Genotypic variations and frequencies of OPRM1 by race/ethnicity group in an orthopedic trauma population

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Specific Aims: The complex nature of pain, composed of both subjective and objective components, makes its proper and effective management difficult for every patient. In many instances factors such as race/ethnicity, which affect culture distort the objective nature of self-reported pain levels. Management is further compounded by opioid analgesics, which exert a range of adverse affects that vary in presentation and intensity between individuals. Therefore research in genetics, particularly the OPRM1 gene, may expose more reliable and successful methods for individual pain management. This study sought to accomplish this goal by evaluating the following specific aims: 1) Describe the distributions of genotypes and alleles for the variants, A118G and C17T, 2) Evaluate the distributions of the genotypes for A118G and C17T between races/ethnicities, 3) Evaluate the distribution of self-reported pain scores by genotype, and 4) Evaluate the distribution of opioid use by genotype.

Methods: Eighty-three subjects were recruited from Presbyterian Hospital, University of Pittsburgh Medical Center. Variables such as race/ethnicity, pain report, and opioid use were collected from patient report or from the medical record. Genotypes were determined through DNA extraction from saliva samples.

Results: The proportions of C17T variant genotypes, CT and TT, were significantly higher in the African-American group as compared to the Caucasian group ($p<.001$). The pain scores at 15 minutes post-operatively were significantly lower in participants with either the CT and TT genotypes than participants having the CC genotype ($p=.039$). No significant differences between the genotypes, A118G and C17T, were found for other categories of race/ethnicity, self-reported pain levels, or amount of opioid use.

Conclusions: The finding of higher proportions of the variant CT and TT genotypes in African-American patients relative to Caucasian patients is consistent with the literature. The findings of lower pain scores within the immediate post-operative time frame for those with CT and TT genotypes has not been as well documented in the literature and may support the need for further research in this area. Therefore this study does not support a change in practice for pain management, but it does provide the basis for further research into the SNPs of OPRM1, particularly C17T.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 CHAPTER 1: INTRODUCTION

1.1 PURPOSE

The proper management of pain has been recognized by many healthcare facilities as such a complex and important initiative that it is often considered the "fifth vital sign" (Lynch 2001). The ubiquitous nature of pain makes it a significant factor for management in many patients, yet despite this commonality there are stark discrepancies in both the presentation and management of pain. These discrepancies vary by demographics, but recent research has indicated genetics as a substantial factor in the variations related to pain management. In order to find methods to better manage pain, researchers are looking at the genetic influence on pain response and other associated outcomes.

Determining the genotypes of opioid receptors--mu, kappa, delta, and the most recently discovered ORL_1-- has been of particular importance as a variety of therapeutic agents act on these receptors (Corbett 2006). The majority of research has focused on the mu opioid 1 receptor. The genotype for this receptor, OPRM1, is located on chromosome 6q24-25 and at positions 17 and 118 it has two single nucleotide polymorphisms (SNPs). The wild type of the A118G SNP is AA with AG and GG being the heterozygous and homozygous variants. The wild type for the C17T SNP is CC with CT and TT being the heterozygous and homozygous variants (Lotsch et al 2004).

Researchers are finding that these variations in genotype may differ amongst races/ethnicities and may result in distinct differences in the perception of pain and opioid use. Caucasians, Hispanics, and Asians exhibit more frequent genetic variation in the A118G SNP, while African-Americans exhibit more frequent genetic variation in the C17T SNP (Bond et al 1998). The phenotypic presentation of this variation in the A118G genotype has been found to result in increased pain and opioid use. Evaluating outcomes associated with opioids and the SNP at A118G within a sample of post-cesarean women, Sia et al (2008) found increased pain scores amongst those with the homozygous variant genotype, GG, as compared to those with the homozygous wild-type genotype, AA. A substantial amount of evidence has found that variant genotypes of C17T are associated with higher levels of addiction. Bond et al (1998) found that opioid-dependence was marginally more significant amongst those with the variant allele, T. The literature, however, is insubstantial in evaluating additional outcomes such pain and opioid use associated with the C17T SNP. Therefore exploring the frequency of variation in A118G and C17T genotypes for OPRM1 amongst variables such as race/ethnicity, pain, and opioid use may provide support for the incorporation of genotypic evidence within a more individualized pain management plan.

