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THE ASSOCIATION OF CYP2D6 AND μ-OPIOID RECEPTOR GENOTYPES AND POSTOPERATIVE NAUSEA AND VOMITING IN ADULT ORTHOPEDIC PATIENTS WITH SINGLE EXTREMITY FRACTURES

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Susan Watters Wesmiller, PhD, RN

Often considered the "big little problem," postoperative nausea and vomiting (PONV) is a common surgical complication. Treatment of pain with opioids is the primary cause of PONV although other risk factors include female gender, non smoking status and history of PONV or motion sickness. Research has focused on medications to prevent or treat PONV, and risk factors that contribute to PONV. Genetics may also play a role. The purpose of this study was to explore the association of CYP2D6 and μ-opioid receptor genotypes with PONV in patients with single extremity fractures.

Subjects (n=143), aged 18-70 were recruited for this exploratory, descriptive study. Informed consent was obtained. PONV was collected by self-report and chart audit. Saliva samples were collected for DNA extraction. Results of Taqman® allele discrimination were used to assign a CYP2D6 classification of poor metabolizer (PM), intermediate metabolizer (IM) extensive metabolizer (EM) and ultrarapid metabolizer (UM). Two SNPS of the μ-opioid receptor gene were analyzed, A118G and C17T by Polymerace Chain Reaction (PCR). Due to genetic differences within ethnic groups, only Caucasians (n=112) were included in the CYP2D6 analysis. The incidence of PONV in the PACU was 38%, increasing to 50% when assessed for 48 hours. CYP2D6 classification results were: 7 (6%) PM group; 34 (30%) IM group; 71 (63%), EM group; and no ultrarapid metabolizers. Gender and history of PONV were

significant risk factors in this study ($p<.05$). There was a trend for age ($p=.071$), but smoking was not significant ($p=.505$). The CYP2D6 EM group served as the reference for binary logistic regression analysis which revealed a significant difference with the CYP2D6 PM group for presence of PONV ($p =.003$). The sample size for the μ-opioid receptor genotype analysis was 82, the genotype distribution was 58 (70%) AA or CC (wild type) and 24 (30%) polymorphism (AG, GG, CT, or TT were combined). No statistical differences were found in the μ-opioid receptor genotype groups for PONV. Ultimately personalized medicine will allow health care providers to treat all patients individually, so it is important for clinical genetic research to identify those risks that may lead to a negative outcome.

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PREFACE

Because my dissertation journey has spanned over twenty years, I have many family, friends and colleagues to acknowledge. First, to my husband Steve for always being on my side, and providing me the opportunity to fulfill this dream, to my children Ashley and Matthew for their constant love and encouragement. Second, to my life-long mentor, Dr. Leslie Hoffman, who introduced me to the wonderful world of nursing research almost 30 years ago. To Dr. Ellen Rudy who helped me stay the path even though she does not know it, to Dr. Judith Erlen for her constant support and to Dr. Andrea Schmid-Mazzaccoli for not accepting the status quo.

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1.0 INTRODUCTION

Recently called the "big little problem" by several authors (Lichtor & Glass, 2008; Nelson, 2002), postoperative nausea and vomiting (PONV) is one of the most common and disturbing complications after surgery and general anesthesia (Apfel, Roewer & Korttila, 2002, and Gan, 2007). The use of opioids for postoperative pain is recognized as the primary cause of PONV (Watcha & White, 1992). PONV occurs primarily within the first 24 hours of surgery, may cause significant morbidity, delayed discharge from the post anesthesia care unit (PACU), increased length of hospital stay (LOS), increased hospital costs (Habib, Chen, Hu, & Gan, 2006; Mace, 2003) and perhaps most importantly in today's world of health care; poor patient satisfaction (Silva, O'Ryan, & Poor, 2006; Myles, Williams, Hendrata, Anderson, & Weeks, 2000). Much research has focused on the use of combinations of medications to prevent or treat PONV, based on the multiple pathways that can cause PONV (Gan, 2007). These new combination strategies have improved the management of PONV but have not eliminated the problem (Ho & Gan, 2006). Other researchers have focused on identification of risk factors in order to predict which patients are most likely to experience PONV (Apfel, et al., 2008; White, O'Hara, Robertson, Wender, & Candiotti, 2008; Murphy, Hooper, Sullivan, Clifford, & Apfel, C., 2006; Meng & Quinlan, 2006). Despite volumes of research, the evidence continues to show that 20-30% of postoperative patients experience PONV (Kranke, Roewer, Smith, Piper, Wallenborn, & Eberhart, 2009). The emerging question becomes, are there genetic differences to

why patients respond so differently, and can these differences be identified? Kranke and colleagues (2009) believe that instead of considering PONV the "big little problem," that it should be viewed as a "big little opportunity." They believe that just as postoperative pain has come to be totally unacceptable, so should PONV. This exploratory, descriptive study will provide pilot data for further research to determine the association between PONV, genetic variants and pain.

1.1 PURPOSE

The purpose of this study was to explore the association of CYP2D6 genotypes and μ–opioid receptor genotypes with PONV in adult patients admitted for surgery to repair a single, isolated orthopedic injury.

1.2 SPECIFIC AIMS

The primary aims of this study were:

1. To explore the association between CYP2D6 genotypes and PONV in patients admitted for orthopedic surgical repair of a single fracture to an extremity and to explore if CYP2D6 genotypes moderate the relationship between the amount of opioids administered and the incidence of PONV

2. To explore the association between μ–opioid receptor genotypes and PONV in patients admitted for orthopedic surgical repair of a single fracture to an extremity and to explore if

μ–opioid receptor genotypes moderate the relationship between the amount of opioids administered and the incidence of PONV

The secondary aims of this study were:

3. To explore the association of CYP2D6 genotypes and the amount of rescue anti-emetics in the first 48 hours post-operatively in patients admitted for orthopedic surgical repair of a single fracture to an extremity

4. To explore if patients who experience PONV report a higher level of pain than those subjects who do not experience PONV

1.3 RESEARCH FRAMEWORK

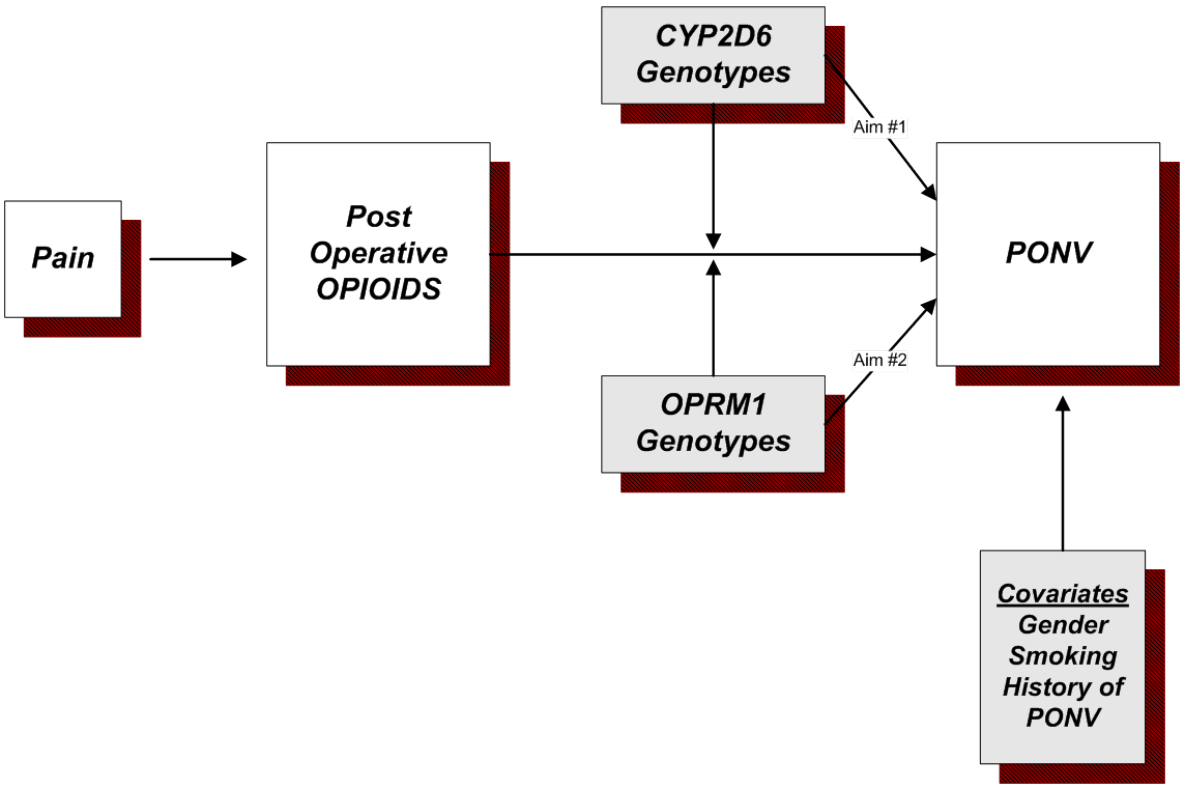

Figure 1: Diagram of the conceptual framework used for this study

1.3.1.1 PONV

PONV is usually addressed in three different stages, nausea, retching and vomiting that occur within 24 hours of surgery. Some researchers designate the first two hours after surgery as early PONV as compared to later PONV that extends to the first postoperative day (Apfel et al., 2002). Nausea is defined as a subjective, personal feeling associated with awareness of the urge to vomit. Vomiting is defined as the forceful expulsion of gastric contents through the mouth (Watcha & White, 1992). Retching is the muscular movement of the abdomen that precedes vomiting, and is also considered the non-productive vomiting (Habib, Chen, Taguchi, Hu, & Gan, 2007) that occurs when the stomach is empty. For the purpose of this study, PONV is the combination of nausea and/or vomiting as measured by the need for rescue ondansetron. Ondansetron is the PRN anti-emetic of choice at the University of Pittsburgh Medical Center. In addition, because the PONV data was in some cases collected retrospectively, it was the only consistent way to determine if a patient had PONV on chart review. Two values for PONV were measured, the total amount received in the PACU and also total amount received 48 hours after surgery. The time span of forty eight (48) hours was selected in order to have a more comprehensive picture of the PONV experience. It also allowed for a truer picture of PONV because all patients are given prophylactic ondansetron prior to leaving the operating room (OR) preventing some PONV that may have occurred in the PACU.

1.3.1.2 Postoperative opioids

Total amount of opioids given during the procedure and in the PACU were recorded. Amount of opioids were converted to morphine equivalence (Table 1) for comparison (McCaffery & Pasero, 1999), and then adjusted by weight for the score used in this study.

Table 1: Morphine equivalents table used for definition of total opioids in study.

Opioid	Dosage	Route
Morphine	10 mg	IV
Morphine	30 mg	PO
Fentanyl	100ug	IV
Dilaudid	1.5 mg	IV
Dilaudid	7.5 mg	PO
Oxycodone	20 mg	PO
Oxymorphone	1 mg	IV

1.3.1.3 CYP2D6

CYP2D6 is a member of the cytochrome P450 (CYP) superfamily, comprised of the most important drug-metabolizing enzymes. The CYP450 superfamily includes 57 functional CYP genes and 58 pseudo genes within 18 families. Members of the CYP family are exceptionally polymorphic and allelic variants result in a significant effect on drug metabolism. To date, the

gene CYP2D6, located on chromosome 22, has been the most extensively studied of the CYP enzymes, and is responsible for the metabolism of up to 25% of common medications (Zhou, S., 2009). Specifically, CYP2D6 can alter the metabolism of some opioids and is responsible at least in part, for the metabolism of many of the anti-emetic drugs given postoperatively, including ondansetron, tropisetron, palonosetron and dolasetron (Janicki, Schuler, Jarzembowski, & Rossi, 2006). The CYP2D6 single nucleotide polymorphisms (SNPs) examined for this study are listed on Table 2. The SNPs listed were chosen for this study based on the high frequency in which they occur in North American populations. CYP2D6 classifications are assigned based on alleles identified. Individuals are considered poor metabolizers (PMs) when there are two nonfunctioning alleles, intermediate metabolizers (IMs) with two decreased functioning alleles or one non functioning allele and one allele that leads to decreased activity, extensive metabolizers (EMs) if two functional wild type alleles are present and ultrarapid metabolizers (UMs) in the presence of three or more functional wild-type alleles (Owen et al, 2009). Multiple gene copies will cause a patient to posses the extra functional wild-type alleles that usually leads to the UM classification. Increased metabolic activity leads to lower serum concentration of the drugs, leading to potential under-treatment. Conversely, a poor metabolizer may not clear the drug as easily and is at risk to develop toxic side effects due to prolonged plasma exposure. However, depending on the drug, a poor metabolizer may not be able to metabolize medications into active metabolites, so they are also at risk for being under-medicated.

1.3.1.4 μ-Opioid Receptor Gene

The genetic code for the μ-opioid receptor (OPRM1) is located on chromosome 6q24-q25. Mutations in OPRM1 are reflected in protein sequences in the μ receptors and have been

associated with variation in agonist binding. Two of the more clinically relevant polymorphisms that are associated with the OPRM1 gene were identified for the parent study. They are A118G, a substitution that occurs in 10.5 to 18.8% of the population, and C17T, a substitution that occurs in 1-10% of the population (Lötsch & Geisslinger, 2005). Specifically, the polymorphisms include the A to G transition at nucleotide 118 (rs1799971) and the C to T transition at nucleotide 17 (rs1799972) (Bond et al., 1998). Both of these single nucleotide polymorphisms (SNPs) are non-synonymous, resulting in amino acid differences. Differences in genotypes are reflected in the protein structure and function of mu receptors, potentially influencing the ability of opioid binding.

1.3.1.5 Ondansetron

Ondansetron is the anti-emetic drug of choice at the University of Pittsburgh Medical Center. A serotonin or 5-hydroxytryptamine type 3 ($5\text{-}HT_3$) receptor antagonist, ondansetron has a well established role in the prophylaxis and the treatment of PONV. Compared to earlier, more traditional anti-emetic medications, the 5-HT3 receptor antagonists have greater efficacy and fewer side effects (Ho & Gan, 2006). Ondansetron, like most of the 5HT3 receptor antagonists, are more effective in preventing vomiting than nausea, but continues to be the first line of defense in both situations (Janicki, Schuler, Jarzembowski & Rossi, 2006). Because it is the drug of choice, it has been selected as the measure to determine the presence of PONV in this study. All postoperative patients are ordered ondansetron for relief of PONV. It is not a routine medication order, but a "PRN" order, to be used in the event the patient experiences nausea and or vomiting.

1.3.1.6 Pain

Pain is operationally defined for this study by an 11-point verbal pain rating score. Subjects are asked to describe their pain with 0 designating no pain and 10 meaning the worst pain ever experienced. A verbal pain score is considered more reliable when assessing postoperative patients in the Post Anesthesia Care Unit (PACU) who may be too drowsy to mark a line on a visual analog scale (Loos, Houterman, Scheltinga & Roumen, 2008; Cork, Isaac, Elsharydah, Saleemi, Zavisca, & Alexander, 2004).

