidence regarding identification of risk factors for opioid induced respiratory depression will provide guidance for development of clinical interventions and protocols aimed at increasing vigilance in patients at heightened risk for adverse outcomes. Understanding of the etiology and mechanisms of opioid induced respiratory depression can lead to the development of targeted interventions based on identification of risk factors including genetic variants identified on candidate genes. Identification of risk factors could be clinically useful for all surgical patients. Postoperative patients observed for limited periods of time, particularly those that are ambulatory surgery patients or outpatients, as well as the inpatient population in the hospital setting will benefit from pain management strategies targeted to decrease the occurrence of opioid induced respiratory depression secondary to opioids.

Effective pain management is considered a patient right during treatment. Pain has been recognized as the “fifth vital sign” by the American Pain Society (American Pain Society, 2008) to encourage frequent and thorough assessment by healthcare providers. Adequate pain control is crucial because healthcare providers aim to minimize patient suffering as well as the negative consequences that unrelieved pain has on the body. Unrelieved pain is known to cause other negative consequences on physiology, development, psychology, future pain and quality of life

(Pasero & McCaffery, 2011). Adequate pain control can aid in avoiding these negative effects, making pain management central to achieving the best possible patient outcomes.

In the hospital setting opioids are the most common type of pain medication administered for pain. Respiratory depression is a dangerous adverse effect of opioid use that must be considered to achieve safe opioid pain management. Nurses play a central role in pain management as well as in the identification and treatment of respiratory depression. Exploration of risk factors for opioid induced respiratory depression will aid in safe and effective management of pain. Fear of opioid induced respiratory depression and other adverse effects can lead to under treatment of pain. Conversely, if excess opioid is administered, respiratory depression, a potentially fatal adverse effect may occur. Exploring this topic to achieve a better understanding of opioid induced respiratory depression will enable healthcare providers to treat pain safely and effectively.

Variability of patients' experience of pain, can make treatment of pain challenging. Patient report of pain has been found to be the most effective means available for pain measurement. "Pain is whatever the experiencing person says it is, existing whenever he/she says it does" (McCaffery, 1968). This is the gold standard definition of pain used in clinical practice. Efficacy of pain medication given is also measured by patient report of pain score during pain reassessment. Identification of significant risk factors for opioid induced respiratory depression can aid in the clinical management of patients receiving opioids for pain management. Establishment of risk factors for opioid induced respiratory depression will contribute significantly to healthcare professionals' goal of achieving safe and adequate pain management.

Data collected from 152 adult patients that received surgery for orthopedic trauma will be used for analysis of respiratory depression with associated risk factors. Potential risk factors will include age, sex, height, weight, body mass index, smoking status, race, amount of opioid received in the operating room, amount of opioid received in the PACU, sedation score postoperatively, American Society of Anesthesiologists (ASA) Physical Status and genotypes. Specific genetic variants will be evaluated in candidate genes of interest including: *OPRM1*, *COMT*, *ABCB1*, and *CYP2D6*. Orthopedic trauma patients undergoing surgery are an optimal patient population for studying pain management, due to the severe pain associated with orthopedic trauma and history of under treatment of pain in this patient population (Sinatra et al., 2002). Analysis of these data will seek to answer the question; what are the risk factors for respiratory depression in orthopedic trauma patients receiving opioids for pain management postoperatively?

1.2 SPECIFIC AIMS

Patient factors were evaluated for potential associated risk for opioid induced respiratory depression in 152 adult patients that received surgery for orthopedic trauma. Both demographic and clinical factors including age, gender, height, weight, body mass index, smoking status, race, sedation score postoperatively, amount of opioid received in the operating room, amount of opioid received in the PACU, ASA Physical Status and genetic risk factors including genetic variants on candidate genes, specifically *OPRM1*, *ABCB1*, *COMT*, and *CYP2D6* were evaluated for risk of opioid induced respiratory depression. The purpose of this study was to identify those

subject risk factors that contributed to risk of opioid induced respiratory depression postoperatively.

Aim 1. Explore the patient demographics; age, sex, height, weight, body mass index, smoking status, race, sedation score postoperatively, amount of opioid received in the operating room, amount of opioid received in the PACU and ASA Physical Status for presence of a significant relationship in the development of opioid induced respiratory depression after surgery for orthopedic trauma.

Aim 2. Explore genetic variants for identified candidate genes; *OPRM1*, *ABCB1*, *COMT*, and *CYP2D6* to determine if specific genotypes contribute to increased risk for opioid induced respiratory depression after surgery for orthopedic trauma.

Identification of risk factors including genetic variants for opioid induced respiratory depression will aid in the individual management of respiratory depression in the context of opioid pain treatment.

2.0 CHAPTER 2: LITERATURE REVIEW

2.1 ASSESMENT OF PAIN

The International Association for the Study of Pain defines pain as " An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (International Association for the Study of Pain, 1994). Although pain is an intense sensation for affected individuals, variation exists in the way pain is experienced and expressed. Pain does not always have obvious direct physical manifestations or physiologic indications, necessitating that healthcare providers assess pain. Patient report is the most accurate method of pain assessment. "Pain is whatever the experiencing person says it is, existing whenever he/she says it does" (McCaffery, 1968). Treatment is initiated in response to pain assessment, followed by reassessment to evaluate effectiveness of this treatment. In recent years assessment of pain has been emphasized and healthcare providers have been encouraged to aggressively treat pain (American Pain Society, 2008). As part of the movement to closely monitor pain in the hospital setting, pain has been referred to as the fifth vital sign by the American Pain Society (American Pain Society, 2008), encouraging assessment of pain as frequently as other vital signs (American Pain Society, 2008). Regular assessment of pain by the health care provider is essential because many patients will not spontaneously express pain (American Pain Society, 2008).

In order to accurately assess pain, the appropriate pain scale according to the developmental level and communication abilities of the patient must be used. Currently, the most widely used pain scale is the 0-10 scale (Krebs, Carey, & Weinberger, 2007). When using the 0-10 scale to assess pain, the patient is asked to assign their current pain a number rating on a scale from 0-10, 0 being no pain and 10 being the worst pain imaginable. Treatment decisions are made based on patient reported pain level. The way that each individual rates their pain may differ depending on pain tolerance and pain perception. Certain patients may be more prone to subscribing higher or lower ratings to their pain depending on perception of their pain. Regardless of this individual variation in pain rating healthcare providers should take patient reported ratings as the truth. Comparison of pain ratings and looking at trends of pain scores over time is a useful way to evaluate the effectiveness of the patient's pain treatment (Pasero & McCaffery, 2011).