1.2 SPECIFIC AIMS

The goal of this study was to explore genotypic variations in the SNPs, A118G and C17T, of the OPRM1 gene in order to determine its association and effect on relevant outcomes. Identifying these associations was believed to lead to a better understanding of prescription of opioids and management of pain in the acute care orthopedic trauma population. In order to accomplish this

goal the study examined the following specific aims:

1. Describe the distributions of genotypes and alleles for A118G and C17T.
2. Evaluate the distribution of genotypes, A118G and C17T, between races/ethnicities.
3. Evaluate the distribution of self-reported pain score by genotype.
4. Evaluate the distribution of opioid use by genotype.

It was expected that differences in race/ethnicity, pain report, and amount of opioids administered would be found between each wild-type and variant SNP for A118G and C17T. No particular association, was qualified between these variables and the genotypes, however, it was predicted that these differences would offer further insight into the mechanism of pain and therefore allow for the advancement of individualized pain management.

2.0 CHAPTER 2: LITERATURE REVIEW

2.1 HISTORICAL PERSPECTIVE OF PAIN

Amongst the myriad of disease processes there usually exists one common component: pain. Historically scientists have viewed pain as both the result of emotional and physiological responses. Aristotle, the 4th century BCE philosopher, proposed that pain was the result of an emotional response emerging from the heart (Pearl 2007). Scientists throughout the centuries, however, disagreed with the purely emotional component of pain and developed formal proposals for pain's association with specific neurological pathways and sense organs such as the brain. Therefore pain has been debated as a physiological symptom with a subjective presentation that differs by disease process and by person. Recently, however, brain imaging studies have provided the objective evidence that pain results in activation of certain cortical areas of the brain and matches very closely with the intensity of the subjective patient report (Coghill & Eisenach 2003). This finding substantiates the need to examine and treat pain as more of a quantifiable outcome.

2.2 PHYSIOLOGY OF PAIN

According to the International Association for the Study of Pain, pain is defined as: "…an unpleasant sensory end emotional experience associated with actual or potential tissue damage…" (Merskey & Bogduk 1994). Pain is essentially a defense mechanism meant to alert an organism that defense barriers have been compromised and an adaptation must be made to prevent further tissue damage. Therefore understanding the mechanism of pain ultimately leads to an improvement in management. The systems responsible for the sensation, transduction, and modulation of pain are the central and peripheral nervous systems. Although there are still some uncertainties about the process of pain, a general model has been accepted.

In order for a pain response to be felt, it must first be transduced from the periphery to the higher order levels of functioning. Transduction is the result of compromised tissue releasing chemical and mechanical stimuli that produce voltage changes in the cell. The influx of sodium ions into first order neuronal cells, results in an impulse traveling along the neural axon to presynaptic membranes where it terminates in the dorsal horn of the spinal cord. This termination, more specifically, depends upon the type of pain. Sharp, well defined pain and dull, visceral pain, are carried along different afferent pathways. Superficial somatic injury such as that resulting from an object piercing the skin is carried along fast myelinated afferent A fibers that terminate in the substantia gelatinosa in the II, III, and IV lamina. Deep pain, felt in result to injury of the ligaments, tendons, bones, organs, and other similar tissues, results in an aching, undifferentiated pain carried along slow unmyelinated afferent C fibers that terminate in the I and II lamina within the substansia gelatinosa.

The overall result of transduction between first order neurons located in the periphery and second order neurons located in the spinal column is the release of chemicals from presynaptic

membranes that regulate the transmission of the pain signal. Excitatory neurotransmitters like Substance P, which binds with neurokinin-1 (NK-1) receptors, result in activation of second order neurons that transmit the pain signal to the brain and other higher order centers. A majority of these signals ascend contralaterally via the spinothalamic tract to synapse with third order neurons located in the thalamus. This is where a multitude of physiological factors affecting pain converge and result in pain discrimination. Connections to the primary somatosensory cortex, result in specific physical processing of the size, location, and type of noxious stimuli whereas other connections with the amygdala, hypothalamus, and other regions result in the emotional, psychomotor, and autonomic responses to pain (Almeida et al 2004).