Table 2: Identification and definition of CYP2D6 alleles examined for this study

Allele Name	SNP	Change	Metabolic Activity	Assignment	Notes
CYP2D6*1		Reference allele	Normal	Extensive Metabolizer	Defined by absence of variation in other alleles
CYP2D6*2	rs16947	1661G>C 4180G>C	Normal, if multiple copies increased activity	Extensive Metabolizer	Ultrarapid Metabolizer if multiple copies
CYP2D6*3	rs35742686	2549delA	None	Poor Metabolizer	
CYP2D6*4	rs3892097	1846G>A	None	Poor Metabolizer	Most frequent allele - Caucasians
CYP2D6*5		Whole gene deletion	None	Poor Metabolizer	
CYP2D6*9	rs5030656	2615-2617del AAG	Decreased	Intermediate Metabolizer	
CYP2D6*10	rs1065852	100C>T and the absence of 1846G>A	Decreased	Intermediate Metabolizer	As high as 50% in Asian populations
CYP2D6*17	rs28371706	1023C>T	Decreased	Intermediate Metabolizer	Marked variability
CYP2D6*39	rs1135840	1661G>C 4180G>C	Normal	Extensive Metabolizer	

1.4 BACKGROUND AND SIGNIFICANCE

Postoperative nausea and vomiting has long been a concern for nurses caring for surgical patients. In the "Ether Days," PONV was consistently as high as 80% (Watcha & White, 1992). Despite new medications, and new technology the incidence of PONV continues to be 20-30%, thus affecting as many as one third of the over 71 million surgical patients annually (ASPEN, 2006). PONV is one of the strongest predictors for prolonged hospital stay and unanticipated admission for outpatient surgical patients (Pradham, Crichton, Edmonds, (1999), costing what could account for millions of dollars of health care costs annually (Apfel, Kranke, & Eberhart, 2004). Potential adverse effects of PONV in addition to causing extreme discomfort for the patient can include aspiration, wound dehiscence, bleeding, hematoma, dehydration, electrolyte imbalance, delay in ability to begin oral medications, exhaustion and general delay in mobilization and recovery (Miaskowski, 2009; Jolley, 2001).

1.4.1 Pathophysiology

Essential to the central mechanism in the cause of PONV is the area postrema, a part of the brain that controls vomiting. It is located on the dorsal surface of the medulla oblongata at the caudal end of the fourth ventricle. Because it lacks a blood-brain barrier, it detects toxins in both blood and cerebral spinal fluid (Gan, 2007). The use of opioids for postoperative pain is recognized as being the primary cause of PONV (Watcha & White, 1992). There are three different mechanisms that lead to PONV, all eventually stimulating the emetic center in the medulla.

First, opioids cause a reduced gastrointestinal mobility that generates a release of serotonin from the enterochromaffin cells in the gastrointestinal tract. The enterochromaffin cells produce approximately 90% of the body's store of serotonin (Nielson & Olsen, 2008). Serotonin then binds to the visceral $5HT_3$ receptors, stimulating vagal afferent neurons that in turn activate the vomiting center via the nucleus tractus solitarius (NTS) which is connected to the area postrema. Second, opioids stimulate the chemoreceptor zone in the brainstem, which causes a release of serotonin and dopamine, which directly activate the emetic center by binding to receptor sites (Gan, 2007; Nielson & Olsen, 2008). A third mechanism develops when opioids enhance the sensitivity of the middle ear to movement. This action activates the emetic center by the release of histamine and acetylcholine from vestibular fibers (Jolley, 2001; Silva et al., 2006). Another neurotransmitter that has received attention in the past decade is Substance P, a member of the tachykinin family of neuropeptides (Gan, 2007). Substance P may be released from enterochromaffin cells or from sensory neurons and is tied to at least three neurokinin 1 (NK_1) subtypes that are located in the area postrema. Currently, the mechanisms of NK_1 activity are the focus of much research. Blocking these multiple neurotransmitter receptor sites is the mechanism of most anti-emetic drugs, and many agents have been developed to act against one or more of these receptors (Nielson & Olsen, 2008). Each of these pathways provides opportunities to reduce PONV as they all function independently. Because of their independence, there is significant empirical evidence that support the use of multiple drugs to prevent or treat PONV. Guidelines established by an expert panel recommends the use of combination therapy with 2 or 3 prophylactic agents from difference classes for patients who are considered to be high risk for PONV (Gan et al., 2007).

Not only is PONV the most common postoperative side effect (Apfel, Laara, Koivuranta, Greim, & Roewer, 1999; White et al., 2008), it is also the most bothersome for patients. When patients are asked to identify the side effect they most want to avoid, they rank PONV above pain. Macario and colleagues (1999) gave one hundred subjects play money to "buy" those symptoms they most wanted to avoid postoperatively. The "winning" symptom, that symptom that patients were most willing to spend their money to prevent was vomiting. In addition to vomiting, gagging on the endotracheal tube, incisional pain and nausea were the other top four symptoms that patients wanted to avoid, literally at all costs. A decade later, Macario's study was replicated in Germany and Turkey (Kerger et al., 2007). In the second study, Kerger and colleagues gave 100 German and 100 Turkish patients 100 Euros to spend. Patients were willing to spend their money to prevent PONV more frequently than pain or other postoperative complications. Subjects who had not experienced PONV in the past spent an average of 68 Euros to prevent postoperative vomiting. However, patients who had experienced PONV in the past were willing to use all of their money to preventing PONV, using an average of 99 of the100 allotted Euros.

1.4.2 Risk Factors

Risk factors have been identified by multiple studies trying to predict those patients most vulnerable to develop PONV. The risk factors most frequently identified include gender, smoking status, history of PONV or motion sickness and use of opioids for postoperative pain (White et al., 2008; Apfel et al., 2008; Murphy et al., 2006). On the basis of two independent studies, Apfel and colleagues (1999) developed a simplified risk score for predicting PONV. In a study of 1,137 subjects, they assigned one point for each risk factor known, so a non-smoking

female with a previous history of PONV that was treated with opioids for postoperative pain would be assigned a score of four. Using forward stepwise regression, they found that the presence of none, one, two, three or four of these risk factors increased the incidence of PONV by 10%, 21%, 39%, 61%, and 79%, respectively. The Risk Factor Assessment Tool has been further tested and continues to successfully give physicians and nurses the opportunity to provide guidance for prophylactic anti-emetic therapy, especially focusing on those patients who are considered high risk (Apfel, Korttila, et al., 2004; Apfel). Silva and colleagues (2006) studied retrospectively 514 patients in an attempt to determine predictors of PONV. Among their patients, 40 % experienced PONV during the first 24 hours after surgery. In their study, the most important predictive factors associated with an increased risk of PONV were female gender, young patients (15 to 25 years old), nonsmoking status, presence of predisposing factors (i.e., prior history of motion sickness and/or PONV, vertigo or migraine headaches), use of volatile general anesthetics, maxillary surgery, postoperative pain level in the PACU and the use of postoperative analgesic opioid drugs. They also found a directly proportional relationship between the number of risk factors and the prevalence of PONV. Not surprisingly, Kerger and colleagues (2007) also found in their study of the 100 German and Turkish patients, that the amount of money patients were willing to pay to prevent PONV, was also related to female gender, history of motion sickness and non-smoking status.

1.4.3 Smoking Advantage

Among these studies, smoking has consistently been identified as a protectant of PONV. There are two explanations offered in the literature to support these findings. There is a general sense that patients who smoke initially have to develop a tolerance to nausea if they continue to smoke

(Pomerleau, Pomerleau, Mehringer, Snedecor, Ninowski, & Sen, 2005). So smokers, used to the smoke from cigarettes, are able to exhibit cross tolerance behaviors to other noxious stimuli. The second enzymes explanation is chronic exposure to cigarette smoke produces changes in the liver microsomal that metabolize nicotine (Sweeney, 2002). Specifically, there are two enzymes in the P450 family that are affected by cigarette smoke; CYP1A2 and CYP2E1. Tar, the condensate of cigarette smoke, is a mixture of polycyclic aromatic hydrocarbons (PAH's). There is some thought that PAHs have a positive effect on the hepatic enzymes, increasing their efficiency. Both CYP1A2 and CYP2E1 are involved in the metabolism of drugs used for anesthesia, especially CYP2E1 which is responsible for the metabolism of volatile anesthetics, considered one of the major causes of early PONV (Sweeney, 2003). More efficient enzymes are able to more quickly metabolize the anesthetics, decreasing risk of PONV. Smokeless tobacco has also been shown to have a protective effect on PONV. In a study of 355 postoperative patients, Brattwall and colleagues (2009) found that smoking or snuffing had an equal influence on PONV, which was decreased by 50% in the tobacco group when compared to the nonsmokers and "non snuffers". The use of the nicotine patch has been suggested as a strategy to prevent PONV (Ionescu, Badescu, & Acalovschi, 2007), and is currently the focus of an NIH funded study.

Given that despite the best research, identification of new medications and risk factors, 20% of postoperative patients still experience PONV, the next logical direction is to explore if there are genetic components that could help predict who will experience PONV.

1.4.4 CYP450 Genotypes and PONV

CYP2D6 is located on chromosome 22q1 where it forms part of the CYP2D6 gene cluster with two non-functional pseudo-genes, CYP2D7P and CYP2D8P (Arneth, Shams, Hiemke, & Hartter, 2009). CYP2D6, which localizes primarily to the liver, is now believed to be involved in the metabolism of 25% of the common drugs administered today, including many opioids and anti-emetics (Owen, Sangkuhl, Klein, & Atman, 2009; Zhou, S., 2009). CYP2D6 was first known to effect drug metabolism in a pharmacokinetic study of the antiarrhythmic drug, sparteine. Researchers observed serendipitously that some subjects experienced symptoms of nausea and diplopia, which are indicative of toxic doses of Group 1 antiarrhythmics. They went on to report that between 5-10% of Caucasians had a defect in their ability to metabolize this drug (Eichelbaum, Spannbrucker, Steincke, & Dengler, 1979). It is now known that the defective metabolism they observed is inherited as an automsomal recessive trait (Sweeney, 2003). CYP2D6 is believed to be involved with up to 25% of commonly used medications (Owen, Sangkuhl, Klein, & Atman, 2009). Among the CYP 450 family, CYP2D6 is the only noninducible enzyme, which results in a large contribution of genetic variation to the interindividual variation noted in enzyme activity (Ingelman-Sundbert, Sim, Gomez, & Rodriguez-Antona, 2007). CYP2D6 is highly polymorphic, with over 90 known allelic variants identified (Daly, Brockmöller, Broly, Eichelbaum, Evans, & Gonzalez, 1996). Some alleles led to a complete loss of CYP2D6 function, other allelic variants resulted in hyperfunction. Knowing the number of functional gene copies of CYP2D6 per genome has become an important determinant of drug clearance for many substrates of this enzyme, in addition to genotyping for nonfunctional and variant alleles (Schaeffeler, Schwab, Eichelbaum, & Zanger, 2003).

Based on the identification of alleles, a system was developed that assigned patients into four classifications: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultrarapid metabolizers (UM), where the extensive metabolizers are considered to be normal (Ho & Gan, 2006). In a study that compared the results of 76 liver biopsies, average microsomal CYP2D6 protein and enzymatic activity of the four classifications, IM and EM were found to be significantly different. The immunologically quantified CYP2D6 for the UM group was 23.8 $\pm$ 7.7 pmol/mg as compared to 2.64 $\pm$ 2.67 pmol/mg in the IM group ($p<.01$). Genotype distribution (n=76) was 5% UM, 78% EM, 9% IM and 6% PM (Zanger et al., 2001). This distribution is consistent with other studies (Candiotti et al., 2005).

The polymorphisms identified in the CYP2D6 gene are very different across ethnic groups. CYP2D6*10, an allele that leads to decreased functioning, is the most common allele (Zhou, Q. et al., 2009), and in fact CYP2D6*10 occurs in almost 50% of Chinese. CYP2D6*4 is the most common allelic variant in Caucasians occurring in 20-24% of the population (Arneth et al., 2009), yet in Chinese populations is found in only one out of 100 individuals. CYP2D6*17 is an allele that occurs frequently in the Afro-American population causing decreased CYP2D6 activity.

The clinical impact of the CYP2D6 enzyme can be significant. Patients who were classified UM experienced more vomiting compared with the EM or PM groups in subjects treated for chemotherapy induced nausea and vomiting (Kaiser et al., 2002). The same study found that patients with CYP2D6*1 allele duplications had lower 5-HT3 drug concentrations. In another study that compared 250 patients in terms of their metabolic status, the highest percentage of subjects with nausea and vomiting was in the UM group (Candiotti et al.., 2005). In their study, when activity was analyzed by genotype, the incidence of PONV in poor,

intermediate, extensive and ultra rapid metabolizers was 1/12 (8%), 5/30 (17%), 26/176 (15%) and 5/11(45%), respectively. The UMs vs all other groups combined resulted in a significant difference ($p<.01$). Janicki and colleagues (2006) compared treatment of PONV with granisetron and dolasetron in relation to the CYP2D6 genotype in 150 subjects considered to have moderate to high risk for PONV. Granisetron is not metabolized by CYP2D6, but a less polymorphic CYP3A4, so it not surprisingly that they found those subjects treated with granisetron had a more frequent complete response. Among the subjects treated with dolasetron, the greatest incidence of nausea and vomiting was in the subjects who were phenotyped as ultra metabolizers.

1.4.5 Mu-opioid receptor gene

Opioid receptors belong to the G-protein coupled transmembrane protein class and are present in both the central and peripheral nervous systems (Piestrzeniewicz, Fichna, & Janecka, 2006). Multiple opioid receptors have been identified (μ, δ, κ) and though they have similarities in structure, each has distinct clinical effects and tissue distribution. In the case of the opioid class of drugs, OPRM1 (the μ-opioid receptor gene) has been identified as having the greatest relevance in determining a patient's relative risk of experiencing adverse effects from opioids and is most related to analgesia (Reynolds, Ramey-Hartung, & Jortani, 2008). Several naturally occurring single nucleotide polymorphisms (SNPs) have been identified in the μ–opioid receptor gene located at chromosome 6q24-25; they are rs1799971 (A118G) and rs1799972 (C17T) (Bond et al., 1998). All of these single nucleotide polymorphisms (SNPs) are non-synonymous, resulting in amino acid differences. The A118G polymorphism results in an asparagine being replaced by an aspartic acid. The C17T polymorphism results in an alanine being replaced by a

valine. Therefore differences in genotype are reflected in the protein structure, potentially influencing the binding of opioids to these receptors. In previous work done by Bond and colleagues (1998) μ-opioid receptor binding with endorphins was found to be 3 times greater in patients with the A118G polymorphism, although there was no difference in binding of exogenous opioids. Bond's work has not been confirmed and there is some evidence that the polymorphism A118G changes the functional properties of the μ-opioid receptor (Kroslak et al., 2007) but the precise mechanism of the allelic effect is still considered a mystery (Beyer, Koch, Schroder, Helmut, & Hoiit, 2004). The μ-opioid SNP that has been studied most frequently is the A118G. Several studies have reported the association of the μ-opioid receptor gene polymorphism (A118G) with variations in morphine consumption for analgesia after total knee arthroplasty and after total abdominal hysterectomy (Chou, Yang, Lu, & et al., 2006; Chou, Wang, Liu, Liu, Tseng, & Jawan, 2006). Chou and colleagues (2006) in both studies did not find statistical differences in the incidence of PONV, but in both studies report a higher incidence of PONV in patients who have the AA (wild-type) genotype. That trend was confirmed by the work of Sia and colleagues (2008). In their study of women post cesarean-section, they report that genetic variation at A118G of the μ-opioid receptor is associated with individual differences in pain score, amount of self-administered morphine and the incidence of postoperative nausea. Specifically, they found that those subjects who carried the AA (wild-type) for A118G had significantly more PONV, despite a lower consumption of PCA morphine postoperatively. They speculated that the greater sensitivity to morphine attributed to the AA (wild-type) genotype might also be the reason for the increased PONV (Sia et al., 2008).