2.2 HISTORICAL METHODS OF OPIOID PAIN TREATMENT

Opium, the latex derived from the opium poppy flower, was originally used for recreational purposes in ancient times (Hamilton & Baskett, 2000). The application of opioids to postoperative pain management was first noted in the 1700s (Hamilton & Baskett, 2000). Although incapable of providing surgical anesthesia, it was found that oral opioids were a quality means of postoperative pain relief. The first published report of the systemic effect of morphine was by Charles Hunter in the Medical Times Gazette in 1858 (Hamilton & Baskett, 2000). Prior to this it was thought that morphine exerted mostly local pain control effects.

2.3 OPIOID PAIN MANAGEMENT

Endogenous opioids are naturally occurring peptides in the body that are thought to play a role in opioid analgesia. The precise physiologic purpose of endogenous opioids is unknown, but it is known that opioid medications produce their effects by imitating endogenous opioids (Pasero & McCaffery, 2011). Opioid receptors are the sites where endogenous opioids and opioid medications bind and the mechanisms for exerting pain relieving effects (Pasero & McCaffery, 2011).

Detrimental effects on physiology, development, psychology, future pain and quality of life have been noted as a result of untreated pain (Pasero & McCaffery, 2011). Undertreated postoperative pain can lead to decreased immune function due to activation of hypothalamus-pituitary-adrenal hormonal changes (Hutchison, 2007; Pasero & McCaffery, 2011). Increased sympathetic nervous system activation secondary to untreated pain may have negative effects on the cardiac, gastrointestinal, and renal systems (Hutchison, 2007; Pasero & McCaffery, 2011). These negative systemic effects of untreated pain can cause impaired or delayed healing, leading to poor patient outcomes (Hutchison, 2007; Pasero & McCaffery, 2011).

One of the barriers to effective pain treatment is the presence of breakthrough pain, additional pain despite treatment. This pain may be brought on by engaging in an activity that causes additional pain or the onset of pain towards the end of the dose of pain medication. The presence of breakthrough pain indicates the need for changes in pain treatment. Once a patient starts to experience breakthrough pain it can be difficult to regain control of pain. In efforts to prevent breakthrough pain patients may be placed on continuous scheduled opioid doses to ensure that the patient stays pain free. The goal of pain treatment is to keep the patient pain free and prevent the occurrence of breakthrough pain.

Over time as the uses of opioids were explored and different forms of opioids were developed, public misconceptions were also formed. A prevalent misconception about opioids is that taking opioids for pain relief causes addiction (Pasero & McCaffery, 2011). This fear of opioid addiction may be held by the patient, the healthcare provider or both, and creates a significant barrier to effective pain management (The Joint Commission, 2012). Taking opioids for pain relief purposes results in addiction at a rate of less than 1 percent (Pasero & McCaffery, 2011).

2.4 RESPIRATORY FUNCTION

The respiratory system requires the orchestration of many body systems in order to properly function. The pulmonary system and the central nervous system (CNS) function in conjunction with one another to achieve effective respiration (Levitzky, 2013). The lungs, a critical organ in respiratory system, perform breathing, the inhalation and exhalation of air, and gas exchange, taking oxygen from the air and transporting it into the bloodstream, and removing carbon dioxide from the body and expelling it into the air (Weinberger, Cockrill, & Mandel, 2008). The impulse and drive to breathe is created by muscles of the chest wall and diaphragm (Weinberger et al., 2008). The contraction of these muscles is triggered by signals transmitted by the CNS from neurons in the medulla through the spinal cords to the nerves that innervate the respiratory muscles (Levitzky, 2013).

2.5 OPIOID INDUCED REPIRATORY DEPRESSION

The depression or reduction in respiratory functioning is a dangerous adverse effect of opioids that can be potentially fatal (Yaksh & Wallace, 2011). Opioids affect the relationship between the respiratory and neurologic systems to create a depressant effect. Drive for rhythm of respirations is decreased by the effect of opioid on mu opioid receptors and delta opioid receptors (Yaksh & Wallace, 2011). This is manifested by a decreased respiratory rate and altered respiratory pattern (Yaksh & Wallace, 2011). Opioids also alter respiratory function by diminishing the respiratory response to increased carbon dioxide and decreased oxygen (Yaksh & Wallace, 2011). The excitability of chemoreceptors that normally facilitate communication of the presence of high carbon dioxide or low oxygen is lessened by the presence of opioids (Yaksh & Wallace, 2011). The presence of high amounts of carbon dioxide or low amounts of oxygen can have detrimental effects on the body in the regulation of acid-base balance (Levitzky, 2013). The situation in which a patient is experiencing respiratory depression this acid-base balance may be altered and cause a respiratory acidosis state in the body (Levitzky, 2013). Other effects of opioids on the pulmonary system include increased stiffness of the chest wall and lowered patency in the upper airway (Yaksh & Wallace, 2011).

There is no exact definition of respiratory depression that may be used for all patients. Determination of respiratory depression must be based on evaluation compared with the patient's baseline. (Pasero & McCaffery, 2011). The relative definition of respiratory depression is part of what makes this such a dangerous adverse effect. In order to correctly identify a patient as experiencing respiratory depression the health care provider must know the patient's baseline respiratory status. Thorough respiratory assessment and comparison with the patient's baseline status must be performed to find if the patient is experiencing respiratory depression.

2.6 GENETIC INFLUENCE

Advances in genetics have had major influence on patient care in many different healthcare specialties. In the context of opioid pain management, genetic variation may explain differences in analgesic and adverse effects in individual patients. These differences in the way that opioids exert effects may produce changes in an individual's risk for opioid induced respiratory depression. If the way in which genetics causes changes in an individual's risk for respiratory depression can be identified, genetic testing could be used to assess patient risk for opioid induced respiratory depression. The candidate genes examined in this study were *OPRM1*, *COMT*, *ABCB1*, and *CYP2D6*.

OPRM1 is a gene that will be analyzed in the subjects in this analysis for association between a specific genotype and risk for respiratory depression. The gene *OPRM1* encodes for the mu opioid receptor, where endogenous opioids and opioid analgesics bind (Romberg et al., 2005). Variation in the mu-receptor has the potential to cause variation in the way that opioid binds in the body. Differences in the way that an opioid binds to the mu-receptors may cause potential differences in the effects of opioid medication in the body. This variation of the behavior of opioids in the body secondary to differences in the mu-receptor has the potential to effect an individual's risk of opioid induced respiratory depression. The fact that the *OPRM1* gene codes for the mu-receptor means that differences in genotype at the *OPRM1* gene can cause variation in the mu-receptor. This function of the *OPRM1* gene creates interest for potential differences in risk of opioid induced respiratory depression associated with individual differences in the *OPRM1* gene. Individual variations in *OPRM1* genotype may have implications for associated risk for opioid induced respiratory depression. It has been noted that

individual variations in *OPRM1* genotype and associated respiratory risk secondary to opioids and analgesic effects do not correlate (Romberg et al., 2005).