Inhibitory neurotransmitters, like endorphins and enkephalins provide analgesia as they inhibit pain transmission to and from higher processing centers. Opioid receptor ligands, both endogenous and exogenous, bind receptors and produce a conformational change in the receptor site that activates a secondary messenger, G-protein. This activation leads to an electrolyte imbalance as calcium is prevented from moving into the cell and potassium moves out of the cell (Purves et al 2001). The ultimate result is hyperpolarization of the cell and hence a decrease in neurotransmitter release. The opioid receptors that produce this response are primarily located in the dorsal horn of the spinal column and the periaqueductal grey, PAG, matter located in the midbrain. Those located in dorsal horn prevent the ascending transmission of pain whereas those located in the PAG result in the cessation of the pain stimuli descending to the periphery (Dossey 2004).

Repetitive activation of this system, however, can result in complex mechanisms of dysregulation that sensitize some nociceptors and desensitize others resulting in an overall lowered threshold for pain stimulation. Nociceptors in the periphery may have a lowered pain

threshold after the release of inflammatory mediators such as cytokines (Carr & Goudas 1999). Long-term changes, however, result from neuronal remodeling of the nervous system, which is often attributed to the phenomenon of plasticity that allows for alternative neuronal connections. For example remodeling of opioid receptors after neuronal injury may increase the influx of calcium and therefore the transmissions of pain. Other neurotransmitter imbalances may also lead to results of pain dysregulation such as neuropathic pain, complex regional pain syndrome, and other chronic pain disorders (Nalin & Sinatra 2005). Understanding the relationship between the mechanisms of pain and its consequences will therefore better elucidate alternative forms of treatment.

2.3 PAIN RESPONSE

The effective relief of pain first starts with understanding its profound effects on healing. Pain elicits numerous responses that may initially support the body in finding the source of the noxious stimuli and help to combat it. After an extended period of exposure to these noxious stimuli, however, pain responses may have detrimental affects on the body. These systemic effects can result in everything from hormonal dysrgulation and compromise in cardiac function to psychological stress, and ultimately transition into chronic pain.

In postoperative populations experiencing acute pain, physiological responses result in both specific tissue injury related to the site of surgery and also a wide range systemic effects. Localized tissue injury activates the inflammatory response, which stimulates flooding of inflammatory mediators such as cytokines into the area. These mediators result in sensitized nociceptors that either transmit pain spontaneously or in response to a lower threshold of

chemical, mechanical, and other stimuli (Fabien et al 2005). The systemic effect of this compromise in tissue function results in increased plasma concentrations of hormones such as catecholamines, beta-endorphins, and cortisol. Ineffective anesthesia and analgesia management intraoperatively and postoperatively further enhance the neuroendocrine stress response by increasing fat and muscle breakdown, hyperglycemia, and decreasing immune function (Halter et al 1977). This effect spirals as the overproduction of catecholamines stimulates the sympathetic response and leads to an increased consumption of oxygen and therefore increases the work demands on the heart. These cardiovascular effects may present as hypertension, tachycardia, and increased systemic vascular resistance. Overall the effect of pain on the heart may increase the possibility for myocardial ischemia. The body's response to pain also results in a compromise in respiratory function. An overall decrease in pulmonary performance and hence oxygenation has been found with injurious processes and results in atelectasis, intrapulmonary shunting, and hypoxemia. The physiological effects of pain are numerous, but early and effective prevention and treatment of pain can minimize their extent (Berry et al American Pain Society).