1.4.6 Pain and PONV

Chia and colleagues followed over 600 women undergoing gynecological surgery to determine if those subjects who experienced postoperative pain also had increased incidence of emesis postoperatively. They reported that patients who experienced postoperative emesis had significantly higher pain scores ($p<.05$). They also found, that for those subjects still experiencing PONV three days postoperatively, pain was the main risk factor (Chia et al., 2002). Patients often describe the very uncomfortable feeling of nausea as being worse than pain, so it is important to understand if this uncomfortable feeling may increase the perception of pain. Pain clinical nurse specialists have many anecdotal observations that patients who experience PONV also report higher levels of pain; however, the evidence that supports these observations is very limited.

1.4.7 Innovation and Significance

Further research is needed to better understand the genetic component of PONV so this negative side effect of postoperative opioids can be treated more effectively, if not eliminated all together. This exploratory study should lead to future research that would address the interactions between multiple genetic variants and also be the framework from which other populations of surgical patients can be studied. It is interesting that the medical literature focuses on assessment of PONV in the immediate postoperative period, up to 24 hours postoperatively. The American Society of PeriAnesthesia Nursing (ASPAN, 2006) describes the problems associated with PDNV (post discharge nausea and vomiting – which is PONV that occurs after the patient has been discharged from the hospital setting), yet there is little research in the literature that

describes what happens after the first 24 hours postoperatively. Few studies have followed patients for 48 hours postoperatively, and doing so will provide an increased understanding of the potential problem of post discharge nausea and vomiting.

1.5 RESEARCH DESIGN AND METHODS

1.5.1 Design

This study was designed to explore the association between candidate gene genotypes (OPRM1 and CYP2D6) and PONV in patients undergoing surgery for orthopedic trauma for a single extremity fracture. A secondary analysis was conducted using data from a larger, parent study; "The Association between μ-receptor Genotypes and Postoperative Pain Response." The primary study, under the direction of Richard A. Henker, RN, PhD, was supported by two grants: 1) AANA Foundation, and 2) Clinical Translational Science Institute grant funded by NIH, NCRR 1 UL1 RR024153 and also funded by a Special Grant from the Office of the Senior Vice Chancellor for the Health Sciences, University of Pittsburgh. The parent project is a descriptive, prospective study designed to explore the association of μ-opioid receptor genotypes and postoperative pain. In the proposed secondary analysis, an exploratory, prospective design was proposed, with two additional variables collected retrospectively from chart audit.

1.5.2 Clinical Setting

The setting for this study was the University of Pittsburgh Medical Center (UPMC) Presbyterian Hospital, an 816 bed hospital designated as a Level I Regional Trauma Center fully accredited by the Pennsylvania Trauma Systems Foundation. UPMC-Presbyterian has 43 operating rooms and 2 post anesthesia care units. Approval was obtained to conduct the secondary analysis from the Principal Investigator and the Co-Investigators of the parent study, and IRB modification approval was obtained to collect additional data both retrospectively and prospectively.

1.5.3 Sample

Subjects for this study were recruited for the parent study from the UPMC – Presbyterian preoperative holding area and medical/surgical units. UPMC-Presbyterian schedules a minimum of 2-5 orthopedic procedures per day, 5 days a week, by orthopedic surgeons. Inclusion criteria for the parent study were patients who were at least 18 years and no more than 70 years old, received general anesthesia or general and regional anesthesia for surgery planned to be no more than 4 hours, had an isolated orthopedic injury and an American Society of Anesthesiologists (ASA) physical status of I, II or III. The physiology, experience and treatment of pain are different in children < 18; therefore, 18 years was selected as starting age for study inclusion. Due to the effect age has on patients' response to opioids; the age of 70 was used for the upper limit for age criteria. Surgical time of greater than four hours has shown to be associated with postoperative complications that might have confounded the measure of pain and PONV. Using 4 hours of surgical time or less as inclusion criteria controlled for this potential confounder. An isolated fracture and an ASA score $\leq$ III provided a single cause of pain. An ASA score greater

than III signifies an extremely acute situation that requires surgery within 24 hours for survival. Multiple trauma patients were excluded because they have more than one source of pain.

Exclusion criteria are from the parent study and included treatment with opioids during the six months prior to surgery, alcohol use in the 24 hours preceding surgery; recreational drug use in the preceding 6 months; and any documented history of alcohol abuse, mental illness, hepatic disease, renal disease or neurologic conditions such as stroke, head injury, spinal cord injury or intracerebral hemorrhage or previous history of arthritis or bone disease. Any of these situations would be potential competing explanations for the association of opioids and postoperative pain and sedation, the focus of the parent study. There were no additional inclusion or exclusion criteria for this study when compared to the parent study.

1.5.4 Recruitment

Subjects were recruited as part of the parent study being conducted at the University of Pittsburgh, School of Nursing. Study personnel reviewed the operating room schedules each morning to determine if orthopedic procedures were planned. Potential patients with a single extremity fracture were reviewed for study eligibility. Patients were then approached by one of the investigators in the preoperative holding area based on the Institutional Review Board accepted protocol of the parent study (Appendix A). If patients agreed to participate verbally, informed consent was obtained.

1.5.5 Research Setting

All genetic analysis occurred at the University of Pittsburgh, School of Nursing, under the direction of Dr. Yvette Conley. The School of Nursing has a 3200 square foot molecular genetics laboratory located in the Victoria Building. It is divided into three sections, a culture room facility, a pre-Polymerace Chain Reaction (PCR) room and a post-PCR/equipment room. Major equipment available in the laboratory include an AB1377 automated sequencer/genotyper with all the necessary computer equipment and software for analysis of data, the WAVE^{R} Nucleic Acid Fragment Analysis System from Transgenomic to perform denaturing high performance liquid chromatography (dHPLC), and ABI7000 for TaqMan^{R} real time PCR and allele discrimination assays, a Turner Designs Luminometer, various horizontal/vertical electrophoresis units and power supplies, several SSCP apparatus, a gel documentation system, centrifuges, a spectrophotometer, a cold room for DNA storage, ultra-low freezers, culture room equipment and several 96-well thermal cyclers. Dr. Conley's laboratory is completely OSHA compliant and up to date for all required inspections. Two technicians with advanced degrees are employed full time in the laboratory.

1.5.6 Procedures for Data Collection

All demographic data were collected as part of the parent study. Pain and vital signs data were collected preoperatively, 15 minutes after the subject had been admitted to the PACU postoperatively and again at 45 minutes after admission to the PACU. Total amount of opioids during the OR and in the PACU were recorded. The presence of PONV was measured preoperatively and again 45 minutes postoperatively. Smoking history, history of previous

PONV or motion sickness, and total amount of ondansetron for the 48 hours postoperatively were new variables for this study, and were obtained retrospectively from the patients' medical records.

1.5.7 DNA Specimen

Saliva samples were collected from all subjects following surgery, usually the morning after so rehydration could occur. The saliva samples were collected using the Oragene DNA self collection kit from DNA Genotek corporation. DNA were extracted from the saliva/buffer combination utilizing the protocol and reagents for extraction supplied with the Oragene kit.

CYP2D6 SNP genotypes rs16497 (CYP2D6*2), rs3572686 (CYP2D6 *3), rs3892097 (CYP2D6 *4), rs5030656 (CYP2D6*9), rs1065852 (CYP2D6 *10), rs28371706 (CYP2D6*17), rs1135840 (CYP2D6 *39) and whole gene deletion (CYP2D6*5) were determined using the TaqMan® Allelic Discrimination Assay (Applied Biosystems, Foster City, CA). An allelic discrimination assay is a multiplexed, endpoint assay that detects variants of a single nucleic acid sequence (SNP), which means that it includes more than one primer/probe pair (multiplexed) and data is collected at the end of the PCR process (endpoint) (Applied Biosystems, 2010).

Using the extracted and purified DNA, 1 µl of each DNA sample was placed into a 96-well optical plate. Added to each well was 12.5 µl of reaction mixture (Taqman® Universal PCR master mix, SNP genotyping assay and sterile water). The polymerase chain reaction and fluorescence measurements were performed using the Applied Biosystems Prism 7000 Sequence Detection System® with cycling conditions as follows:

1. Two minutes at 50º C
2. 10 minutes at 95º C
3. 15 seconds at 95º C
4. 1 minute at 60º C
5. Step three is repeated 40 times – 15 seconds at 95º
6. Hold at 60ºC

After processing, the ABI Prism 7000 Sequence Detection System software produced a plotted graph of the results with each allele as an axis of the graph. Individual cluster plots show strong fluorescent signals for each allele, with a clear separation between the three clusters that discriminate the variant, heterozygous, and homozygous allelic status. A plot was generated for each CYP2D6 allele included in the analysis for this study.

Quantitative real time-PCR assays were used to determine the CYP2D6 gene copy number using the Taqman® copy number assay (Applied Biosystems.) In this procedure, extracted and purified DNA is added to a reaction mix comprised of TaqMan® Universal master mix, Taqman® copy number assay, Taqman® copy number reference assay and sterile water. In this procedure it was imperative that the percentage of DNA is exactly the same for all samples so the amount of mix was computed for each individual sample. After the plates were prepared they are loaded into the real-time PCR instrument with cycling conditions as follows:

1. Hold at 95º C for 10 minutes
2. 40 cycles for 15 seconds at 95º C
3. 40 cycles for 60 seconds at 60º

The process used to quantify the real time-PCR results was the comparative threshold method, also known as the [delta] [delta] C_t method. This process involves comparing the C_t values of samples of interest with a control or calibrator such as a non-treated sample or RNA from normal tissue. By employing this method, the [delta]$C_{tsample}$ is the C_t value for any sample normalized to the endogenous housekeeping gene and [delta]$C_{t,\ reference}$ is the C_t value for the calibrator also normalized to the endogenous housekeeping gene. For the [delta][delta]C_t calculation to be valid, the amplification efficiencies of the target and the reference must be approximately equal. If the plot of cDNA dilution versus delta C_t is close to zero, it implies that the efficiencies of the target and housekeeping genes are very similar (Applied Biosytems Inc., 2010). To determine OPRM1 genotypes a more complicated process was completed starting with the design of forward and reverse primers to cover the two SNPS of interest located in exon 1 of the gene; rs799971 (C17T) and 199972 (A118G). A polymerase chain reaction (PCR) was completed consisting of three major steps, denaturation, annealing and extension. Cycling conditions began with 10 minutes at 95º C to activate the process using Amplitaq Gold as a thermal stabler, followed by 35 cycles of 1 minute at 95º C, 30 seconds at 59º C and 1 minute at 72º C. When this is completed, the amplified DNA can be held indefinitely at 10º C. To continue the process, the PCR product was mixed with a 1% Agarose gel (10µl) plus 10µ of orange dye to verify that the samples were amplified and gel electrophoresed, Before sequencing could begin, the PCR product was cleaned using the EXO SAP It® under very specific conditions to remove any uncorporated primers and dNTPs (deoxynucleotides triphosphates). Sequencing is the process of determining the exact order of the bases A, T, C and G in a single SNP. The sequence data were collected using the ABI Prism 377 ® data collection software. The data were analyzed by GeneScan ® ABI Prism 77 analysis software. The computerized results print the nucleotide

sequence across the top of plotted traces of each base for detection of base substitutions within a SNP. Validity was achieved by checking the sequencing traces for each section several times. All DNA are stored in 1X TE buffer at 4°C. All analysis was performed in the genetics laboratory at the University of Pittsburgh, School of Nursing.

1.5.8 Study Variables

The primary dependent variable for this study is PONV. It is defined as presence of post-operative nausea and post-operative vomiting that has occurred within 48 hours of surgery, and was measured by the presence of rescue ondansetron given during that time. Ondansetron was the most consistently found evidence that PONV had occurred during retrospective chart reviews. PONV was measured as a nominally scaled, binary variable (Yes/No), but the total amount of ondansetron given in the 48 hour postoperative time period, a ratio scaled variable was also collected. The total amount did not include the 4mg of ondansetron given in the OR before admission to the PACU. Because all patients that experienced vomiting also experienced nausea, PONV was considered one variable for this study.

CYP2D6 genetic variances and μ-opioid receptor genetics variances are the primary independent variables for this study. After alleles were identified for each subject, assignments to the CYP2D6 classifications were completed following the definitions in Table 2. There were no ultrarapid metabolizers in this study sample, so all subjects were classified as poor metabolizers (PM), intermediate metabolizers (IM), or extensive metabolizers (EM) based on the combination of the alleles. Subjects with two fully functional alleles were considered EMs, subjects with one functional and one non functional or two decreased function alleles was considered an IM. Subjects with two non functional alleles were considered PMs.

For the μ-opioid receptor gene, analyzed from same DNA sample, two single nucleotide polymorphisms (SNPs) were studied based on evidence from previous research that suggests the alleles in this area are associated with postoperative pain levels (Chou, Yang, et al., 2006). A118G and C17T were classified into one of three genotypes. These genotypes will be homozygous for the wild-type, homozygous for the variant, or heterozygous. A118G, is a substitution that occurs in 10.5 to 18.8% of the population and, C17T, a substitution that occurs in 1-10% of the population (Lötsch & Geisslinger, 2005). Pain is a secondary dependent variable for Specific Aim 4. Pain was operationally defined in this study as the level of pain reported by the patient using a verbal pain scale consisting of 11 points – with 0 being the absence of pain and 10 being the highest level of pain ever experienced. Subjects were instructed to rate their current pain based on this scale, and were not limited to whole numbers (for example a subject may say 5.5) Pain was measured 15 minutes post-operatively and 45 minutes post-operatively. The pain scale is a highly ordinal level of measurement and should approximate an interval scaled variable allowing for the use of parametric statistics.

1.5.9 Covariates

The four patient factors consistently identified in the literature as risk factors related to the occurrence of PONV are smoking status, gender, history of PONV and/or motion sickness and opioids uses for postoperative pain. Opioids were included in the study model as an independent variable. Smoking status, history of PONV and gender were treated as covariates in the data analysis plan, with the expectation that they would be ruled out as confounders. Gender, smoking status and history of PONV are dichotomous variables that were measured on a nominal scale. Subjects were considered smokers or nonsmokers based on current activity; therefore a

former smoker was considered a nonsmoker. History of PONV was assessed from documentation on the Anesthesia Preoperative Assessment note that is completed by the anesthesiologist or nurse anesthetist prior to all surgical procedures at the University of Pittsburgh Medical Center.

1.5.10 Data Management

SPSS (Version 17.01, SPSS, Inc. Chicago, IL.) was used for data management. Form design, data entry, and data verification was performed as per the parent study. Data were entered into the database and visually verified by a member of the research team. Erroneous responses identified during data verification were checked and corrected. Once data were fully verified, scores were computed, variables and values were labeled, and missing values identified to create the data files for analysis. All data were stored in password protected computers and the locked offices of the primary investigator for the parent study. The new CYP2D6 genotype data and new variables collected for the current study were stored in a database within a password protected computer with no other personal identifiers.

1.5.11 Preliminary Data Analysis Plan

Data analyses were performed using SPSS (Version 17.01, SPSS, Inc. Chicago, IL). Initially, descriptive and exploratory statistical techniques were employed to describe the data, assess for underlying statistical assumptions, and identify any anomalies that would invalidate the results from the planned analysis. Extreme effort was made to retrieve missing data. When outliers were detected, they were investigated and, if necessary, corrected values were retrieved by

further chart review. Descriptive statistics (frequencies, percentages, means and standard deviations) were computed for all variables and summarized for the entire sample based on genotypes, risk factors, PONV, pain, total opioids, and ondansetron doses.