The *COMT* gene codes for the production of an enzyme called catechol-O-methyltransferase (U.S. National Library of Medicine, 2007). The function of the enzyme catechol-O-methyltransferase in the body is to break down chemical messenger substances called neurotransmitters (U.S. National Library of Medicine, 2007). The brain plays an integral role in respiratory function. Individual variation in the *COMT* gene will lead to variation in individuals' catechol-O-methyltransferase enzymes. Differences in individuals' catechol-O-methyltransferase enzymes has the potential to alter the regulation of neurotransmitters in the brain. The alteration of neurotransmitters has the potential to affect respiratory function, as well as the behavior of opioid medications in the body. These potential differences in respiratory function and effects of opioid medications may affect risk for opioid induced respiratory depression. The gene *COMT* has been associated with differences in postoperative pain and response to opioids (Henker et al., 2013). One of the single nucleotide polymorphisms (SNPs) on the *COMT* gene analyzed in this study was rs4680. This rs4680 SNP on the *COMT* gene has been associated with a greater opioid dose requirement in cancer patients (Rakvag et al., 2008; Reyes-Gibb et al., 2007). These differences in opioid response and consumption associated with the *COMT* gene have a potential effect for risk of opioid induced respiratory depression.

The function of the *ABCB1* gene is to code for the formation of a membrane-associated protein that belongs to the MDR/TAP subfamily of membrane transport proteins (U.S. National Library of Medicine, 2014). This protein serves a role in the transport of drugs efflux out of cells (U.S. National Library of Medicine, 2014). The function of the *ABCB1* gene creates interest for the potential effect of differences in *ABCB1* genotype on an individual's risk for opioid induced

respiratory depression. The function of the membrane-associated protein from the MDR/TAP subfamily of membrane transport proteins is to facilitate the efflux of drugs out the cell (U.S. National Library of Medicine, 2014). Variation in this membrane-associated protein may potentially change the effects of opioid in the body. Due to the function of the *ABCB1* gene in coding for this membrane-associated protein, differences in the *ABCB1* gene may impact risk for opioid induced respiratory depression. Differences in an individual's genotype on the *ABCB1* gene have been identified by Park and colleagues (2007) as a risk factor for respiratory depression secondary to opioid pain management (Park et al., 2007).

One of the genes that influence the metabolism of opioid in the body is *CYP2D6*. The gene *CYP2D6* encodes for the enzyme that affects the way that a variety of opioids and antiemetics are metabolized (Wesmiller et al., 2013). Individual variations in the *CYP2D6* gene account for differences in metabolism and resulting contrasts in the noticeable effects that the opioids produce (Wesmiller et al., 2013). The CYP2D6 gene codes for a monooxygenase protein which resides in the endoplasmic reticulum (U.S. National Library of Medicine, 2014). This protein belongs to the cytochrome P450 family of enzymes which are known to aid in the metabolism of over 20% of drugs (U.S. National Library of Medicine, 2014). Over 100 different allelic variants of the CYP2D6 gene are known to exist in the population (Ingelman-Sundberg, Daly, & Nebert, 2009). In efforts to simplify the study of these various types of allelic polymorphisms, a classification system has been developed describing metabolic function yielded from these genetic differences. Based on genotype patients can be classified as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM), (Daly et al., 1996; Lotsch et al., 2002; Oertel, Schmidt, Schneider, Geisslinger, & Lotsch, 2006; Park et al., 2007; Wesmiller et al., 2013). These differences in an individual’s drug metabolism

have the potential to impact opioid metabolism and risk for development of opioid induced respiratory depression.

2.7 CLINICAL GUIDELINES

Evidence based clinical protocols available for determining patients at increased risk for opioid induced respiratory depression are limited. Despite this lack of evidence the importance of monitoring for both sedation and respiratory depression during opioid medication treatment has been noted (Henker & Dunwoody, 2011; Jarzyna et al., 2011; Pasero & McCaffery, 2011). The American Society of Pain Management Nursing determined a set of factors as putting individuals at increased risk for opioid induced respiratory depression (Jarzyna et al., 2011). The priority risk factors found were age above 55 years, obesity (BMI greater than 30), untreated obstructive sleep apnea, history of snoring or witnessed apneas, excessive daytime sleepiness, retrognathia (teeth misalignment which leads to posterior positioning of the maxilla or mandible), neck circumference greater than 17.5 inches, preexisting pulmonary or cardiac disease, major organ failure, smoker, ASA Physical Status 3-5, and increased opioid dose requirement (Jarzyna et al., 2011).

3.0 CHAPTER 3: METHODS

3.1 DESIGN

A secondary data analysis from a prospective comparative study that explored the differences in genotype for the mu opioid receptor, *OPRM1* (The association between mu-receptor genotypes and postoperative pain response, American Association of Nurse Anesthetists Foundation, Dr. Richard Henker) was performed. Data collection was performed by investigators with clinical privileges at Presbyterian Hospital of University of Pittsburgh Medical Center. Specific information that was collected from the participating subjects included; age, sex, height, weight, body mass index, smoking status, race, sedation score postoperatively, amount of opioid received in the operating room, amount of opioid received in the PACU, ASA Physical Status and genetic risk factors including genetic variants on candidate genes, specifically *OPRM1*, *ABCB1*, *COMT*, and CYP2D6. An 11 point verbal pain score was used to evaluate subjects' pain response. Subjects were taught how to use the scale to communicate pain score prior to surgery to aid in increasing the accuracy of patients' use of the scale post operatively. To obtain subjects genetic information, saliva samples for DNA extraction were collected in the clinical unit after the patient had left the PACU. Saliva samples were preserved in Oragene DNA self collection kit from DNA Genotek corporation (Ottawa, ON, Canada). The amount of opioid that subjects received was measured in the operating room and 45 minutes following surgery, while the

subject was in the PACU. Information regarding specific opioid medication administered, amount administered, and time of administration for each subject was gathered from the PACU and perioperative records. Respiratory rates at 15 minutes and 45 minutes postoperatively were collected from the subjects' medical records.