The systemic effects of pain encompass an even wider scope as they also result in psychological disturbances. Beyond the stress from the physical affects of pain, many studies have found that a patient's preemptive mental outlook on surgery and related processes greatly predict mental and behavioral outcomes postoperatively. A positive outlook can reduce pain intensity, compromise in physiological function, and enhance the efficacy of analgesic medications. In order to achieve this mental state, proper information and understanding of the procedure is necessary. Egbert et al (1964) found that preoperative discussion about the surgical procedure, associated discomfort, and the requirements for postoperative medications significantly reduced discharge time. These findings suggest that patient anxiety is relieved by

individualized attention and therefore suggest that an personalized pain management plan that is understandable and properly explained to the patient, may further decrease anxiety levels (Egbert et al 1964).

Physiological and psychological affects, however, are not as detrimental until they reach a persistent state. Pain that last beyond the time of noxious tissue injury is defined as chronic pain. This development of chronic pain results in neuronal remodeling that leads to numerous conditions such as hyperalgesia and neuropathic pain. Hyperalgesia is in part the result of an increase in inflammatory cytokines such as tumor necrosis factor, TNF, that alter the flucuations of ion concentrations in neuronal cells. The increase of calcium into neuronal cells results in the increased transmission of pain signals. Neuropathic pain results in lesions forming on nerve cells and presents with altered perceptions of pain such as wide spread pain or sensations of burning, and also muscle weakness. Pain experienced with non-painful stimuli such as light touch, known as allodynia, is an additional consequence of neuropathic pain (Gordon et al 2005). The mechanism for allodynia is undefined, but it may result from a dysregulation in cytokines that alter the functioning of mechanoreceptors. The pathological progression to chronic pain is varied and extends beyond changes in the periphery as some studies have found activation of areas in the brain involved in the formation of memories are also associated with pain (Lenz et al 1995). Therefore recognizing this complexity, the prompt, effective treatment of acute pain should also be seen as preventing chronic pain, which is not simply a symptom but a disease process itself.

2.4 OPIOID MANAGEMENT

The proper management of pain as defined by the American Pain Society concurs with the following guidelines: 1) prompt recognition and treatment, 2) involvement of patients in their pain management plan, 3) improved treatment patterns, 4) regular reassessment and adjustment of pain management as needed, and 5) measurement of processes and outcomes of pain management (Berry et al American Pain Society). These guidelines serve as an approach to reduce the numerous literature findings of mismanaged pain that often result from improper medication prescription.

Non-prescription analgesics are the common first line treatment for pain management, as over half of Americans have used these "over the counter" medications. Many of these non-opioid analgesics exert similar effects, and they are often used in combination with others or sometimes exclusively for their prevention of adverse effects and/or intolerance amongst certain populations. Non-steroidal anti-inflammatory drugs, NSAIDs, decrease the accumulation of cytokines such as prostaglandins via inhibition of the cyclooxygenase enzyme. This therefore inhibits pain, fever, swelling and other effects of the inflammatory system. Even though, acetaminophen, another non-opioid analgesic, does not provide significant anti-inflammatory relief it is commonly used in conjunction with NSAIDs as it provides fewer side effects such as damage to the gastric mucosa. Although aspirin provides analgesic and antipyretic effects, it is commonly not used in children because of its potentially adverse outcome, Reye's syndrome (Berry et al American Pain Society).

Overall non-opioid analgesic provide mild to moderate pain relief in both acute and chronic populations, yet some pain related to trauma may require a stronger analgesic such as opioids. In these instances it is important to understand when opioid prescription may

necessitate first line treatment. Postoperative pain populations often experience severe pain related to critical tissue damage, organ repair, joint and bone manipulation, and many other procedures. Therefore prompt, effective analgesia is necessary to prevent the physical and psychological consequences of pain and the further development into chronic pain.