1.5.12 Justification of sample size for each specific aim

Specific Aim #1 explores the association between CYP2D6 genotypes and PONV. Because the study is a secondary data analysis, the sample size was fixed at 143 patients that had been genotyped for the parent study. Originally, it was determined that a logistic regression of a binary response variable on a binary independent variable with a sample size of 150 would achieve 80% power at 0.05 significance level with a minimal odds ratio as small as 4.750. Odds ratio values lower than this likely will be clinically meaningful, even if not statistically significant. The preferred effect size measure when comparing predictor variables is the odds ratio (Bland & Altman, 2000). The final sample size used for analysis for this question was 112 Caucasian patients due to the ethnic differences in genetic studies. With 112 subjects an odds ratio of 3.35 is necessary to achieve 78% power.

Specific Aim #2 was to explore the association between μ–opioid receptor genotypes and PONV. For specific aim # 2, the final sample size for analysis was 82 due to the number of subjects with completed genotyping for the μ-opioid receptor gene in the parent study. An odds ratio, as small as 3.35, in this situation will result in only 66% power.

Specific Aim #3 was to explore the association of CYP2D6 genotypes and the need for rescue anti-emetics in the 48 hours postoperatively. The planned analysis was an analysis of variance (ANOVA) or Kruskal Wallis if parametric assumptions were not met. The proposed sample size of 150 achieves 100% power to detect differences among the means versus the

alternative of equal means using an f test with a 0.05 significance level. The final sample size of 112 achieves a lower but still moderate power of 78% to detect the differences among the means versus the alternative of equal means using an F test with a 0.05 significance level.

Specific Aim #4 was to explore if subjects who experience PONV report a higher level of pain. For this analysis, a t-test was planned, or a Mann Whitney procedure if parametric assumptions were not met. The final sample size of 112 (PONV = 56, No PONV = 56) achieved only 17% power to detect a difference of 0.6 with a significant level of 0.05 using a two- sided two-sample t-test.

1.5.13 Data analysis for specific aims

Specific Aim #1: To explore the association between CYP2D6 genotypes and PONV. Frequency counts were used to describe the frequency distribution and percentages of the different CYP2D6 classification assignments. Bivariate correlations were computed among all predictor variables and covariates to assess for the risk of multicollinearity. Suspected covariates were the known risk factors of smoking, history of PONV and gender. The association between suspected covariates and all independent variables of interest and the dependent variable were computed to determine that they were not also confounders. Unadjusted (crude) odds ratios were first computed using univariate logistic regression. Hierarchical binary logistic regression was then used to determine the relationship for a predictive model of PONV, with the known risk factors of smoking, history of PONV and gender, included in the first block, CYP2D6 classifications, age and opioids were added in the second block and the interaction of CYP2D6 classifications and opioids in the third. The study model suggests that CYP2D6 could be a moderator between the relationship of opioids and PONV so an interaction between total opioids

by weight and CYP2D6 classification was investigated. The Hosmer Lemeshow test was employed to evaluate the goodness of fit of the model and the Omnibus test of model coefficients (traditional chi-square method) was used to determine the overall significance of the predictors in the model.

Logistic regression has two main uses; it predicts group membership and also can help understand the relationship and strengths among categorical variables. Logistic regression does not assume linearity of relationship between the independent and dependent variables. It does not require normally distributed variables and it does not assume homoscedasticity.

Specific Aim #2: To explore the association between μ–opioid receptor genotypes and PONV. The same statistical methods were originally planned for this aim, as planned for Specific Aim #1. However after the descriptive data were reviewed, one group (the homozygous variant [GG] of the μ–opioid receptor gene) had only one observation. The decision was made to combine the heterozygous AG group with the homozygous GG group and term it polymorphisms so it is a two group comparison rather than three groups.

Specific Aim# 3: To explore the association of CYP2D6 and the need for rescue anti-emetics in the first 48 hours post-operatively. If the assumption of a normally distributed sample from a normally distributed population is met, parametric statistics can be used. Because of the multiple levels of metabolizers, these data were analyzed using analysis of variance (ANOVA). The *F* statistic was used to test the hypothesis that there would be a difference among the group means, with a p-value of $<.05$ considered statistically significant. If the assumptions for parametric evaluation were not met, a Kruskal-Wallis procedure would have been performed to determine group differences.

Specific Aim #4**:** To explore the association of pain and PONV, a two sample t-test was used to compare the level of pain in those subjects who experience PONV and those subjects who do not. The *t* statistic was used to test the hypothesis that the groups had significantly different levels of pain with a p-value of <.05 considered statistically significant. If the data were not normally distributed, a non parametric procedure, the Mann Whitney U-test would have been employed to compare the two groups.

1.6 LIMITATIONS

There are several limitations for this study that require discussion. As a secondary data analysis, the sample size was fixed and thus ability to increase effect size was not possible. Another limitation was based on the fact that some data were collected retrospectively. Retrospective research suffers from the risk of missing data, mistakes during interpretation of data or incorrect documentation. A prospective study would allow for real time assessment of PONV, and also the measure of the magnitude of PONV possibly employing a visual analog scale. Prospective data collection would be stronger than relying on the documentation of rescue anti-emetics for the determination of PONV. Finally, CYP2D6 knowledge has grown exponentially in the past five years, with the identification of new alleles. Though the CYP2D6 alleles that occur most frequently were included in this analysis, there are others that have been identified recently that may be significant in the study of postoperative nausea and vomiting and may have influenced the outcome.

1.7 HUMAN SUBJECTS

1.7.1 Human Subjects Involvement and Characteristics

IRB Approval was granted for 200 subjects to be recruited for the parent study which continues to accrue subjects. Inclusion criteria for selection were: 1) Male or female, 2) 18-70 years of age, and 3) diagnosis of orthopedic injuries of tibia and/or fibula. The exclusion criteria were: Second orthopedic trauma site, abdominal or thoracic trauma, history of mental illness (e.g., depression, bipolar disorder, schizophrenia), currently taking phenothiazines, history of hepatic disease (e.g., history of hepatitis C), history of renal disease, ASA physical status >3, previous history or neurologic conditions such as stroke, head injury, spinal cord injury, intracerebral hemorrhage and previous history of arthritis or bone disease. No special classes of subjects (e.g., fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals or others) who may be considered vulnerable populations were recruited into the parent study.

1.7.2 Sources of Materials

Baseline and postoperative data were collected on all subjects from the medical record that included demographic and medical variables such as age, gender, race, medical history and condition and amount of medications administered. Pain scores and assessment of nausea and vomiting (PONV in the PACU) were collected directly from the patient in the PACU. All biological specimens were labeled with a unique study identification number. The saliva sample was collected after the subject's surgery for genotyping and was delivered to Dr. Conley's lab for DNA extraction.

1.7.3 Potential Risks

Confidentiality of the genetic and personal health information collected for this study was assured in three ways. First, all subjects were assigned a unique identification number which was used to identify data and samples. Second, data are reported in aggregate form only. Finally, all data files are maintained in a locked office in a locked department in the School of Nursing. All records connecting subjects to data are in a separate locked file. Electronic data are stored on password- protected computers. All samples and data collected for this study were used exclusively for research purposes.

1.7.4 Recruitment and Informed Consent

Recruitment was conducted by approaching subjects either in their hospital room or in the preoperative holding area of the PACU prior to surgery. An explanation of the study details, including all risks and benefits were provided, with subjects given an opportunity to ask any questions. Participation comprehension was assessed by asking subjects why they were being asked to be part of the study. After informed consent was obtained, subjects were provided with a copy of the signed document.

1.7.5 Protection against risk

The risks to subjects are limited to possible unknown risks related to breach of confidentiality related to disclosure of genetic information. Risk to a breach of confidentiality was minimized by

entry of all data immediately into a database where the individual data were linked by a study number rather than by personal identifiers.

1.7.6 Potential benefits of the proposed research to subjects and others

Potential Benefits of the Proposed Research to the subjects and others – Subjects are likely to receive no direct benefit from taking part in this study; however, the information obtained from their participation may eventually lead to better methods to prevent or treat PONV.

1.7.7 Importance of the knowledge to be gained

The importance of the knowledge to be gained is significant. This study could advance nursing research in the areas of PONV and genetics. The results of this study could help nurses caring for postoperative patients, understand the differences in how patients respond to both pain and anti-emetic medications, and the importance of having alternative strategies. The knowledge gained from this exploratory study will be significant in the development of future protocols for the treatment and prevention of PONV.

1.7.8 Data safety and monitoring plan

A Data Safety and Monitoring Committee for the parent study is comprised of the principal investigators, co-investigators and statistician. The committee meets quarterly to evaluate the progress of the research study, including assessments of data quality, timeliness of participant

recruitment, accrual and retention of subjects. The committee assesses external factors that may have an impact on the safety of study participants or the ethics of the research.

1.7.9 Inclusion of women and minorities

The majority of patients with the medical diagnosis of orthopedic trauma are men. In the parent study, there have been 44 females (39%) and 99 males (61%) recruited to participate. The parent study currently has an ethnic mix that includes Caucasians (79%), African Americans (18%), Asians (3%) and Native American Indians (2%). For the parent study, there are no exclusion criteria based on race or gender and every attempt is made to recruit all minority patients admitted with a single orthopedic fracture. Children between the ages of 18-21 years are included in the parent study. Children less than 18 years are not included. Subjects younger than 18 years are excluded because there is evidence that the physiology of pain and PONV in children is not the same as adults.

2.0 SUMMARY OF STUDY

The study followed the terms of the proposal in all aspects, with three exceptions. The original conceptual framework was revised to better guide the research, and the planned analyses for Specific Aims 1 and 2 were altered due to genotype distribution. Specific Aim #1 and #3 are addressed in the data based manuscript found in Section 4.0. It was decided due to the known differences in allelic variation among ethnic groups, and due to the small number of non-Caucasian subjects in the sample, that only Caucasians would be included in the evaluation of these specific aims. That decision resulted in a sample size of 112. When this sample was compared to the larger sample in terms of smoking, gender, history of PONV and age there were no significant differences. Genotyping resulted in no ultrarapid metabolizers (UM) in this group of subjects, so data analysis compared EMs to IMs and PMs. Specific Aims #2 and #4 are restated below, followed by results and discussion.

Specific Aim #2: To explore the association between μ–opioid receptor genotypes and PONV in subjects admitted for orthopedic surgical repair of a single fracture to an extremity.

Results: A total of 82 subjects were included in this analysis. The sample was based on the number of subjects who were genotyped for μ-opioid receptor gene as part of the parent study. There are no patterns in the selection of these subjects, and when compared to the larger sample they were similar in terms of gender (69% men) and smoking status (45% smokers). The mean age was also the same (38.9 years) and 47% percent experienced PONV (as compared to

50% of the larger sample). The distribution of the A118G SNP alleles was as follows: 63 (77%) subjects were AA (wild-type), 18 (22%) subjects were heterozygous AG, and only 1 (<1%) subject was GG. The distribution of the C17T SNP alleles was as follows: 76 (93%) subjects were CC (wild-type), 18 (22%) subjects were heterozygous CT, and only 1(<1%) subject was TT. Due to only one subject in the GG and TT groups, the polymorphism groups were combined for statistical comparison as shown in Table 3. Twenty seven subjects (42%) of wild type groups experienced PONV as compared to 10 (58%) of the combined polymorphism group. The unadjusted odds ratio and confidence intervals comparing the wild type group with the combined polymorphism group were OR = 0.641 (95% CI= 0.261- 1.674), p value = .362. Logistic regression, controlling for the known covariates of smoking, history of PONV and gender did not reveal a significant interaction between opioids and OPRM1 genotypes as a predictor for PONV.

Table 3: Cross tabulation for OPRM1 genotypes and PONV

		PONV		
		NO	YES	Total
Wild type (AA and CC)	n	33	25	58
	%	56.9%	43.1%	100.0%
Polymorphism (CT, TT AG or GG)	n	11	13	24
	%	45.8%	54.2%	100.0%
Total	n	44	38	82
	%	53.7%	46.3%	100.0%

Discussion: A lack of significant findings is not totally surprising, based on the mixed results reported in the literature (Chou, Yang et al., 2006, Chou, Wang et al., 2006). It is surprising that the trends, noted by other authors that showed a greater incidence of PONV in the AA (wild-type) group could not be confirmed in this study. A larger sample size may allow for a stronger comparison.

Specific Aim # 4 was to explore if subjects who experience PONV report a higher level of pain than those subjects who do not experience PONV.

Results: The sample for this comparison was the Caucasian sample of 112 subjects described in the first specific aim. To address this specific aim, first descriptive statistics were computed. The mean pain score at 15 minutes after admission to the PACU for those subjects who experienced PONV was 6.54 $\pm$ 3.23 compared to the mean for subjects who did not experience PONV; 7.13 $\pm$ 2.93. A t-test for independent groups was computed to compare the means. The results did not show a significant difference ($p = .308$). Pain scores at 45 minutes post admission to the PACU were also compared. The mean pain score at 45 minutes after admission to the PACU for those subjects who experienced PONV was 5.80 $\pm$ 2.76 compared to the mean for subjects who did not experience PONV; 6.60 $\pm$ 2.84. Results are depicted in Figure 2.

Discussion: The genetics of pain and PONV are actually working in opposite directions, with subjects that experience PONV reporting less pain than those who do have PONV. This is consistent with the findings noted in the u-opioid receptor gene literature where patients who are more sensitive to opioids and require less morphine for pain management have an increased incidence of PONV (Sia et al., 2008). To examine this aim further, a t-test was computed to compare the total amount of opioids received by patients with PONV compared to patients who

did not experience PONV. Once again the results were not significant (p =.364). Though the differences were not significant, subjects who experienced PONV required less amounts of opioids than those subjects who did not experience PONV.

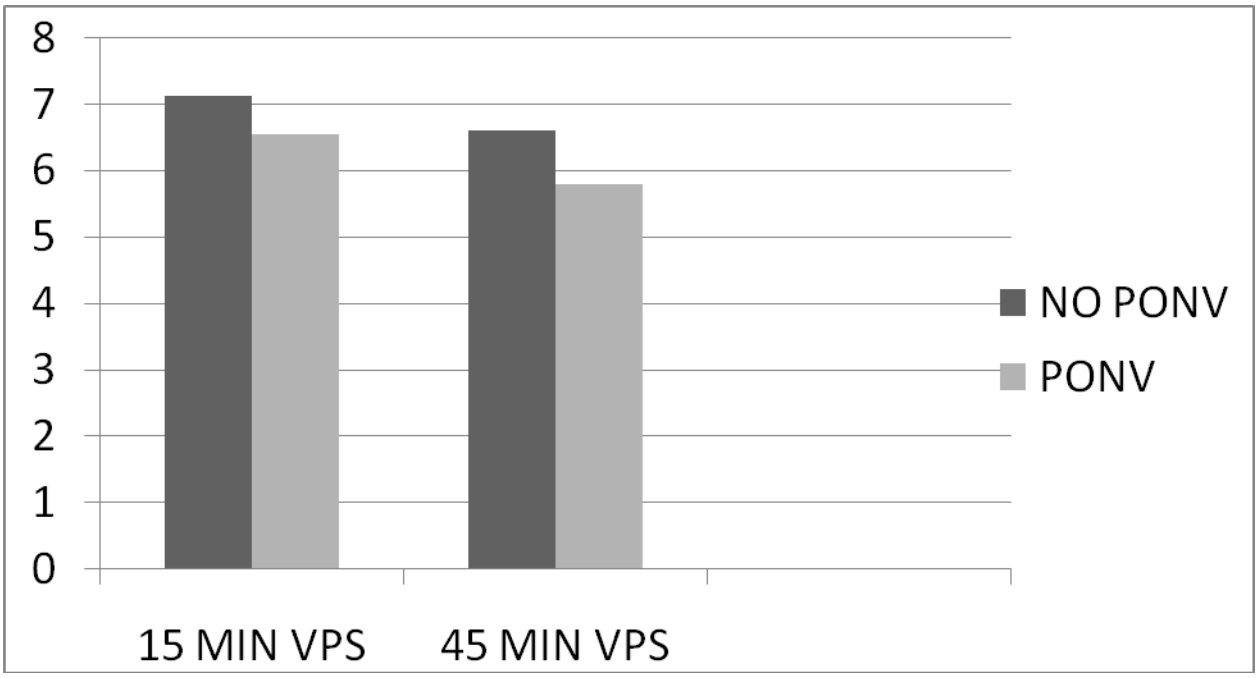

Figure 2: Comparison of mean pain scores in subjects with and without PONV

Pain level as measured by 11 point verbal pain scale (VPS) in subjects who experienced and did not experience postoperative nausea and vomiting. The VPS score means are not significantly different at either point in time, though at both time points those patients who experienced PONV reported lower pain scores.