3.2 SAMPLE

A sample of 152 adult subjects that received surgery for orthopedic trauma were used for analysis of differences in genotype for the mu opioid receptor, *OPRM1* (The association between mu-receptor genotypes and postoperative pain response, American Association of Nurse Anesthetists Foundation, Dr. Richard Henker) in the parent study. In this secondary analysis 152 of the subjects from the original sample from the parent study were included for analysis of risk factors for opioid induced respiratory depression. Due to missing data, 8 subjects from the original sample from the parent study were not included in this secondary analysis.

Inclusion criteria were: diagnosis of single isolated extremity fracture, and age between 18-70 years. Exclusion criteria included: opioid consumption within the past 6 months (not including the current hospital admission), history of alcohol abuse, alcohol use within the last 24 hours (3 drinks or greater), history of mental illness, current use of phenothiazines, history of hepatic disease, history of renal disease, history of neurological trauma, and having an American Society of Anesthesiologists Physical Status rating greater than 3 (American Society of Anesthesiologists, 2014).

Orthopedic trauma patients undergoing surgical repair were chosen for this study due to the reports of high pain levels reported with these types of injuries. The high pain levels that

these patients experience require rigorous and comprehensive pain management, for which the gold standard of treatment includes opioids. The high frequency of opioid pain control used in this population provided ample data for analysis of risk factors for opioid induced respiratory depression. In the parent study a sample of opioid naive subjects was chosen intentionally to limit the influence that opioid tolerance has on opioid pain control. Opioid tolerance goes hand in hand with changes in opioid receptors that alters the course of opioid pain control as compared to opioid naive subjects. An opioid naive population was chosen with the intention of minimizing threats to internal validity of the study. Single isolated extremity fracture was the specific orthopedic trauma diagnosis included in this sample, due to the variation of ages that present with this type of injury. Inclusion of a wide range of ages is preferred to limit influence on results from the unique characteristics of any one specific age group. Incorporation of a variety of ages in the sample aims to minimize threats to external validity of the study.

3.3 DATA COLLECTION

3.3.1 Recruitment

Subjects were recruited in the preoperative holding area and inpatient units at Presbyterian hospital of University of Pittsburgh Medical Center. Data from patient reports and medical records were gathered to ensure that the subject met the selection criteria for the study. Subject demographics, co-morbidities, physical status, laboratory values, preoperative and intraoperative medications were all evaluated. The subject was enrolled in the study only if criteria for participation were met and informed consent was obtained.

3.3.2 Anesthesia Management

Intraoperative anesthesia management included: Midazolam <4 mg; Propofol (<3mg/kg) or Sodium thiopental 4-6mg/kg; Succinylcholine or Rocuronium; Fentanyl; hydromorphone; vasoactive medications as needed; and inhaled agents such as isoflurane, sevoflurane, or desflurane. Amount of phenylephrine administered did not exceed 400 mcg. Dose of ephedrine administered did not exceed 25 mg during surgery.

3.3.3 Pain Scale

Pain was measured in the PACU at 15 minutes and 45 minutes postoperatively utilizing the 11-point verbal pain response scale. Education regarding how to use the 11-point verbal pain response scale was provided to patients prior to surgery. Utilizing the verbal pain response scale patients were asked to rate their pain from 0 (no pain) to 10 (the worst pain imaginable).

3.3.4 Opioids

All opioid medications that subjects received were collected from the perioperative and PACU medical records. Medication type, dosage, and time of administration were all collected. Fentanyl was recorded both separately as well as included in total opioids. Dosage of fentanyl was reported in mcg/kg. In efforts to analyze fentanyl in the context of standardized opioid amount, dosage of fentanyl was converted to mg of morphine equivalents, and divided by kg of body weight.

3.3.5 Demographic Information

Age, sex, height, weight, body mass index, smoking status, and race were all obtained from both the medical record and patient report. Race/ethnicity categories used are the following as defined by the NIH; Caucasian, Black or African-American, Asian or Pacific Islander, Hispanic, and Native American (Office of Management and Budget, 1997). ASA (American Society of Anesthesiologists) Physical Classification is an additional aspect of subject demographic data that was collected. The ASA Physical Classification categorizes patients according to level of acuity on a 1-6 scale; 1 is a normal healthy patient, 2 is a patient with mild systemic disease, 3 is a patient with severe systemic disease, 4 is a patient with severe systemic disease that is a constant threat to life, 5 is a moribund patient who is not expected to survive without the operation, and 6 is a declared brain-dead patient whose organs are being removed for donor purposes (ASA, 2014). If the case is deemed emergent, an E is used to denote the emergency nature of the case. (ASA, 2014). The subjects' health history of comorbidities including diabetes mellitus, hypertension, cardiac heart failure, myocardial infarction, and chronic obstructive pulmonary disease, past surgical history, and alcohol use were gathered from both the medical record and patient report.

3.3.6 Saliva Samples

The Oragene self-collection kit from DNA Genotek corporation (Ottawa, ON, Canada) was used for collection of subject saliva samples for DNA extraction. Vials used for collection of saliva contained a stabilization buffer with which the saliva mixed when added to the vial. The mixture of saliva and stabilization buffer in the vials was stable at room temperature for extended periods

of time. Dr. Yvette Conley's lab extracted DNA from the saliva samples using protocol and reagents included with the Oragene self-collection kit. The quantity of DNA extracted from each saliva sample was approximately 100ug. This amount of DNA was more than required for the genotypic analysis used in this study.

3.3.7 Genotype Data

The candidate genes that were genotyped and analyzed for this study were; *OPRM1*, variants A118G (rs1799971), and C17T (rs1799972), *COMT* SNPs; (rs6269), (rs4633), (rs4818), and (rs4680), *ABCB1* SNPs; (rs1045642), (rs2032582), (rs1128503), (rs1202168), and (rs3213619) and *CYP2D6* metabolizer groups. The A118G polymorphism of *OPRM1* at position 118 has the AA genotype which is the homozygous wild type while the AG, and GG genotypes are the genetic variants. The C17T polymorphism of *OPRM1* at position 17 has the CC genotype which is homozygous wild type, while CC and CT at this position are the genetic variant genotypes. The SNP (rs6269) of the *COMT* gene has the AA genotype which is the homozygous wild type while the AG, and GG genotypes are the genetic variants. The SNP (rs4633) of the *COMT* gene has the CC genotype which is the homozygous wild type while the CT, and TT genotypes are the genetic variants. The SNP (rs4818) of the *COMT* gene has the CC genotype which is the homozygous wild type while the CG, and GG genotypes are the genetic variants. The SNP (rs4680) of the *COMT* gene has the GG genotype which is the homozygous wild type while the GA, and AA genotypes are the genetic variants. The *ABCB1* gene SNP (rs1045642) has the GG genotype which is the homozygous wild type while the GA, and AA genotypes are the genetic variants. The *ABCB1* gene SNP (rs2032582) has the CC genotype which is the homozygous wild type while the CT, and TT genotypes are the genetic variants. The *ABCB1* gene SNP