Opioid anaglesics often provide this relief as they bind specific receptors that prevent the transmission of pain from the periphery to the central nervous system and also modulate the response from the higher function cognitive areas. These receptors are distinguished by four different types: mu, kappa, delta, and, the most recently discovered ORL_1 (Berry et al American Pain Society). Martin and colleagues first formally proposed the existence of these receptors in the 1970s, and the first receptor, the mu opioid receptor, was discovered in nervous tissue by Pert and Snyder in 1973. In the 1990s they were genotyped by Henderson and McKnight, (Pearl 2007). Since then research has been highly concentrated on the mu opioid receptor and more specifically on its subtype, mu opioid receptor 1, OPRM1, as many pharmacological agents such as morphine assert their affects on this receptor. This receptor (similar to the other receptors) functions as a G-protein that is activated by the binding of ligands such as endogenous opioids, beta-endorphins and enkephalins, or exogenous opioids, such as morphine and fentanyl. Activation results in a decreased influx of calcium and the increased efflux of potassium, which ultimately hyperpolarizes the cell and inhibits pain transmission via action potentials (Pearl 2007).

The effect of endogenous and exogenous ligands on the opioid receptors can be adjusted by many different factors. One innate function of opioid receptors, especially the mu and delta opioid receptors, is the formation of homo- and heterodimers. This formation changes the pharmacologic characteristics of the receptors, as in heterodimers their expression is a

combination of the two receptors' properties (Pearl 2007). Therefore, drug manipulation can target these homo and heterodimer formations and possibly improve therapy.

Manipulation of the opioid receptor's effect can also be achieved with simply adjusting the exogenous opioid dosages, potency, and other properties. Morphine and codeine are extracted from the opium poppy, Papaver somniferum, and therefore are the most pure (Freye & Levy 2008). In the lab, however, their dosages are adjusted for drug administration and synthetic derivatives, such as fentanyl, hydromorphone, and meperidine, are produced. In order to measure their effects, dosages, and other properties against one another, morphine is used as the standard. The affinity of the mu opioid receptor 1 for fentanyl is much stronger than that for morphine and the onset of action is also much faster, making this medication ideal for treatment in acute pain and intraoperative populations. Hydromorphone is commonly used as an alternative to morphine as it provides moderate to severe pain relief and results in the accumulation of less active metabolites that cause adverse effects associated with opioids such as renal failure. Meperidine was originally prescribed as both an analgesic and antispasmodic, yet its particularly adverse neurological effects of seizures and delirium hinder its prescription (Freye & Levy 2008).

Although exogenous opioids have an essential role in pain relief, repetitive treatment can lead to noxious side effects, adverse reactions, increased tolerance and dependence, and decreased efficacy. Side effects include constipation, dry mouth, pruritus, sedation, nausea and vomiting, and confusion. Adverse reactions may include respiratory depression and organ toxicity (Gordon & Dahl 2003). These effects are most often controlled by careful prescription of appropriate dosages, combination therapy between opioids or non-opioid analgesic, and other alternative methods. Tolerance and dependence are two interrelated conditions, which result in the need for large dosages of opioids. Tolerance most often results from opioid receptor

desensitization, yet the mechanism for this remains unclear (Pearl 2007). One accepted theory for the mu and delta opioid receptors is that they are phosphorylated by G-protein coupled receptor kinases (GRKs) and this results in the binding of arrestin, which prevents them from coupling with G-proteins. Arrestin-bound mu opioid receptors are then internalized into endosomes and then re-introduced to the plasma membrane with an altered level of sensitivity for opioid ligands. Dependence is characterized by symptoms of opioid withdrawal (Bailey & Connor 2005). Some of these symptoms include restlessness, insomnia, and anxiety, and tachycardia. There are no defined mechanisms for these symptoms of hyperexcitation, but some have found an upregulation of cyclic adenosine monophosphate (cAMP) in association with these symptoms. The upregulation of cAMP results in an increase in protein kinase A (PKA), which sets off a chain reaction of phosphorylation within the cell and ultimately leads to the increase in neurotransmitter release (Williams et al 2001). The increases in PKA may also activate GABA transporter-1 (GAT-1) that is present in the PAG and result in a cation current, which leads to an increased firing of action potentials (Bagley et al 2004). Understanding the association of these effects of tolerance and dependence in conjunction with frequent opioid use, an opioid naïve population was selected for this study.

Although the mechanisms underlying morphine tolerance and dependence are still uncertain, finding more effective methods to prescribe opioids within each individual may prevent a wide range of negative effects associated with opioids. Thus increasing the efficacy of opioid prescription through the use of genetic testing may counteract the side effects, adverse reactions, and physiological effects of both tolerance and dependence.