The results of this study support further research in patients with PONV. There are still well documented gaps in our knowledge of this surgical complication. All subjects in this study received 4 mg of ondansetron before they left the OR, yet 68 (47.5%) patients of the total sample of 143 experienced PONV within 48 hours of surgery requiring rescue anti-emetic ondansetron.

The strengths of this study can be defined by the differences we see in those subjects who experience PONV compared to those subjects who do not experience PONV. When the

CYP2D6 classification groups are compared to incidence of PONV, there was a significant difference between the PMs and the EMs, and also a significant interaction between the CYP2D6 classification and total opioids received. This is the first nursing research project known that has examined PONV in terms of CYP2D6 classifications. The significant results add to the body of knowledge that will be helpful as personalized medicine becomes more prominent.

There are several limitations to this study that should be addressed, and they are consistent with the limitations described in the original proposal. The inherent problems of retrospective research are a severe limitation. Going back in time to retrieve missing data can be very challenging, and the researcher is at the mercy of the nurse or physician who provided the original documentation that was scanned into the medical records. Sometimes it is easy to decipher, other times it is very difficult to interpret. A prospective study will would allow for real time assessment of PONV, not relying on rescue medications to define PONV. Another limitation is the sample size of this study, and the lack of UMs in the sample obtained. The lack of UMs prevented this study from the ability to compare UMs with PMs in regards to total amount of ondansetron received for PONV. A larger future study would hopefully be able to address that question. Finally, the increased number of CYP2D6 alleles that have been identified in the past several years is also a limitation of the study. Though the analysis for this study compared the most frequently occurring alleles, there are others not identified in this study that may have an association with PONV.

Future studies are needed in the area of postoperative complications, especially to determine genetic risks for PONV. A stronger prospective study with a larger sample would include urine drug levels to compare drug metabolite ratios with CYP2D6 allele assignments.

3.0 MANUSCRIPT 1: A REVIEW OF THE GENETICS OF POSTOPERATIVE NAUSEA AND VOMITING

3.1 ABSTRACT

Postoperative nausea and vomiting (PONV) is recognized as one of the most common side effects of surgery, affecting as many as one third of all surgical patients. Previous studies have successfully established the major patient risk factors However, despite the best research, identification of new medications and risk factors, as many as 30% of postoperative patients still experience PONV. The purpose of this article is to review recent studies that have presented a potential genetic association and the incidence of PONV that may further explain this gap.

3.2 THE NOT SO LITTLE PROBLEM

Postoperative nausea and vomiting (PONV) is the most common complication after general anesthesia. Despite new medications, and new technology the incidence of PONV continues to be 20-30%, thus affecting as many as one third of the over 71 million surgical patients annually (ASPEN, 2006). Potential adverse effects of PONV in addition to causing extreme discomfort for the patient can include aspiration, wound dehiscence, bleeding, hematoma, dehydration, electrolyte imbalance, delay in ability to begin oral medications, exhaustion and general delay in mobilization and recovery (Miaskowski, 2009; Jolley, 2001). PONV is one of the strongest predictors for prolonged hospital stay and unanticipated admission for outpatient surgical patients (Pradham, Crichton, Edmonds, 1999) costing what could account for millions of dollars of health care costs annually (Apfel, Kranke, & Eberhart, 2004. Researchers and physicians have referred to PONV as the "big little problem," (Lichtor & Glass, 2008; Nelson, 2002), but nurses who care for postoperative patients know that PONV is not a little problem, and know

that it can be more worrisome than pain. In fact, when patients are given dollars to hypothetically "buy" away potential postoperative complications, vomiting was chosen above pain, with nausea also a major concern for patients (Kerger et al., 2007).

3.2.1 Pathways of PONV

Understanding the mechanism behind PONV helps one understand the treatment guidelines, and also why it can be such a complicated problem. The use of opioids for postoperative pain is recognized as being the primary cause of PONV (Watcha & White, 1992). Opioids cause PONV through three distinct mechanisms; yet all eventually stimulate the emetic center in the medulla which triggers nausea and vomiting. The first mechanism occurs when opioids cause a reduction in gastrointestinal motility. The reduced motility generates a release of serotonin from the enterochromaffin cells in the gastrointestinal tract. Enterochromaffin cells produce approximately 90% of the body's store of serotonin (Nielson & Olsen, 2008). Serotonin then binds to the visceral $5HT_3$ receptors, stimulating vagal afferent neurons that in turn activate the vomiting center via the nucleus tractus solitarius (NTS) which is connected to the area postrema. The second mechanism occurs when opioids stimulate the chemoreceptor trigger zone in the brainstem, which causes a release of serotonin and dopamine that directly activate the emetic center by binding to receptor sites (Gan, 2007; Nielson & Olsen, 2008). A third mechanism develops when opioids enhance the sensitivity of the middle ear to movement. This action activates the emetic center by the release of histamine and acetylcholine from vestibular fibers (Jolley, 2001; Silva, O'Ryan, & Poor, 2006).

3.2.2 How do we treat PONV?

Blocking these multiple neurotransmitter receptor sites is the focus of most anti-emetic drugs, and many agents have been developed to act against one or more of receptors (Neilson & Olsen, 2008). Each of these pathways provides an opportunity to reduce PONV as they each function independently. Though, because of their independence, there is significant empirical evidence that supports the use of multiple drugs to prevent or treat PONV. Guidelines established by an expert panel recommends the use of combination therapy with 2 or 3 prophylactic agents from difference classes for patients who are considered to be high risk for PONV (Gan et al., 2007).

Based on the published guidelines for the management of PONV, the first drug of choice is a $5HT_3$ receptor antagonist such as ondansetron. In higher risk patients the addition of other anti-emetics is recommended. Frequently, dexamethasone, a glucocorticoid, is given to augment the effectiveness of the HT_3 receptor antagonists. Other anti-emetics which are used include the histamine receptor antagoinist, promethazine; dopamine receptor antagonists, metoclopramide or prochlorperazine; and the scopolamine patch which adds anticholinergic properties (Gan et al., 2007).

3.2.3 So who is high risk?

Risk Factors have been identified by multiple researchers studying the ability to predict those patients most vulnerable to develop PONV. The patient risk factors most frequently identified include female gender, smoking status, history of PONV or motion sickness and use of opioids for postoperative pain (White, O'Hara, Roberson, Wender, & Candiotti, 2008; Apfel et al., 2008; Murphy, Hooper, Sullivan, Clifford, & Apfel, 2006). On the basis of two independent

studies, Apfel and colleagues developed a simplified risk score for predicting PONV (Apfel, Laara, Koivuranta, Greim, & Roewer, 1999). In a study of 1,137 subjects, they assigned one point for each major identified risk factor, so a non-smoking female with a previous history of PONV that was treated with opioids for postoperative pain would be assigned a score of four. Apfel and colleagues found that the presence of none, one, two, three or four of these risk factors translated to an increased incidence of PONV by 10%, 21%, 39%, 61% and 79% respectively.

The Risk Factor Assessment Tool has been further tested and provides support for physicians and nurses who need to decide upon prophylactic anti-emetic therapy. It seems especially valuable for those patients who are considered high risk (Apfel et al., 2008; Weilbach, Rahe-Meyer, Weissig, Scheinichen, & Piepenbrock, 2006). Apfel's work on risk factors continues to be validated by further research. In a recent observational cohort study that examined variations in the serotonin receptor genes (HTR_{3A} and HTR_{3B}), 95 subjects with PONV were compared to 95 subjects that did not experience PONV in the PACU following surgery. When the demographics of the two groups were compared they found that the PONV group had more women ($p < .001$), less smokers ($p = .03$) and had a history of PONV ($p < .001$). Both groups were comparable in amount of opioids received postoperatively (Reuffert et al., 2009).

Given the evidence, it appears that the successful PONV management strategy should include the identification of patients at risk; keeping the baseline risk low; and when risk is high; using a combination of anti-emetics acting on different receptors. However, anyone who spends time in a postoperative acute care unit (PACU) quickly realizes that there are patients not considered high risk that develop PONV.

3.2.4 Even the best strategies do not always work: A case study

MC is a 37 year old male who was admitted for an open reduction and internal fixation of his left femur. He had no history of motion sickness or previous PONV and he is a two pack a day smoker. He did receive opioids for pain. Yet postoperatively, despite having a risk factor score of one, and having been medicated with ondansetron an hour before leaving the operating room, he was extremely ill with nausea and vomiting in the PACU. MC was considered to have a relatively low risk for PONV, and in fact there is evidence that his smoking status alone potentially should have provided protection (Brattwall et al., 2009). So what made this patient so very sick? Despite the best research, identification of new medications and risk factors, as many as 20% of postoperative patients still experience PONV.

3.2.5 Could there be a genetic link causing this patient to develop PONV?

The purpose of this review was to explore the established association of the CYP2D6 genotypes and the OPRM1 genotypes with PONV in adult patients. CYP2D6 and OPRM1 are the genes of focus, because they are the genes that are thought to hold the greatest relevance to determining a patient's relative risk of experiencing PONV following opioid therapy (Reynolds, Ramey-Hartung, & Jortani, 2008). However, due to the limited number of studies found in this area, studies looking for the association of other candidate genes and PONV were included. All studies reviewed were published between 2002 and 2010. Inclusion criteria were quantitative studies limited to adult patients that measured nausea and vomiting as side effects of treatment, most commonly surgery. Key words used were PONV, CYP2D6, OPRMI1, postoperative complications, opioids and genetics. PUBMED, OVID, CINAHL databases were searched using

all possible combinations of the key words. The literature review resulted in 16 studies (Table 4) from the United States, Sweden, Denmark, Switzerland, France, Germany, Taiwan, Denmark and Japan.

3.2.6 How do differences in CYP2D6 genotypes affect PONV?

CYP2D6, a member of the CYP450 superfamily, was first shown to effect drug metabolism in a pharmacokinetic study of the antiarrhythmic drug, sparteine. Researchers serendipitously observed that some subjects experienced symptoms of nausea and diplopia, which are indicative of toxic doses of sparteine. Thirty years later researchers have demonstrated that the defective metabolism first localized by Eichenbaum and colleagues (1979) is inherited as an autosomal recessive trait affecting the CYP2D6 gene (Sweeney, 2003). It is now recognized that the enzyme defect begins from a mutation leading to a faulty expression of the enzyme. CYP2D6 is highly polymorphic, with over 100 known allelic variants identified, and more are discovered every year. Based on CYP2D6 genotyping, patients are assigned into one of four classifications: poor metabolizers (**PM**), intermediate metabolizers (**IM**), extensive metabolizers (**EM**) and ultrarapid metabolizers (**UM**) (Table 2). Patients that fall in the UM group have a gene copy number variant, and possess at least three functional wild-type alleles which cause a too rapid drug metabolism. Extensive metabolizers are considered to be normal and have the expected response to medications (Ho & Gan, 2006). The IM group may experience some or lesser degree of drug response, and the PM group will have an extremely slow or no drug metabolism. PM status is caused by a faulty expression of the enzyme with either complete deletion of CYP2D6 or more commonly, a replacement of a single nucleotide leading to aberrant gene splicing (Gaedigk, Blum, Gaedigk, Eichelbaum, Meyer, 1991). In the case of pro-drugs, that require

CYP2D6 for conversion to active drug status, PMs may not respond at all (Ingelman-Sundberg, Oscarson, McLellan, 1999).

Several studies focused on subjects who were classified as UM believing those patients would be at greater risk for developing PONV. Candiotti and colleagues (2005) compared 250 female patients in terms of their CYP2D6 metabolic status and incidence of PONV. When the 88 subjects who experienced PONV were analyzed by genotype, 45% (5 out of 11 patients) who were classified as UM experienced PONV; compared to 8% who were PMs, 17% who were IMs, and 15% in the EM group. Janicki and colleagues (2006) compared treatment of PONV with granisetron and dolasetron in relation to the CYP2D6 genotype in 150 subjects considered to have moderate to high risk for PONV. Granisetron is not metabolized by CYP2D6, but a less polymorphic CYP3A4, so it would be expected that those subjects treated with granisetron had a more frequent complete response. Among the subjects treated with dolasetron (a drug that is metabolized by CYP2D6) the greatest incidence of nausea and vomiting occurred in subjects who were classified as UM. Kirchheiner, Jan-Tobias, Bauer, Roots, & Brockmöller (2008), studied the response to opioids in healthy subjects who were predetermined to have either the UM or EM classification. Patients classified as UM were more sensitive to opioids than those who are classified as EM. In a different population of patients, Kaiser and colleagues (2009) also found significantly more nausea and vomiting in their oncology patients who were defined as UMs. They studied 270 patients receiving their first day of chemotherapy to compare the relationship between CYP2D6 genotypes and effectiveness of anti-emetic treatment. Nausea and vomiting were assessed within the first 4 hours and then 5-24 hours after treatment. Patients with 3 active CYP2D6 genes (classified UMs) demonstrated the highest incidence of vomiting

and nausea after chemotherapy and the lowest serum concentrations of tropisetron from blood drawn the same day (Kaiser et al., 2002).

The current anti-emetic drug of choice is ondansetron; a serotonin receptor antagonist that is metabolized by CYP2D6, so it is not surprising that several of the research articles reviewed focused on the metabolism of ondansetron, or the association of CYP2D6 genotypes on the effectiveness of ondansetron. Stamer, Rauers, Eun-Hae, Mubhoff, & Stüber (2009) found that surgical subjects that possessed three or greater active CYP2D6 alleles (and were thus classified as UM) had reduced ondansetron plasma concentration compared to those subjects that had zero to two active alleles. Candiotti and colleagues (2005) also observed a higher incidence of ondansetron failure for vomiting in their subjects with three or more copies of the CYP2D6 gene. There is strong evidence that those subjects who are genotyped as UMs will experience rapid drug metabolism and be at an increase risk for PONV, though based on the population, as few as 2% of patients will be categorized as UMs (Zhou, S., 2009).

Other researchers have looked more closely at the larger number of patients classified as IM or PM. Zwisler and colleagues (2009) compared only PMs and EMs in their study of hypo-analgesic effects of oxycodone in 33 healthy volunteers. They found statistical differences in the analgesic effects of oxycodone but they did not find differences in the opioid side effects of nausea and vomiting. In a study from Korea, Kang and colleagues looked only at CYP2D6 allele 10 (CYP2D6*10) in 135 women hospitalized for gynecologic surgery. CYP2D6*10 (common in Asian populations) would result in classification of IM. All patients were treated with 4 mg of ondansetron before extubation. The study results showed no statistical differences in the incidence of PONV in patients who were wild-type, heterozygous or homozygous for CYP2D6*10 (Kang et al., 2006).