(rs1128503) has the GG genotype which is the homozygous wild type while the GA, and AA genotypes are the genetic variants. The *ABCB1* gene SNP (rs1202168) has the GG genotype which is the homozygous wild type while the GA, and AA genotypes are the genetic variants. The *ABCB1* gene SNP (rs3213619) has the AA genotype which is the homozygous wild type while the AG, and GG genotypes are the genetic variants. Subjects were classified into *CYP2D6* metabolizer groups according to genotype (Wesmiller et al., 2013).

One sequencing reaction was needed to identify the subjects' genotypes at A118G and C17T due to the fact that these are within 101 base pairs of each other on exon 1. Primers were designed to flank the genetic variants, which were then amplified using polymerase chain reaction (PCR). The PCR fragments were cleaned using exoSAP reagents (US Biochemicals, Cleveland, OH). Sequencing was performed using Big Dye Cycle Sequencing reagents (Applied Biosystems Inc, Foster City, CA). The resulting data was converted into viewable data and assigned genotypes using Sequencer software (Gene Codes Corporation, Ann Arbor, MI).

TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) was used to genotype the *COMT*, *ABCB1*, and *CYP2D6* genes. The subjects' single nucleotide polymorphisms were determined utilizing 5' exonuclease Assay on Demand TaqMan assays (ABI). The genetic variants were flanked with the use of primers. Fluorescent oligonucleotide probes in a homogeneous assay were used to amplify the fragments using PCR. Genetic variants were amplified and identified using ABI Prism7000 sequence detection system and SDS 2.0 software. All genotype data were stored in a secure data file on SPSS (SPSS 17.0, Chicago, IL) on a password protected computer.

Before testing for association with phenotypes, all the genetic variants (SNPs) were tested for Hardy-Weinberg equilibrium (HWE) using the exact method (Wigginton et al., 2005)

implemented in the PLINK software (Purcell et al., 2007). All the SNPs with a HWE p-value of less than 0.05 will be flagged for extra attention in explanations of any association findings. To investigate the additive, dominant, and recessive genetic effect of variant allele (minor allele) on phenotypic variation of our outcome measures, we also recoded raw genotypes of all SNPs into three new variables that are consistent to three genetic models. That is, the homozygous major allele genotype, heterozygous genotype, and homozygous variant (minor) allele genotype will be coded as 0, 1, and 2 under additive model, coded as 0, 1, 1, under dominant model, coded as 0, 0, 1 under recessive model, respectively.

3.3.8 Data Cleaning

Data were stored and analyzed utilizing the program SPSS. Categorical variables were assigned a number code in SPSS for organizational and analysis purposes. For variables of interest in which data was unavailable during prospective data collection, the data was collected retrospectively. Data was reviewed and re-checked frequently to ensure that the data was entered correctly into SPSS from the subjects' medical records.

3.4 ANALYSIS

Descriptive statistics were used to determine the minimum, maximum, range, mean and standard deviation of all continuous variables. Frequencies were computed for all categorical variables. Univariate analyses were first performed to assess association of non-genetic risk factors with outcomes (i.e., respiratory rate at 15 and 45 minutes, and change in respiratory rate between 15

and 45 min, respectively). For continuous risk factors, simple linear regression method was used. For categorical risk factors, one-way analysis of variance (ANOVA) method was used to compare the mean difference of outcome between or among each category.

Single-marker genetic association analysis was then performed by multiple linear regression analyses of each outcome that modeled the specific genetic inheritance model (additive, dominant, recessive) of each SNP, after adjusting for these non-genetic variables which are at least marginally significant at $p<0.20$ in univariate analyses. Bonferroni correction method will be applied to adjust from multiple comparisons. A P-value of less than 0.05 will be considered to statistically significant. All statistical analyses were performed using the software SPSS (SPSS 17.0, Chicago, IL).

4.0 CHAPTER 4: RESULTS

4.1 SUBJECT CHARACTERISTICS

Descriptive statistics were used to summarize subject characteristics that were continuous variables (See Table 1). The sample consisted of a total of 152 subjects ranging from age 18-70. The average height of subjects was 173.01 cm with a standard deviation of 18.25, ranging from 64 cm to 206cm. The average weight of the sample was 89.13kg with a standard deviation of 20.81, ranging from 48 kg to 170 kg. Body mass index of the sample ranged from 18.42 to 69.86 with an average of 29.04 and standard deviation of 7.38. Amount of opioid received in the operating room ranged from 0 mg to 103.33 mg with an average of 34.81 mg and standard deviation of in 18.07 mg of Morphine equivalents. The amount of opioid that subjects consumed in the operating room by weight ranged from 0 mg/kg to 1.35 mg/kg with an average of 0.40 mg/kg and standard deviation of 0.22. The amount of opioid consumed in the PACU within the first 45 minutes postoperatively ranged from 0mg to 31.33mg with an average of 7.77mg and standard deviation of 6.02. The amount of opioid consumed by weight in the PACU within the first 45 minutes postoperatively ranged from 0mg/kg to 0.37 mg/kg with an average of 0.09 and standard deviation of 0.07. Frequencies were used to summarize subject characteristics that were categorical variables (See Table 2). American Society of Anesthesiologists (ASA) physical status of the subjects was as follows; 30 subjects had an ASA physical status of 1, 87 subjects had an

ASA physical status of 2, and 17 subjects had an ASA physical status of 3. There were 104 males and 48 females in the sample. There were 65 subjects that were smokers and 86 that were nonsmokers. Smoking status data was unavailable for 1 subject. The racial makeup of the sample was as follows, there were 121 White subjects, 24 African American subjects, 4 Asian subjects, 2 Hispanic subjects, and 1 American Indian subject. American Society of Anesthesiologists (ASA) Physical Status Classifications of the sample are as follows; 30 subjects had an ASA classification of 1, 87 subjects had an ASA classification of 2, 17 subjects had an ASA classification of 3, and data regarding ASA classification was unavailable for 18 of the subjects. Subject sedation score was reported in frequencies (See Table 3). At 15 minutes postoperatively; 38 subjects had a sedation score of 0, 79 subjects had a sedation score of 1, 10 subjects had a sedation score of 2, 0 subjects had a sedation score 3, 2 subjects had a sedation score of 4, and 1 subject had a sedation score of 5. At 45 minutes postoperatively; 58 subjects had a sedation score of 0, 58 subjects had a sedation score of 1, 3 subjects had a sedation score of 2, 0 subjects had a sedation score of 3, 9 subjects had a sedation score of 4, and 0 subjects had a sedation score of 5.