2.5 GENETIC COMPONENT

As new developments within the field of genetics have been made, medical research seeks to make the connection between genotypic variations and the discrepancies seen in the presentation of disease and symptoms. In particular, pain research has found variations in the genetic coding for the OPRM1 gene may have significant effects on modulating pain response. Located on chromosome 6q24-q25, this polymorphic receptor exhibits single nucleotide variations at the locations 118 and 17. At the position of 118 a single nucleotide polymorphism (SNP) converts the nucleotide base adenine (A) to guanine (G). The occurrence of this transformation in one base it is known as a heterozygote, coding for the AG variant genotype; the occurrence of this transformation in two bases it is known as a homozygote, coding for the GG variant genotype. At position 17, this SNP results in the nucleotide base cytosine (C) being changed to thymine (T). The heterozygote and homozygote forms of this mutation exhibit the same pattern as the A118G SNP, resulting in the heterozygous and homozygous variant genotypes of CT and TT. The A118G SNP results in the non-synonymous amino acid change of asparagine to aspartic acid and C17T results in alanine being replaced by valine (Bond et al 1998). These changes ultimately affect the protein structure in the extracellular domain, with aspartic acid adding a negative charge and valine adding extra methyl groups (Matthews et al 2003).

It is uncertain exactly how these molecular changes affect the phenotypic presentation of pain, but differences have been found between the wild-type and variant groups. The SNP at A118G has been found to alter opioid binding. Bond et al (1998) found the mu opioid receptor has an increased affinity for endogenous opioids with the genotypes that contain the variant allele G, but this same allele has a decreased or even no effect on the binding of exogenous opioids. Unlike A118G, the C17T SNP has not been shown to affect binding of opioids. This

may be attributed to the similarity in the biochemical structure of the amino acid change; however, no studies have documented this evidence. Both the C17T and A118G genotypes have, however, been evaluated for addiction, and Bond et al (1998) reported a slightly higher rate of addiction amongst those with the variant allele T but inconsistent results for addiction have been found amongst those with the variant allele G. There are also discrepancies in the literature findings regarding the effect of A118G and C17T SNPs on pain reports and opioid use. A meta-analysis by Kim et al (2009) showed there is no significant difference in pain intensity between those individuals with the wild-type A118G genotypes versus those with the variants, yet many studies have found that persons those having the G allele require larger amounts of opioids. The findings for the C17T SNP have been limited and many have not found an association between this SNP and pain and/or opioid use.

The numerous literature findings for the A118G genotype perhaps reflect the distribution of alleles and genotypes. The NCBI database reports the A118G variant allele G present in approximately 10%-17% of the population whereas the frequency of variant allele T for C17T is more rare and found in approximately 5%-7% of the population. The genotypic frequencies are similar to allelic frequencies. The variant genotypes for A118G, AG and GG, are found in approximately 10.5% to 18.8% of the population and the variants for C17T, CT and TT, are found in approximately 1%-10% of the population (NCBI-rs1799971 and rs1799972 1998). Variations in the presentation of SNPs for OPRM1 by race/ethnicity have been reported in the literature. The variant genotypes for the C17T SNP, CT and TT, have been found more frequently within African-Americans as compared to other racial/ethnic groups. Bond et al (1998) found the variant allele T present in 14% of the African-American population, whereas Gelernter et al (1999) found the variant present in frequencies as high as 21%. The NCBI

database reports the genotypes CT in frequencies between 16.7%-23% amongst African-Americans. These findings are significantly higher than those of other races/ethnicities such as Caucasians who express the allele T around a frequency of 1%. On the other hand, the G allele is more frequent amongst Caucasians, Hispanics, and Asian populations (LaForge et al 2000). According to NCBI the highest frequencies for the G allele are found in the Asian race with allelic frequencies as high as 49%. The variant AG and GG genotypes are therefore also more frequently expressed in these populations. The NCBI database reports the AG genotype as high as 58% in Asians (NCBI- rs1799971 1998). Interestingly, however, Gelernter et al (1999) studied multiple ethnicities and found the G allele varied as much as 14% between African-Americans and Ethiopians. This finding indicates the variations between not only races but also ethnicities and therefore supports the proper classification of participants by ethnicity for genotypic analyses.