A published case study about a CYP2D6 poor metabolizer demonstrates the potential of personalized medicine. The report describes an 85 year old woman recovering from hip surgery. The patient had a long standing intolerance to codeine, and during her first admission was ordered oxycodone combined with tramadol. The initial oxycodone dose was 5mg combined with 500 mg of acetaminophen every 12 hours. The dosage was increased to 10 mg every 12 hours and then 7.5 mg every 6 hours. Analgesia was still not achieved, and the patient suffered severe nausea and vomiting. The patient was discharged on famotidine for the persistent PONV. A year later the same patient was readmitted for further hip surgery. Prior to surgery she was genotyped for CYP2D6 classification and was found to be a poor metabolizer. Knowing this, the physicians report that they effectively adjusted postoperative medications so that pain relief was obtained, without the very uncomfortable side effects of PONV (Susce, Murray-Carmichael, & de Leon, 2006).

3.2.7 OPRM1 and PONV

The μ-opioid receptor gene single nucleotide polymorphism (SNP) that has been studied most frequently in terms of opioid response is A118G. Several studies have reported the association of the μ-opioid receptor gene (A118G) with variations in morphine consumption for analgesia after total knee arthroplasty and after total abdominal hysterectomy (Chou, Yang, et al., 2006; Chou, Wang, et al., 2006). In both studies, Chou and colleagues did not find statistical differences in the incidence of PONV, but in both studies report a higher incidence of PONV in patients who have the homozygous (AA) variation. The strong trend reported was based on observing 22% of subjects in the AA group with PONV, as compared to 12% and 7% in the heterozygous (AG) and homozygous variant (GG) groups respectively. This trend was

confirmed by the work of Sia et al., (2008). In their study of women post cesarean-section, they report that genetic variation at A118G of the μ-opioid receptor is associated with individual differences in pain score, amount of self administered morphine and the incidence of postoperative nausea. Specifically results showed that those subjects who carried the AA (wild-type) for A118G had significantly (p =.02) more PONV, despite a lower consumption of PCA morphine postoperatively. Sia and colleagues believe that a greater sensitivity to morphine may be attributed to the AA allele and thus lead to the increased PONV that was observed in the wild-type group.

3.2.8 Other candidate genes that influence the occurrence of PONV?

Several articles focusing on other candidate genes and PONV were identified in recent literature. Coulbault and colleagues (2006) investigated the genetic factors on morphine dose requirements and adverse events after colorectal surgery in a pilot study of 74 patients. The study compared the association of μ-opioid receptor gene (SNP A118G), the gene UGT2B7 (SNP T802C), the gene ABCB1, and gene MDR1 (G2677T/A & C3435T) with patient characteristics. Morphine side effects were measured in this study by the use of ondansetron for PONV. Study results revealed only one borderline (p=.07) predictive factor, which was the ABCB1 GG-CC diplotype. Another study that examined the association of the ABCB1 and μ-opioid genes and adverse opioid drug reactions focused on oxycodone. In this study, Zwisler and colleagues (2009) recruited 33 healthy volunteers who were exposed to experimental pain. In this small sample, there were no significant differences between the genotypes for the μ-opioid A118G, however they observed a significant difference (p=.02) in adverse reactions (nausea and vomiting)

between the ABCB1 C3435T wild-type group when compared to patients possessing the variant alleles.

Two additional articles were reviewed that address the association of genetics with PONV. Reuffert and colleagues (2009) studied the variations in the 5-HT_{3A} and 5-HT_{3B} serotonin receptor genes to see if they influence the occurrence of postoperative vomiting. Comparing 95 postoperative patients that experienced PONV with 94 control patients, they observed significantly higher risk (p=.003) in those patients who were carriers for the HTR_{3A} variant c1377A>G. The study results revealed several HTR_{3B} variants that were associated with a lower risk for PONV, though these significant genetic variants were located in the noncoding regions of the gene (Reuffert et al., 2009). The final article reviewed studied the relationship between Dopamine D2 receptor TaqIA polymorphisms and PONV. Nakagawa and colleagues (2008) enrolled 1070 subjects who were scheduled for elective surgery under general anesthesia. They found that the relative risk for the A2A2 allele in comparison to the A1A1 or A1A2 was a significant risk factor for the development of early onset PONV. Understanding the multiple pathways that can lead to PONV, both studies provide support for additional research.

3.2.9 Discussion

Many of the studies reviewed have moderate sample sizes with several extremely large (Sia et al., 2008 and Nakagawa et al., 2008). Of the 16 studies reviewed, eleven used postoperative patients for their populations. One study looked at patients beginning their course of chemotherapy; one study used patients from a Pain Clinic and the other three recruited healthy volunteers.

In the studies that sought an association between CYP2D6 and PONV results are mixed depending upon the metabolic classification examined. Results observed from the studies that focused on the ultra metabolizers report strong evidence that patients classified as UM are at greater risk for developing PONV (Candiotti et al., 2005; Janicki et al., 2006; Kirchheiner et al., 2008). Though as Kaiser noted, at least fifty patients would have to be genotyped to protect one patient from severe emesis (Kaiser et al., 2002). In the study that explored the association of CYP2D6 EMs and PMs with PONV, no significant differences were found, though the sample as noted above was relatively small and they were healthy volunteers, not surgical patients (Zwisler, et al., 2009). The case study (Susce, et al., 2006) provides a good example of why PMs may have as much risk as those subjects who are UMs. If drugs are used that must be metabolized by CYP2D6, patients will have no response instead of a rapid response causing PONV to persist. Kang and colleagues (2006) looked only for CYP2D6*10, an allelic variant that is found more frequently in the Asian population (Zhou, S. 2009). CYP2D6*10 is known to cause reduced metabolic activity and patients with this allele are considered to be IMs. Ondansetron was given to all subjects (all women) prior to leaving the OR, yet there was no variability in PONV when genotypes were compared.

The articles reviewed that studied the μ-opioid receptor gene (A118G) consistently reported a higher incidence of PONV in the A118G AA (wild-type) groups as compared to those patients who were AG or GG. Though only one study found statistical significance (Sia, A., et al., 2008), Chou and colleagues (2006) found in their patients recovering from knee surgery and abdominal hysterectomy, that patients, who were in the wild-type group experienced more PONV despite the fact that they required less morphine. Patients who are AA could be more

sensitive to opioids, requiring lower doses for pain relief, yet due to a more intense response have increased PONV.

Lötcsh and colleagues (2009) recruited their subjects from patients who were followed at a Pain Center, so their sample included both patients with chronic and acute pain, in fact the average time on pain therapy was over five years. Perhaps they did not find significant differences in opioid side effects because these chronic pain patients have developed a tolerance to the noxious opioid response that leads to PONV. Patients who are sensitive to opioids would also request alternative pain medication, so the sample could be in some ways self selected. Both scenarios might explain the low occurrence of nausea and vomiting (2.9%) overall noted in this paper (Lötcsh et al., 2009).

The articles that addressed the association of PONV and dopamine receptor and serotonin receptor genes (Rueffert et al., 2009 & Nakagawa et al., 2008) are not strong enough to stand alone. However, due to the multiple pathways that lead to PONV, they provide evidence that the influence of these genes should be investigated in additional studies with appropriate sample sizes.

3.2.10 Summary

The candidate genes that have been considered as suitable targets for the study of PONV based on articles published in the past 8 years were the aim of this review. The results of all studies provide sufficient evidence for further research in this area, even with inconsistent and sometimes contradictory findings.

Table 4: Studies included in literature review

Author, Year, Title	Sample Description	Study Design	Study Intervention/Analysis	Study Findings/Outcomes
Candiotti, K. A.et al. (2005). The impact of pharmacogenomics on postoperative nausea and vomiting: do CYP2D6 allele copy number and polymorphisms affect the success or failure of ondansetron prophylaxis?	250 female patients	Prospective observational study	All subjects pre-medicated with Ondansetron 30 minutes before intubation. PONV assessed at	The incidence of vomiting in UM subjects (5/11 or 45.5%) was significantly increased as compared to the PM (8.3%), IM (16.7%) or EM (14.7%) groups. Patients with 3 normally functioning CYP2D6 alleles might benefit from administration of anti-emetics not metabolized by CYP2D6.
Janicki, P., et al., (2006). Prevention of Postoperative Nausea and Vomiting with Granisetron and Dolasetron in Relation to CYP2D6 Genotype.	150 adults	Randomized Double Blind Study	Compared effectiveness of two 5-HT3 antagonists (Granisetron and Dolasetron) in relations to CYP2D6 genotype. Granisetron is metabolized independently of CYP2D6.	Findings suggest that the difference in the anti-emetic response may be associated with differences in the carrier status for duplication of the CYP2D6 allele, with subjects in the UM classification experiencing more vomiting episodes. The Granisetron group required less rescue medication as compared to the Dolasetron group.

Table 4 (Continued)				
Kirchheiner, J. et al. (2008). Effects of the CYP2D6 Duplication on the Pharmacokinetics and Pharmacodynamics of Tramadol.	11 subjects who were classified as UM, 11 subjects classified as EM, Healthy male volunteers, 18-65 years.	Observational, cohort study	Comparison of results of cold pressure test, pupillometry and standardized adverse event recording in subjects who were UM vs EM.	5/11 (45%) of the UM group experienced nausea after tramadol compared to 1/11 (9%) in the EM group.
Stamer, U., et al. (2009). Ondansetron for the treatment of opioid induced nausea and vomiting: Impact of Cytochrome Polymorphisms.	92 adults scheduled for abdominal surgery	Prospective, observational study	Comparison of metabolism of Ondansetron and association of CYP2D6 alleles.	Carriers of $\geq$3 active CYP2D6 showed reduced plasma concentrations of ondansetron compared to carriers of <2 active alleles. Increasing the dosage of ondansetron from 4mg to 8mg did not significantly increase the ondansetron plasma concentration.
Kaiser, R., et al. (2002). Patient-tailored anti-emetic treatment with $5HT_3$ receptor antagonists according to cytochrome P-450 2D6 genotypes.	270 cancer patients receiving their first day of chemotherapy.	Prospective, observational study	Comparison of anti-emetic activity of ondansetron or tropisetron as related to functional polymorphisms of e CYP2D6. Nausea and vomiting were assessed within the first 4 hours and then 5-24 hours after first chemotherapy treatment.	Patients with 3 active CYP2D6 genes (UMs) demonstrated the highest incidence of vomiting and nausea after chemotherapy when ondansetron or tropisetron was given.

Table 4 (Continued) Susce, M., et al. (2006). Response to hydrocodone, codeine and oxycodone in a CYP2D6 poor metabolizer. A case study.	85 year old female (n=1)	Case Study		Severe history of PONV not controlled with anti-emetics. Following hip surgery, rehabilitation was complicated by continued multiple episodes of nausea and vomiting. CYP2D6 genotyping resulted in poor metabolizer classification.
Zwisler, S. et al. (2009). The hypoanalgesic effect of oxycodone in human experimental pain models in relation to the CYP2D6 oxidation polymorphism.	33 healthy volunteers aged 22-32 years genotyped either CYP2D6 EM or PM.	Randomized, placebo controlled, double blinded crossover design with washout	Pain tests were performed 1, 2, 3 and 4 hours after medication. Side effects were observed by self report using a 4 point verbal rating scale.	There were no differences in number of subjects in each group who experience nausea or in the average nausea score (2.5 in the PM group vs 2.9 in the EM group). No treatment for nausea was offered.
Chou, W. et al. (2006). Human opioid receptor A118G polymorphism affects intravenous patient-controlled analgesia morphine consumption after total abdominal hysterectomy.	80 female patients admitted for total abdominal hysterectomy.	Prospective, observational study	Subjects were genotyped for A118G polymorphism at the μ– opioid receptor gene. Morphine (via PCA) consumption, pain and side effects were measured for 48 hours following surgery.	The incidence of PONV was higher in patients of the AA and AG group than the GG group, though the differences were not statistically different. 7 (16%0 of the AA group were observed to experience vomiting as compared to 6% in the GG group

Table 4 (Continued)				.
Sia, A, et al. (2008). A118G single nucleotide polymorphism of human mu-opioid receptor gene influences pain perception and patient-controlled intravenous morphine consumption after intrathecal morphine for post cesarean analgesia.	588 healthy women who received intrathecal morphine for post cesarean analgesia.	Prospective, observational study	All subjects were genotyped for the A118G polymorphism. Pain scores, PONV, and total morphine were measured for the first 24 hours.	Subjects who were wild type (AA) for the A118G polymorphism reported the lowest pain scores. The same group had the highest incidence of postoperative nausea versus the AG and GG groups ($p<.02$)
Chou, W., et al. (2006). Association of μ-opioid receptor gene polymorphism (AI18G0 with variations in morphine consumption for analgesia after total knee arthroplasty.	147 patients admitted for total knee arthroplasty	Prospective, observational study	Subjects were genotyped for A118G polymorphism at the μ– opioid receptor gene. Morphine (via PCA) consumption, pain and side effects were measured for hours following surgery.	Though there were no significant differences found among the groups for PONV, the wild type (AA) group had a higher incidence as compared to the allelic variant groups.
Coulbault, L., et al. (2006). Environmental and genetic factors associated with morphine response in the postoperative period.	74 postoperative colorectal surgical patients	Descriptive, prospective pilot study	The purpose of the study was to determine the association of OPRM1, UGT2B7 and ABCB1 with total morphine given in 24 hour period and PONV requiring ondansetron.	This study found that neither UGT2B7 nor OPRM1 genetic polymorphism could be identified as predictive factors for the need for curative ondansetron, though 23% of subjects who were AA for A118G experienced PONV also compared to 8% and 1% of subjects who

Table 4 (Continued)				were heterozygous or homozygous variant. Subjects with the ABCB1 homozygous wild type diplotype required significantly less (p=.028) anti-emetics as compared
Zwisler, S., et al. (2009). The antinociceptive effect and adverse drug reactions of oxycodone in human experimental pain in relation to genetic variations in the *OPRM1* and *ABCB1*genes.	33 healthy volunteers aged 22-32 years	Randomized, placebo controlled, double blinded crossover design with washout	Pain tests were performed 1, 2, 3 and 4 hours after medication. Adverse drug reactions were measured by self report using a 4 point verbal rating scale.	There was not a significant difference between wild-type and variant allele groups for the OPRM1, A118G polymorphism. There was however a significant difference in the ABCB1 G2677T. Nausea and vomiting was significantly different for the volunteers with the wild-type genotype as compared to carriers of the variant allele (p<.0005)
Lötsch, J., et al. (2009). Cross-sectional analysis of the influence of currently known pharmacogenetic modulators on opioid therapy in outpatient pain centers.	352 subjects treated with opioids for pain of various origins.	Cross-sectional, descriptive study	The purpose of this study was to describe associations of genetic variants with opioid dose, pain score and side effects.	No significant genetic associations with opioid side effects were identified, though subjects with more side effects had higher opioid daily doses. Only 2.8% of subjects experienced nausea or vomiting.