Table 1. Subject Characteristics, Descriptive Statistics.

Subject Characteristic	N	Data Unavailable	Range	Minimum	Maximum	Mean	Std. Deviation
Age	152	0	52	18	70	38.48	13.13
Height (cm)	149	3	142	64	206	173.01	18.25
Weight (kg)	152	0	122	48	170	89.13	20.81
BMI	149	3	51.44	18.42	69.86	29.04	7.38
OR Opioid Administered in mg of Morphine equivalents	151	1	103.33	.00	103.33	34.81	18.07
OR Opioid Administered by weight (kg)	151	1	1.35	.00	1.35	.40	.22
Opioid Administered in the PACU within 45 minutes	148	4	31.33	.00	31.33	7.77	6.02
Opioid Administered in the PACU within 45 minutes by weight	148	4	.37	.00	.37	.09	.07

Table 2. Frequencies of Subject Characteristics

Subject Characteristic	Characteristic Subcategory	Frequency	Percent
Gender	Male	104	68.4%
	Female	48	31.6%
	Unavailable	0	-
	Total	152	100%
Smoking Status	Non Smoker	86	57%
	Smoker	65	43%
	Unavailable	1	-
	Total	151	100%
Race	White (non-Hispanic)	121	79.6%
	African-American	24	15.8%
	Asian	4	2.6%
	Hispanic	2	1.3%
	American Indian	1	.7%
	Unavailable	0	-
	Total	152	100%
ASA Physical Status	1	30	22.4%
	2	87	64.9%
	3	17	12.7%
	Unavailable	18	-
	Total	134	100%

Table 3. Frequencies of Subject Sedation Score

Sedation Score at 15 minutes postoperatively	Frequency	Percent
0	38	29.2%
1	79	60.8%
2	10	7.7%
3	0	0%
4	2	1.5%
5	1	0.8%
Unavailable	22	-
Total	152	100%
Sedation Score at 45 minutes postoperatively	Frequency	Percent
0	58	37.9%
1	58	37.9%
2	3	2.0%
3	0	0%
4	9	5.9%
5	0	0%
Unavailable	24	-
Total	152	100%

4.2 SUBJECT GENETIC CHARACTERISTICS

The genes studied were *OPRM1*, *COMT*, *ABCB1*, and *CYP2D6*. Subject characteristics regarding the genes *OPRM1, COMT,* and *ABCB1* are summarized (See Table 4). The A118G SNP on the gene *OPRM1* was analyzed in sample by separating individuals by genotype; 106 subjects had the genotype AA, 28 subjects had the genotype AG, and 2 subjects had the genotype GG. The SNP C117T on the gene *OPRM1* was also studied; 132 subjects had the genotype CC, 5 subjects had the genotype CT, and 1 subject had the genotype TT. For the *COMT* (rs4680) SNP, 26 subjects had the genotype AA, 39 had the genotype GG, and 32 had the genotype AG. For the *COMT* (rs6269) SNP, 31 subjects had the AA genotype which is the homozygous wild type while 38 subjects had the AG genotype and 22 subjects had the GG genotype. For the *COMT* (rs4633) SNP, 38 subjects had the CC genotype which is the homozygous wild type while 31 subjects had the CT genotype and 26 subjects had the TT genotype. For the *COMT* (rs4818) SNP, 37 subjects had the CC genotype which is the homozygous wild type while 37 subjects had the CG genotype and 22 subjects had the GG genotype. For the *COMT* (rs4680) SNP 39 subjects had the GG genotype which is the homozygous wild type while 32 subjects had the GA genotype and 26 subjects had the AA genotype. For the *ABCB1* gene SNP (rs1045642), 39 subjects had the GG genotype which is the homozygous wild type while 51 subjects had the GA genotype and 19 subjects had the AA genotype. For the *ABCB1* gene SNP (rs2032582) 90 subjects had the CC genotype which is the homozygous wild type while 3 subjects had the CT genotype and 5 subjects had the TT genotype. For the *ABCB1* gene SNP (rs1128503), 48 subjects had the GG genotype which is the homozygous wild type while 49 subjects had the GA genotype and 11 subjects had the AA genotype. For the *ABCB1* gene SNP (rs1202168), 41 subjects had the GG genotype which is the homozygous wild type while 48 subjects had the GA genotype and 10

subjects had the AA genotype. For the *ABCB1* gene SNP (rs3213619), 99 subjects had the AA genotype which is the homozygous wild type while 10 subjects had the AG genotype and 1 subject had the GG genotype. Subjects were classified into *CYP2D6* metabolizer groups according to genotype (Wesmiller et al., 2013).

Subjects were separated by *CYP2D6* metabolizer groups for analysis (See Table 5); 7 subjects were poor metabolizers, 54 subjects were intermediate metabolizers, 79 subjects were extensive metabolizers, and 0 subjects were ultrarapid metabolizers.

Table 4. Summary Information of *COMT, ABCB1, OPRM1* SNPs

				Alleles[a]			Genotype			
Gene	**SNP**	**Chr**	**Position**	**A1**	**A2**	**MAF[b]**	**A1/A1**	**A1/A2**	**A2/A2**	**HWE[c]**
COMT	rs6269	22	19949952	G	A	0.4505	22	38	31	0.141
	rs4633	22	19950235	T	C	0.4368	26	31	38	0.0015
	rs4818	22	19951207	G	C	0.4219	22	37	37	0.0388
	rs4680	22	19951271	A	G	0.433	26	32	39	0.0017
ABCB1	rs1045642	7	87138645	A	G	0.4083	19	51	39	0.8426
	rs2032582	7	87160618	T	C	0.0663	5	3	90	1.05E-06
	rs1128503	7	87179601	A	G	0.3287	11	49	48	1
	rs1202168	7	87195962	A	G	0.3434	10	48	41	0.5122
	rs3213619	7	87230193	G	A	0.0546	1	10	99	0.2715
OPRM1	rs1799972 (C17T)	6	154360696	T	C	0.0254	1	5	132	0.0750
	rs1799971 (A118G)	6	154360797	G	A	0.1176	2	28	106	1

[a]: A1: Minor or Variant allele; A2: Major or wide-type allele.
[b]: MAF: Minor allelic frequency.
[c]: Hardy-Weinberg Equilibrium (HWE) test P-value.
(Note: observed heterozygotes less than expected numbers, causing HWE $p < 0.05$)

Table 5. Summary Information of CYP2D6 Metabolizer Groups

	Frequency	Percent
PM (Poor Metabolizers)	7	5.0%
IM (Intermediate Metabolizers)	54	38.6%
EM (Extensive Metabolizers)	79	56.4%
UM (Ultrarapid Metabolizers)	0	-
Data Unavailable	12	-
Total	140	100.0

4.3 SUBJECT DEMOGRAPHICS AND RESPIRATORY RATE AT 15 AND 45 MINUTES POSTOPERATIVELY

The subject characteristics age, sex, race, height, weight, BMI, amount of opioid received in the operating room, amount of opioid received in the PACU and smoking status were not found to have a significant influence on respiratory rate at 15 or 45 minutes post operatively (See Table 6). Sedation scores at 15 and 45 minutes were found to have a significant association with respiratory rate at 15 and 45 minutes postoperatively (See Tables 6 and 7). ASA physical status was found to have a significant influence on respiratory rate (See Table 6). A higher ASA physical status was associated with higher respiratory rate at both 15 and 45 minutes postoperatively (See Table 8). Post Hoc analysis revealed significant difference between ASA Physical Status groups 1 and 3, and 2 and 3 for respiratory rate at 15 minutes postoperatively. At 45 minutes postoperatively Post Hoc analysis revealed significant difference in respiratory rate between ASA Physical Status Groups 1 and 3.

Table 6. Subject Demographics Analysis P-values

	Postoperative Respiratory Rate 15 minutes after admission to the PACU	Respiratory Rate 45 minutes after admission to the PACU	Change in RR Between 15 and 45 min
Age	0.75	0.85	0 .65
Height	0.72	0.76	0.94
Weight	0.59	0.38	0.24
BMI	0.98	0.99	0.91
OR Opioid in mg of Morphine equivalents	0.64	0.52	0.90
OR Opioid by Weight	0.69	0.65	0.97
Sex	0.65	0.99	0.77
Race	0.09	0.19	0.65
ASA Physical Status	0.01	0.01	0.95
Smoking Status	0.12	0.90	0.28
Presby Sedation Score at 15 minutes	0.05	0.01	0.45
Presby Sedation Score at 45 minutes	0.008	0.004	0.11

Table 7. Subjects' Average Respiratory Rate by Sedation Score

Sedation Score at 15 minutes postoperatively	Frequency	Percent	Respiratory Rate at 15 minutes postoperatively, Mean (Standard Deviation)	Respiratory Rate at 45 minutes postoperatively, Mean (Standard Deviation)
0	38	29.2%	16.55 (3.94)	16.58 (4.73)
1	79	60.8%	15.05 (4.45)	14.59 (3.94)
2	10	7.7%	15.30 (3.97)	12.56 (1.33)
3	0	0%	-	-
4	2	1.5%	20.50 (2.12)	20.00 (9.89)
5	1	0.8%	24.00 (-)	20.00(-)
Unavailable	22	-	-	-
Total	152	100%	15.67 (4.35)	15.16 (4.32)
Sedation Score at 45 minutes postoperatively	Frequency	Percent	Respiratory Rate at 15 minutes postoperatively, Mean(Standard Deviation)	Respiratory Rate at 45 minutes postoperatively, Mean(Standard Deviation)
0	58	37.9%	15.78 (3.93)	16.14 (4.49)
1	58	37.9%	14.61 (4.27)	13.86 (3.24)
2	3	2.0%	14.00 (3.61)	12.00 (-)
3	0	0%	-	-
4	9	5.9%	21.44 (3.75)	17.89 (6.47)
5	0	0%	-	-
Unavailable	24	-	-	-
Total	152	100%	15.61 (4.37)	15.17 (4.30)

Table 8. Subjects' Average Respiratory Rate by ASA Physical Status

ASA Physical Status	Frequency	Percent	Respiratory Rate at 15 minutes postoperatively, Mean(Standard Deviation)	Respiratory Rate at 45 minutes postoperatively, Mean(Standard Deviation)
1	30	22.4%	14.70 (2.89)	14.00 (3.20)
2	87	64.9%	15.53 (3.97)	15.22 (4.37)
3	17	12.7%	18.47 (5.41)	17.82 (4.23)
Unavailable	18	-	-	-
Total	152	100%	15.72 (4.10)	15.28 (4.23)

4.4 GENETIC CHARACTERISTICS AND RESPIRATORY RATE AT 15 AND 45 MINUTES POSTOPERATIVELY

Variation in the subject's genotype on genes *OPRM1* and *COMT* was not found to have an association with respiratory rate at 15 or 45 minutes post operatively (See Table 9). Differences in subject genotype on the *ABCB1* gene for the SNP (rs1045642) were associated with variation in respiratory rate at 45 minutes postoperatively (See Tables 9 and 10). *CYP2D6* metabolizer group classification was associated with differences in respiratory rate at 15 minutes postoperatively (See Tables 11 and 12). Post Hoc analysis revealed significant difference among CYP2D6 metabolizer groups in respiratory rate between poor metabolizers versus intermediate metabolizers and poor metabolizers versus extensive metabolizers at 15 minutes postoperatively.

Table 9. Genetic Association Analysis P-values

Gene	**SNP**	Postoperative Respiratory Rate 15 minutes after admission to the PACU	Respiratory Rate 45 minutes after admission to the PACU	Change in RR Between 15 and 45 min
COMT	rs6269	0.52	0.10	0.20
	rs4633	0.16	0.65	0.48
	rs4818	0.62	0.13	0.20
	rs4680	0.28	0.74	0.57
ABCB1	rs1045642	0.19	0.02	0.40
	rs2032582	0.32	0.78	0.58
	rs1128503	0.42	0.91	0.66
	rs1202168	0.28	0.72	0.66
	rs3213619	0.94	0.65	0.61
OPRM1	rs1799972 (C17T)	0.49	0.36	0.72
	rs1799971 (A118G)	0.39	0.94	0.50