Table 4 (Continued)				
Nakagawa, M. (2008). Dopamine D2 receptor Taq IA polymorphism is associated with postoperative nausea and vomiting.	1070 patients scheduled for elective surgery.	Descriptive, observational study.	Patients were assessed for vomiting and/or need of rescue anti-emetics within 6 hours (early PONV) and within 24 hours (total PONV) of surgery.	Early PONV was observed in 11.3% and total PONV in 14.9% of subjects. The relative risk associated with the A2A2 allele in comparison with A1A1 or A1A2 alleles was 1.27 (95% CI, 0.88-1.84) for total PONV.
Reuffert, H. et al. (2009). Do variations in the 5-HT3A and 5-HT3B serotonin receptor genes (HTR3A and HRT3B) influence the occurrence of postoperative vomiting?	95 patients with history of POV and 94 normal controls.	Non-interventional cohort design	Correlation of identified genetic variants with postoperative vomiting by logistic regression then compared to normal controls.	The HTR3A variant c1377A>G was associated with a significantly higher risk (OR=2.97. 95% CI=1.46-6.02) for PONV.
Kang, J. et al. (2006). Influence of 5-HT3b Receptor AAG Deletion Mutation and CYP2D6 *10 on Ondansetron Treatment.	135 women gynecological surgery	Descriptive study	All patients were medicated with Ondansetron 15 minutes prior to extubation.	Study results found the incidence of PONV were not different among the genotypes of CYP2D6*10 and 5HT3b AAG. CYP2D6

4.0 MANUSCRIPT 2: THE ASSOCIATION OF CYP2D6 GENOTYPES AND POSTOPERATIVE NAUSEA AND VOMITING IN CAUCASIAN TRAUMA PATIENTS ADMITTED FOR SINGLE EXTREMITY FRACTURES

4.1 INTRODUCTION

Postoperative nausea and vomiting (PONV) has long been a concern for nurses caring for surgical patients. It is one of the strongest predictors for prolonged hospital stay and unanticipated admission for outpatient surgical patients (Pradham, Crichton, Edmonds, 1999) costing what could account for millions of dollars of health care costs annually (Apfel, Kranke, & Eberhart, 2004). Potential adverse effects of PONV in addition to causing extreme discomfort for the patient can include aspiration, wound dehiscence, bleeding, hematoma, dehydration, electrolyte imbalance, delay in ability to begin oral medications, exhaustion and general delay in mobilization and recovery (Miaskowski, 2009; Jolley, 2001).

4.2 BACKGROUND

Postoperative nausea and vomiting, well known, but not totally explained, is the most common complication for postoperative patients (Apfel et al., 2004). Research on PONV is primarily divided into two major focuses; identification of risk factors and studies evaluating the best treatment options. Yet despite the evaluation of new medications and risk protocols, 20-30% of postoperative patients continue to experience PONV (Kranke, Roewer, Smith, Piper, Wallenborn, & Eberhart, 2009). Based on the multiple pathways that can lead to PONV some researchers have focused on the use of combinations of anti-emetic medications (Gan, 2007; Golembiewski, Chernin, & Chopra, 2005). In high risk patients, combining two or more anti-emetics with different mechanisms of action has been shown to be more effective than using a single agent. These new combination strategies have improved the management of PONV but by

no means have they eliminated the problem (Ho & Gan, 2006). Other research has focused on identification of risk factors in order to predict those patients who are most likely to experience PONV (Apfel et al., 2008; White, O'Hara, Robertson, Wender, & Candiotti, 2008; Murphy, Hooper, Sullivan, Clifford, & Apfel, 2006). In a recent paper addressing the gaps in knowledge regarding the treatment of nausea and vomiting, the authors noted that although there have been important advances in the last twenty years, one of the major and most interesting gaps is the relatively poor ability to treat nausea (Sanger & Andrews, 2006).

4.2.1 Major Risk Factors of PONV

The patient risk factors most frequently identified by previous studies include gender, smoking status, history of PONV or motion sickness and use of opioids for postoperative pain (White et al., 2008; Apfel et al., 2008; Murphy et al., 2006). On the basis of two independent studies, Apfel and colleagues developed a simplified risk score (PONV Risk Factor Assessment Tool) for predicting PONV (Apfel, Laara, Koivuranta, Greim, & Roewer, 1999). In a study of 1,137 subjects, they assigned one point for each risk factor known, so a non-smoking female with a previous history of PONV that was treated with opioids for postoperative pain would be assigned a score of four. They found that the presence of none, one, two, three or four of these risk factors increased the incidence of PONV by 10%, 21%, 39%, 61% and 79%, respectively. The Risk Factor Assessment Tool has been further tested and continues to successfully give physicians and nurses the opportunity to provide guidance for prophylactic anti-emetic therapy, especially focusing on those patients who are considered high risk (Apfel et al., 2008).

4.2.2 CYP450 Genotypes and PONV

CYP2D6 is located on chromosome 22, where it forms part of the CYP2D6 gene cluster with two non-functional pseudo-genes, CYP2D7P and CYP2D8P (Arneth, Shams, Hiemke, & Hartter, 2009). CYP2D6, which localizes primarily to the liver is now believed to be involved in the metabolism of 25% of the common drugs administered today, including many opioids and anti-emetics (Owen, Sangkuhl, Klein, & Atman, 2009; Zhou, S., 2009). CYP2D6 was first known to affect drug metabolism in a pharmacokinetic study of the antiarrhythmic drug, sparteine. Researchers serendipitously observed that some subjects experienced symptoms of nausea and diplopia, which are indicative of toxic doses of Group 1 antiarrhythmics. They reported that between 5-10% of Caucasians had a defect in their ability to metabolize this drug (Eichelbaum, Spannbrucker, Steincke, & Dengler, 1979). It is now known that the defective metabolism they observed is inherited as an automsomal recessive trait (Sweeney, 2003). CYP2D6 is highly polymorphic, with over 150 known allelic variants and subvariants identified (Ingelman-Sundberg, Daly, & Nebert, 2009). Some alleles lead to a complete loss of CYP2D6 function, others lead to reduced activity. Based on allelic variants, a system has been developed to assign patients into four CYP2D6 classifications (Table 5). The classifications are: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM). The extensive metabolizers are considered to be normal (Ho & Gan, 2006). Saschse and colleagues report the prevalence of the different metabolizers within the Caucasian population as: PMs 5-10 %, EMs 70-80%, IMs 10-17% and UMs 2-5% (Sachse, Brockmöller, & Bauer, 1997). Due to potential overlap and clinical similarities of the IM and EM groups, they are sometimes reported as one group labeled EM/IM (de Leon, Armstrong, Cozza, 2006; Arneth, Shams, Hiemke, & Hartter, 2009).

Table 5: Definitions for the four CYP2D6 classifications

Classification	Abbreviation	Definition
Ultrarapid Metabolizer	UM	Possessing at least three fully functional alleles
Extensive Metabolizer	EM	At least one fully functional CYP2D6 allele
Intermediate Metabolizer	IM	Two reduced functional alleles, or one non functional allele
Poor Metabolizer	PM	Two non functioning alleles – no enzyme activity

The clinical impact of the CYP2D6 enzyme can be significant. Patients who were classified UM experienced more vomiting compared with the EM or PM groups in subjects treated for chemotherapy induced nausea and vomiting (Kaiser et al., 2002). The same study found that patients with CYP2D6 wild-type allele duplications had lower 5-HT3 drug concentrations. In another study that compared 250 patients in terms of their metabolic status, the highest percentage of subjects with nausea and vomiting was in the UM group (Candiotti et al., 2005). In their study, when activity was analyzed by genotype, the incidence of PONV in poor, intermediate, extensive and ultra rapid metabolizers was 1/12(8%), 5/30 (17%), 26/176 (15%) and 5/11(45%), respectively. When the UMs were compared to all other groups, a significant difference ($p<.01$) was noted. UMs will clear the body of CYP2D6 substrates more quickly than the other groups, and consequently are at risk for sub-therapeutic plasma levels (de Groot, Wakenhut, Whitlock, & Hyland, 2009).

The most common variant alleles found in the Caucasian population are CYP2D6*4 (23%) which causes a splicing defect resulting in a non functioning allele, and CYP2D6*5 (4%), that results in the total deletion of CYP2D6 (Johansson, Lundqvist, Dahl, & Ingelman-Sundberg,

1996; Arneth et al., 2009). Patients who are homozygous for CYP2D6*5 and CYP2D6*4 are classified as PMs. PMs are actually at risk for two potential types of problems. First, because they metabolize CYP2D6 substrates (drugs that require CYP2D6 to be metabolized) more slowly, they have increased plasma exposure to those drugs, which may increase the risk for a drug induced adverse reaction. PMs are also at risk for reduced efficacy from a medication that requires CYP2D6 for conversion to an active drug. Oxycodone, for example, a medication used frequently for postoperative pain requires CYP2D6 for the creation of oxymorphone, the active metabolite of oxycodone (Holmquist, 2009). Combining the understanding of the known risk factors for PONV and the potential genetic association is very important in the quest to decrease or eliminate this very common and distressing postoperative complication. The primary purpose of this study was to explore if there was an association between the PONV and the CYP2D6 genotypes, and if there was an interaction effect between CYP2D6 classification and amount of opioids given in regard to PONV.

4.3 METHODS

4.3.1 Design and Sample

With Institutional Review Board approval, secondary analysis was conducted using data from a larger study; "The Association between Mu-receptor Genotypes and Postoperative Pain Response". The primary study, under the direction of Richard A. Henker, RN, PhD, was supported by two grants: 1)AANA Foundation, and 2) Clinical Translational Science Institute grant funded by NIH, NCRR 1 UL1 RR024153 and also funded by a Special Grant from the

Office of the Senior Vice Chancellor for the Health Sciences, University of Pittsburgh. In this secondary analysis, a descriptive study design was employed, with two additional variables collected retrospectively by the author from chart audit. The subjects for the primary study were recruited from an 816 bed tertiary hospital that is a certified Level One Trauma Center. Subjects were recruited in the preoperative holding area on the day of the surgical procedure, if they agreed to be take part in the study, informed consent was obtained.

Inclusion criteria for the parent study were subjects who were at least 18 and no more than 70 years old, received general anesthesia or general and regional anesthesia for surgery planned to be no more than 4 hours, had an isolated orthopedic injury and an American Society of Anesthesiologists (ASA) physical status of I, II or III. The physiology, experience and treatment of pain are different in children and in the elderly; therefore 18 and 70 years were selected as starting and ending ages for study inclusion. Surgical time of greater than four hours has shown to be associated with postoperative complications that might have confounded the measure of pain and PONV. Using 4 hours of surgical time or less as inclusion criteria controlled for this potential confounder. An isolated fracture and an ASA score $\leq$ III provided a single cause of pain. An ASA score greater than III signifies an extremely acute situation that requires surgery within 24 hours for survival. Multiple trauma patients have more than one source of pain.

Because there are known differences in CYP2D6 alleles based on ethnic differences, and a limited number of non-Caucasians, only Caucasians were included in this study. Other exclusion criteria are from the parent study and included treatment with opioids during the six months prior to surgery, alcohol use in the 24 hours preceding surgery, recreational drug use in the preceding 6 months, any documented history of alcohol abuse, mental illness, hepatic

disease, renal disease or neurologic conditions such as stroke, head injury, spinal cord injury or intracerebral hemorrhage or previous history of arthritis or bone disease. Any of these situations would be potential competing explanations for the association of opioids and postoperative pain and sedation; the focus of the parent study.

4.3.2 Measures

Demographic and Patient Characteristics

Information on demographic characteristics, current smoking status, history of previous PONV, total amount of ondansetron, and total amount of opioids administered was obtained from the patient's hospital chart. Pain was assessed via self report 15 minutes after the subject had been admitted to the post anesthesia care unit (PACU) postoperatively and again at 45 minutes after being in the PACU. Pain was operationally defined on an 11 point verbal pain score (VPS). Patients were instructed to describe their pain from 0 to 10, with 0 representing no pain and 10 representing the worst pain ever imagined. Data for PONV were collected as binary variables, if the patient reported nausea, or was observed vomiting or if they were given postoperative ondansetron, they were considered to be positive for PONV. PONV was measured at two points, PONV that was observed or reported in the PACU and total PONV that occurred with 48 hours of surgery as measured by the need for rescue doses of onadansetron. The total amount of opioids that were administered during the procedure and in the PACU was recorded. All administered opioids were converted to morphine equivalence (Table 1) for comparison (McCaffery & Pasero, 1999), and then adjusted by weight for the score used in this study.

G***enetic data***

A saliva sample was collected from all subjects following surgery usually the following morning after surgery so that rehydration could occur. The saliva samples were collected using the Oragene DNA self collection kit from DNA Genotek corporation. DNA was extracted from the saliva/buffer combination utilizing the protocol and reagents for extraction supplied with the Oragene kit. Using the prepared DNA, CYP2D6 genotypes were determined by the TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) using the ABI Prism 7000 sequence detection system. Quantitative real time-PCR assays were used to determine the presence of multiple CYP2D6 gene copy numbers. The Allelic Discrimination Assay included rs16497 that defines allele CYP2D6*2, rs3572686 (CYP2D6 *3), rs3892097 (CYP2D6 *4), rs5030656 (CYP2D6*9), rs1065852 (CYP2D6*10), rs28371706 (CYP2D6*17), rs1135840 (CYP2D6 *39) and CYP2D6*5 which is defined by whole gene deletion (Table 2). Based on genotyping results, subjects were classified as EMs, IMs, or PMs as defined in Table 3. Subjects who had two fully functional alleles were defined as EMs. Subjects with one fully functional allele and one decreased function or a non functioning allele were assigned IM status. Subjects with two nonfunctioning alleles were defined as PMs. All DNA are stored in 1X TE buffer at 4°C. All analyses were performed in the genetics laboratory at the University of Pittsburgh, School of Nursing.

4.3.3 Data Analysis

Statistical analyses were carried out using SPSS 17.0 (SPSS, 2009). Descriptive statistics were computed for each variable. Correlations were computed for all predictor variables to assess for the risk of multicollinearity. The association between suspected covariates and all independent

variables of interest and the dependent variable was computed to determine that they were not also confounders. Suspected covariates were the known risk factors of smoking, history of PONV and gender. All patients were administered opioids during surgery or in the PACU so opioids were not considered a covariate in this study. Unadjusted odds ratios were computed using univariate logistic regression.

Hierarchical binary logistic regression was used to determine the relationship for PONV with the CYP2D6 classifications controlling for the known risk factors of smoking, history of PONV and gender by their inclusion in the first block. The study model suggests that CYP2D6 could be a moderator between the relationship of opioids and PONV so an interaction between total opioids by weight and CYP2D6 classification was investigated. The Hosmer Lemeshow test was employed to evaluate the goodness of fit of the model. The Omnibus test of model coefficients (model chi-square) was used to determine significance. ANOVA was used to compare the means of the three CYP2D6 classifications for amounts of ondansetron given in 48 hours and total amount of opioids administered. The level of significance was set at $p \leq 0.05$ for two sided hypothesis testing.

4.4 RESULTS

One hundred and twelve Caucasian patients who underwent surgical repair for a single isolated extremity fracture met the inclusion criteria. Demographic data are summarized in Table 7. There were more men (n= 81 72%) and non smokers (n=66, 59%). The average age was 39 years, and 24 (21%) of subjects reported a positive history of PONV. The incidence of PONV in the PACU was 38% but increased to 50% during the first 48 hours after surgery. The average 15

minute postoperative pain scores were 6.83± 3.08, and 6.20 ± 2.82, 45 minutes after admission to the PACU.