Table 10. Subjects' Average Respiratory Rate by *ABCB1* SNP (rs1045642) genotype

ABCB1 Genotype, SNP (rs1045642)	Frequency	Percent	Respiratory Rate at 15 minutes postoperatively, Mean (Standard Deviation)	Respiratory Rate at 45 minutes postoperatively, Mean(Standard Deviation)
GG	39	25.7%	16.51 (3.91)	14.23 (3.62)
GA	51	33.6%	15.35 (4.39)	16.08 (4.30)
AA	19	12.5%	15.67 (4.33)	16.65 (6.04)
Unavailable	43	-	-	-
Total	152	100%	15.82 (4.21)	15.50 (4.46)

Table 11. CYP2D6 Analysis P-values

CYP2D6, Metabolizer Groups	Postoperative Respiratory Rate 15 minutes after admission to the PACU	Respiratory Rate 45 minutes after admission to the PACU	Change in RR Between 15 and 45 min
P-value	0.001	0.06	0.37

Table 12. Subject's Average Respiratory Rate by *CYP2D6* Metabolizer Group

CYP2D6 Metabolizer Group	Frequency	Percent	Respiratory Rate at 15 minutes postoperatively, Mean(Standard Deviation)	Respiratory Rate at 45 minutes postoperatively, Mean(Standard Deviation)
PM (Poor Metabolizers)	7	5.0%	11.00 (2.45)	13.00 (2.71)
IM (Intermediate Metabolizers)	54	38.6%	15.24 (2.91)	14.44 (3.99)
EM (Extensive Metabolizers)	79	56.4%	16.50 (4.56)	15.95 (4.45)
UM (Ultrarapid Metabolizers)	0	0%	-	-
Data Unavailable	12	-	-	-
Total	152	100.0	15.72 (4.09)	15.21 (4.27)

5.0 DISSCUSSION

This study found that American Society of Anesthesiologists (ASA) physical status classification and differences in *CYP2D6* metabolizer groups were associated with differences in respiratory rate postoperatively. There is limited evidence available regarding the use of ASA physical status classification to gauge risk for opioid induced respiratory depression postoperatively (Jarzyna et al., 2011). Despite the lack of evidence available regarding the use of ASA physical status to determine risk for postoperative opioid induced respiratory depression specify, there are studies demonstrating the efficacy of ASA physical status as a means of predicting operative risk (Albarran, Simoens, van de Winkel, da Costa, & Thill, 2009; Brouquet, Cudennec, Benoist, Moulias, Beauchet, et al., 2010; Chida, Ono, Hoshikawa, & Kondo, 2008; Johnson et al., 2007; Peersman, Laskin, Davis, Peterson, & Richart, 2008; Sanjay, Jones, & Woodward, 2006; Skaga, Eken, Sovik, Jones, & Steen, 2007; Wolters et al., 1996). These studies demonstrated that ASA physical status was associated with a patient's risk of developing a variety of postoperative complications. The findings of this study support the findings of these previous studies demonstrating that ASA physical status is associated with increased risk of postoperative complications. In the present analysis, ASA physical status was associated with differences in respiratory rate at 15 and 45 minutes postoperatively. Although in the present study higher ASA Physical Status score was associated with an increased respiratory rate postoperatively.

ASA physical status is a classification used in standard practice in the management of surgical patients. These findings provide further evidence to support the use of ASA physical status to identify individuals at increased risk for postoperative respiratory depression. The application of ASA physical status to identify individuals at increased risk for postoperative respiratory depression translates readily into clinical practice because of the current widespread use of ASA physical status in clinical practice. The correlation of both these factors with postoperative respiratory depression have potential applications in clinical practice. With the application of genetic testing, patients could be identified by metabolizer group and have drug therapy tailored to decrease the risk of postoperative respiratory depression.

Available evidence of the role of genetic differences in the *CYP2D6* gene and development of respiratory depression is predominantly in the form of case studies of pediatric patients. In examining the genetic differences between pediatric patients that experienced fatal opioid induced respiratory depression postoperatively it was found that genetic differences on the *CYP2D6* gene were not strongly associated with the risk of opioid induced respiratory depression (Racoosin, Roberson, Pacanowski, & Nielsen, 2013). These findings contrast with the findings of this analysis which found that differences in *CYP2D6* metabolizer group classification were associated with differences in respiratory rate postoperatively. One of the possible reasons for the difference would be age of the sample.

It has been noted in the literature that genetic differences on the *ABCB1* gene can result in differences in an individual's reaction to opioid medications (Dahan & Kest, 2001; Mercer & Coop, 2011; Niesters, Overdyk, Smith, Aarts, & Dahan, 2013; Park et al., 2007). Specifically the role of the *ABCB1* gene in risk for opioid induced respiratory depression has been evaluated and found to be significant (Dahan & Kest, 2001; Mercer & Coop, 2011; Niesters, Overdyk, Smith,

Aarts, & Dahan, 2013; Park et al., 2007). The present study found that difference in subject genotype on the *ABCB1* gene was associated with differences in respiratory rate 45 minutes postoperatively. These findings are in agreement with current available literature on this subject. The findings of this study along with the findings of the current literature support the use of genetic data regarding the *ABCB1* gene to asses an individual's risk for opioid induced respiratory depression.

The subject characteristics age, sex, height, weight, body mass index, smoking status, race, amount of opioid received in the operating room, amount of opioid received in the post anesthesia care unit (PACU) and genotype data including: single nucleotide polymorphism (SNPs) from the candidate genes of interest OPRM1 and COMT. There is evidence to suggest that age over 65 years is associated with opioid induced respiratory depression (Auburn & Marmion, 2007; Taylor, Kirton, Staff, & Kozol, 2005).

One of the limitations of this study is inherent in the secondary analysis design. The data used for analysis in this study was originally collected for a prospective comparative study that evaluated the differences in genotype for the mu opioid receptor, *OPRM1* (The association between mu-receptor genotypes and postoperative pain response, American Association of Nurse Anesthetists Foundation, Dr. Richard Henker). Secondary analysis design presents challenges because the data was not collected for the purposes of the current study. An additional limitation to this study was the collection of data retrospectively that was unavailable during prospective data collection. Retrospective data collection is problematic because of the increased risk of error with this method of data collection. One of the other limitations of this study was the sample size. This creates difficulty in ruling out other possible influences on the results such as age. In other studies (Aubrun & Marmion, 2007; Cepeda et al., 2003) age has been found to be associated

with opioid induced respiratory depression. The relatively small size of this sample makes it difficult to attain significance in all factors that may be associated with changes in respiratory rate. In particular in the *CYP2D6* metabolizer groups there were no ultrarapid metabolizers and 7 poor metabolizers. In analysis of genetic characteristics a larger sample size is preferable.

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