The distribution of subjects according to CYP2D6 classification was as follows: there were 7 (6%) subjects who were classified into the PM group, 34 (30%) were IMs and 71(63%) were EMs. No UMs were identified from this sample. The most frequently occurring allele was CYP2D6*2 with 86 alleles followed by CYP2D6*1 with 75 alleles. Thirty-two (34%) subjects had at least one CYP2D6*4 allele, with 6 (5%) subjects being homozygous *4/*4 (Table 6). One subject had two decreased functioning alleles (*9/*9) and based on phenotype was assigned PM status.

Table 6: Demographic data and patient characteristics (n=112)

Variable	n (%) or Mean + SD
Female Sex	31 (28%)
Non Smoker	66 (59%)
History of PONV	24 (21%)
PONV in the PACU	38 (34%)
PONV in 48 hours after surgery	56 (50%)
CYP2D6 – Extensive Metabolizer	71 (63%)
CYP2D6 – Intensive Metabolizer	34 (30%)
CYP2D6 – Poor Metabolizer	7 (6.3%)
Total Opioids+ (mgs)	0.49 $\pm$ 0.26
Total Ondansetron *(mgs)	3.39 + 4.26
PACU Pain Score at 15 minutes	6.83 $\pm$ 3.08
PACU Pain Score at 45 minutes	6.20 $\pm$ 2.82
Age in years	39.1 $\pm$ 12.7

*Does not include ondansetron given in the operating room
+adjusted by weight

Table 7: Classification assignments based on allele combinations

Allele Combination	Number (Percent of sample)	Classification Assignment
*1*1	15(13.5%)	Extensive Metabolizer (EM)
*1/*2	5(4.5%)	Extensive Metabolizer (EM)
*2/*2	18(16%)	Extensive Metabolizer (EM)
*2/*39	25(22.3%)	Extensive Metabolizer (EM)
*39/*39	2(1.8%)	Extensive Metabolizer (EM)
*1/*3	1(0.9%)	Intermediate Metabolizer (IM)
*1/9	3(2.7%)	Intermediate Metabolizer (IM)
*1/*10	4(3.6%)	Intermediate Metabolizer (IM)
*1/*4	32(28.5%)	Intermediate Metabolizer (IM)
*9/*9+	1(0.9%)	Poor Metabolizer (PM)
*4/*4	6(5.3%)	Poor Metabolizer (PM)

Unadjusted (crude) odds ratios derived from univariate analysis are displayed in Table 7. Female gender and history of PONV were significant risk factors in this study, smoking was not a significant factor. Multivariate logistic regression results are displayed in Table 8. The omnibus test of model coefficient $X^{2,}$ for this model, was 31.839 ($p< .000$). The Hosmer and Lemesow goodness of fit test, for this model, was $X^2 = 13.261$ ($p = .130$). Using the EM group as the reference, logistic regression analysis revealed a trend toward a significant difference with the CYP2D6 PM group for presence of PONV ($p = .085$), but did not find significance differences for the CYP2D6 IM group. A significant interaction between total opioids adjusted

by weight and CYP2D6 PMs (p=.010) was observed but significant interactions between total opioids and the other two CYP2D6 classifications were not found to be present. The significant interaction of opioids and CYP2D6 PM in regards to PONV represented the observation that those subjects classified as PM, who received higher doses of opioids were less likely to experience PONV

Figure 3 depicts a comparison of the means of total ondansetron and total opioids adjusted by weight, across the three CYP2D6 classifications. Using an ANOVA procedure there was a significant difference in the amount of total opioids received across groups (p=.007). Results of an ANOVA procedure did not reveal a significant difference in the amount of ondansetron across the same groups (p =.270). However as shown on Figure 3, the PM group received 2.28 $\pm$ 3.15 mgs of ondansetron compared to 3.88 + 4.38 mgs of ondansetron in the EM group. The range of total ondansetron for the PM group was from 0 mgs to 8 mgs, the range for the EM group was 0 – 20 mg of Ondansetron.

4.5 DISCUSSION

Distribution of CYP2D6 classification groups for this study, are consistent with previous studies. The PM group was 6.7 %, falling between the 5-10% usually reported in other studies (Owen et al., 2009; Zhou, S., 2009). The small percentage of UMs typically found in a sample of 112 could yield as low as a group of two, so it is not that unusual a UM was not identified from this sample. The IMs and EMs, if combined, describe 93% of the sample. However, for this study, an attempt was made to differentiate between the EMs and IMs, with evidence that there may be detectable differences between the groups. The mean total opioids adjusted by weight was

significantly different across the three groups, with a modest "intermediate" step between the EM and PM groups, providing some confidence that there was an intermediate group. A recent study examined serum levels of patients who were treated with the antidepressant venlafaxine. They found that serum ratio levels of venlafaxine to ODV (major metabolite from venlafaxine produced by CYP2D6) for patients with one CYP2D6*4 allele were found to be higher than those patients with two *4 alleles, yet significantly less than subjects who were EMs, thus they labeled them IMs (Arneth et al., 2009). The *4 allele is present in high frequency and accounts for >75% of allelic variants in Caucasians (Stamer, Bayerer, Wolf, Hoeft, & Stuber, 2002). Stamer's findings are consistent with the results of this study, in which CYP2D6*4 was the mutant allele detected most frequently (n=38). The two other variant alleles detected were CYP2D6*9 (n=4) and CYP2D6*3 (n=2). The one subject with two decreased functioning alleles (*9/*9) by definition should probably be considered an IM (Owen et al., 22009) but based on phenotype was assigned PM status. This subject required the highest amount of postoperative opioids of all subjects and reported pain levels of 10 both 15 minutes and 45 minutes postoperatively.

Even with the small number of subjects in the PM group, the significant findings from logistic regression between the EM and PM group for PONV, and the significant interaction of the PM group by total opioids given are noteworthy. PMs in this study were less likely to experience PONV as compared to the EM group, yet received higher doses of total opioids adjusted by weight. Most researchers have reported no significant differences in incidence of PONV between EMs and PMs (Janicki et al., 2006; Candiotti et al., 2005) though Susce and colleagues (2006) described a PM who had a very poor postoperative response to both anti-emetics and opioids in a published case report.

Gaedigk and colleagues (2008) have developed a CYP2D6 activity score (AS) in an attempt to simplify genotype interpretation. Using their AS system, an allele that has no metabolic activity is assigned a zero score, an allele that has decreased function is assigned a half of a point (0.5), a normal allele receives one point, and two points are assigned when multiple gene copies are present. Using the AS, CYP2D6*1/*4 would be assigned a score of 1.0. This provided a higher level of discrimination, and they were able to detect small differences between the EM and IM classifications that they confirmed with urinary metabolite data (Gaedigk, Simon, Pearce, Bradford, Kennedy, & Leeder, 2008).

In our study, fifty percent (56) patients were treated with ondansetron during the PACU and during the 48 hours post surgery compared to 38(34%) of patients who reported PONV in the PACU alone. This is consistent with many studies focused on postoperative complications (Meng & Quinlan, 2006; Janicki et al., 2006). It is standard practice in our institution to medicate all surgical patients with 4mg of IV ondansetron prior to leaving the operating room (OR). The dose given in the OR was not included in the amount of ondansetron recorded as part of the study, yet even with the administration of this prophylactic dose of ondansetron in the OR, 34% of our subjects experienced PONV in the PACU.

The American Society of PeriAnesthesia Nursing (ASPAN, 2006) describes the problems associated with PDNV (post discharge nausea and vomiting), yet there is little research in the literature that describe what happens after the first 24 hours postoperatively. This study demonstrated that patients with PONV increased from 34% to 50% during the 48 hours after discharged from the PACU. This is significant when the number of same day surgical procedures is considered, making discharge teaching in regards to PONV of critical importance.

When the three CYP2D6 classifications were compared, the PMs Mean ondansetron was less than 2.5 mgs for the 48 hours postoperative period, with no one in this group requiring more than 2 doses of ondansetron (ondansetron is given in 4mg doses usually every six hours). The range was broader for the IM and EM group in this respect, with patients in both groups requiring up to 20 mg of ondansetron in the 48 hours postoperatively. The results for total opioids are paradoxical compared to the ondansetron. Those patients who required the most opioids had the least PONV, and those subjects who required the most anti-emetics had the lowest amount of opioids for pain (Figure 1). There was a significant difference for PONV in the CYP2D6 PM group when compared to the EM group.

There was not a significant difference in verbal pain scores across the three groups. This may be explained by the subjective nature of pain. Nurses taking care of postoperative patients appropriately accept the patient's subjective report of pain whether the score is 6 or 10, assessing more for changes in their score rather than the reported score. Verbal pain scores are stronger measures of intra-patient variability than inter-patient variability, and validation for verbal pain scores are reported in terms of the ability to measure individual pain scores (Cork, Isaac, Elsharydah, Saleemi, Zavisca, & Alexander, 2004).

It is not surprising that two of the major risk factors for PONV, a positive history of PONV and gender were significant predictors of PONV in this study, they are both well documented from previous research (Apfel et al., 2008) What is surprising, is that smoking, usually a strong predictor was not found to have a significant influence. This may be due to the increased number of smokers in the sample. The American Heart Association (2008) reports that 18-21 percent of Americans are current smokers, compared to the 43% current smokers found in this group of orthopedic trauma patients. Brattwall and colleagues reported that 32% of subjects

in their study were current smokers or snuff users. They reported that PONV was reduced by 50% in both genders on the day of surgery and the first postoperative day in the smoking/snuffing group (Brattwall, Stomberg, Rawa, Segerdahl, Houltz, & Jakobsson, 2009). Interestingly, they reported that smoking or snuffing equally reduced PONV.

Though not included as one of the four major risks in most adult PONV scoring systems (Apfel et al., 1999) age has been identified in other studies as a moderate predictor for PONV (Sinclair, Chung, Mezei, G., 2000; Junger, Hartmann, Genson, 2001). The relatively young age of this adult sample (39 years) might contribute to the strong influence of age in this study. Two factors described in the literature as possible predictors for PONV were controlled for by study inclusion criteria. They are type of surgery and length of surgery (Sinclair et al., 2000; Junger et al., 2001). All study participants were admitted for an orthopedic procedure for single isolated extremity fracture and all subjects with surgery time greater than 4 hours were not included.

4.5.1 Limitations of the study

This study primarily was based upon secondary data analysis; however several new variables were added through retrospective data collection. Retrospective research suffers from the risk of missing data, mistakes during interpretation of data or incorrect documentation. In the future, a prospective, controlled research study that follows patients postoperatively and measures PONV directly is needed to confirm these findings. Another limitation was the sample size. A larger sample not only would provide more power to the study, but would also allow for a stronger comparison if large enough to identify patients that fall into the UM classification.

4.5.2 Conclusion and implications clinical practice

Ultimately personalized medicine will allow health care providers to treat all patients individually. To prepare for that eventuality, it is necessary for clinical genetic research to continue to identify those risks that may lead to a negative outcome. Sweeney (2003) noted that health care providers should consider inter–individual variability in response to medications a major clinical problem. The ability for nurses to recognize those patients who are not responding from treatment and know when to implement appropriate alternative strategies is imperative for successful postoperative recovery. Nurses, who recognize that patients may respond differently to medications due to genetic variants, will be more understanding when a postoperative patient requires more opioids than or anti-emetics than usual. It is also important to recognize that PONV in this study increased after discharge from the PACU. For patients who are admitted to the hospital after surgery, appropriate treatment will be provided for their PONV. This would not be the case for patients admitted for their surgery as an outpatient, thus special attention to PONV when preparing discharge teaching for outpatients is crucial. Finally, the fact that smoking was not a predictor in orthopedic trauma patients, with a higher than average current smoking status, is worthy of further investigation.

Table 8: Results of Univariate Logistic Regressions for PONV in 48 hours

Variable	Number and Percent PONV	Odds Ratio	Confidence Intervals	p-value
Male	34 (43%)			
Female	22 (71%)	3.379	1.384-8.247	.007
Smoker	19 (41%)			
Non Smoker	37 (56%)	1.813	0.846-3.885	.126
NO History of PONV	37 (42%)			
History of PONV	19 (79%)	0.191	0.065-0.558	.002
Age	NA	0.984	0.956-1.014	.296
Total Opioids/Wt	NA	0.339	0.074-1.551	.163
CYP2D6 (Reference=Extensive Metabolizer)	40 (56%)	1.0	NA	NA
Intermediate Metabolizer	13 (38%)	0.581	0.121-2.790	.498
Poor Metabolizer	3 (42%)	0.480	0.208-1.107	.085

Table 9: Multivariate Logistic Regression for PONV within 48 hours of surgery (n = 112)

	Odds Ratio	95% Confidence Intervals		
		Lower	Upper	p-value
History of PONV	5.379	1.574	18.387	.007
Gender	.236	.084	.662	.006
Smoking Status	1.359	.552	3.343	.505
TotalOpioids+	.073	.005	1.046	.054
CYP2D6 Extensive Metabolizer (REF)	1.000			.012
Intermediate Metabolizer	.492	.010	24.171	.721
Poor Metabolizer	.026	.002	.288	.003
Age	.966	.931	1.003	.071
CYP2D6 Extensive MetabolizerbyTotalOpioids (REF)	1.000			.036
Intermediate Metabolizer by TotalOpioids+	4.616	.026	822.837	.563
Poor Metabolizerby TotalOpioids+	207.726	3.525	12240.875	.010

+adjusted by weight

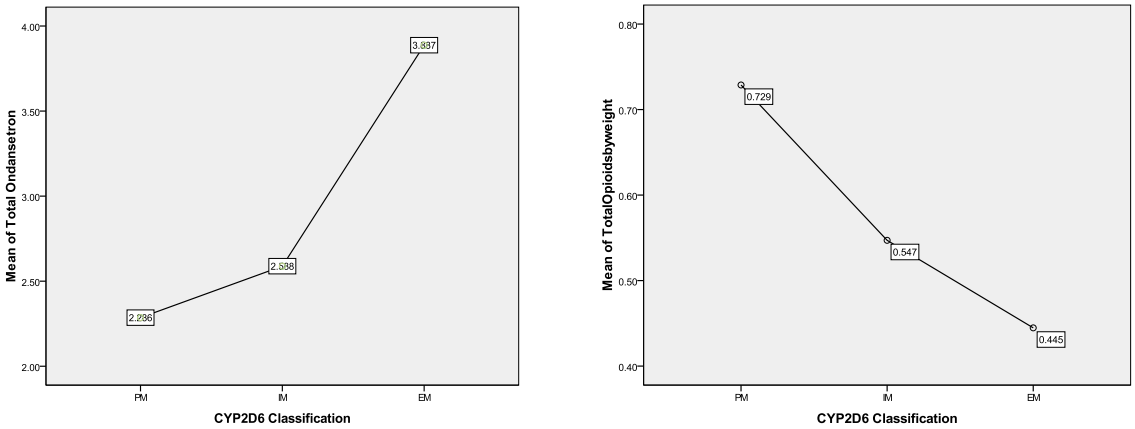

Figure 3: Graph of means of total opioids and total ondansetron

A graph of mean total opioids compared to a graph of the mean total ondansetron for the three CYP2D6 classification groups. The three groups were significantly different in amount of opioids received (p=0.036) when analyzed by ANOVA. Though the groups were not statistically different for total doses of ondansetron (p=0.27), those subjects who required more opioids required less anti-emetic.

APPENDIX A

IRB APPROVAL FOR PARENT STUDY

Universityof Pittsburgh
Institutional Review Board

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Memorandum

To: Richard Henker PHD CRNA

From: Sue Beers PHD, Vice Chair

Date: 8/6/2009

IRB#: REN09070058 / 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)

Approval Date:	8/6/2009
Expiration Date:	8/5/2010

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