2.6 RACIAL AND CULTURAL DISCREPANCIES IN OPIOID MANAGEMENT

Race/ethnicity is often a controversial and sometimes confounding variable within research. Scientific research conducted in the United States has a tumultuous history with experimental designs that have discriminated along the basis of race/ethnicity. Frequently research reflects the social ideology of the time. The most infamous example of the conflict between medical research and race was the design and perpetuation of the Tuskeegee experiment. Conducted for forty years between 1932-1972, it highlighted the discrepancies in disease and symptom management as researchers withheld treatment for the purpose of monitoring the the natural history of syphilis in the African-American male population (NPR 2002). The study's termination followed the

victories for equality won during the Civil Rights Movement of the 1960s. Further victories were won for the field of research as the deplorable of treatment of subjects during the Tuskeegee experiments led to the establishment of standards of protection for all study participants with creation of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (NIH 1979).

This elevation in the ethical practices of research, however, does not completely eliminate or simplify the inclusion or exclusion of races/ethnicities in research. Particularly important to the field of genetics, race/ethnicity has been found to vary between certain genotypes. For example the A118G and C17T genotypes for OPRM1 vary significantly between different races/ethnicities. This variation cannot be disregarded, but it sometimes may act as a confounding variable based upon the study's purpose and/or design. In the area of pain research race/ethnicity has been found to result in different levels of pain tolerance as well as coping mechanisms. These variations may be the result of quantifiable genotypic differences, however, they also may result from differences in cultural practices.

Numerous studies varying in experimental and clinical methods have been conducted in order to examine the difference in pain perception by race. The most common findings for clinical pain have found higher levels of pain report by African-Americans versus their Caucasian counterparts (Edwards et al 2001). Edwards et al (2001) does admit, however, that these findings are not definite as pain report varies with numerous factors such as provider prescription of analgesics. Experimental measures that adjust factors such as pressure and temperature in order to study pain tolerance and severity therefore are usually more robust in substantiating these differences. Most studies have found African-Americans have lowered pain pressure and temperature thresholds in comparison to Caucasians (Mechlin et al 2005). Mechlin

et al (2005) studied these findings in conjunction with blood pressure readings and found that African-Americans did not exhibit the documented inverse relationship between pain and blood pressure that Caucasians exhibited. They hypothesized that this dysregulation may result from differences in molecular functioning of pain regulatory mechanisms (Mechlin et al 2005). In examining Hispanics and Asians reactions to pain thresholds and tolerance studies have found mixed results. A literature review done by Zatzick and Dimsdale (1990) found that Hispanics have lower pain thresholds and lower tolerance levels than Caucasians. Additionally, Walsh et al (1989) noted that there were similarities between the pain reports of Hispanics and African-Americans.

These experimental pain studies support that molecular and genotypic differences may influence discrepancies in the pain report between races/ethnicities, but they often do not evaluate the discrepancies arising from differences in cultural coping mechanisms. In fact culture is more accurately associated with ethnicity as this category includes similar customs and values between people of the same race. In dealing with pain, different ethnicities utilize different coping mechanisms that may alter their perception of pain. The salient use of religious interventions in dealing with pain intensity is most common amongst African-Americans and Latinos. Jordan et al (1998) found that amongst patients with rheumatoid arthritis, African-Americans reported a greater utilization of distraction and praying, while Caucasians reported higher use of controlling their perception of pain through mental manipulation. Some of these mechanisms have obvious benefits. Im et al (2007) found that large support groups are essential to the coping mechanisms of Hispanic cancer patients, and additional research has found that reliance on friends and family leads to lower stress levels. Common in many cultures is the act of stoicism in managing pain. Both the Bariba culture of West Africa and the Hispanic culture

studied in America show reluctance to exhibiting symptoms and complaints of pain (Todd 2005). It is therefore imperative that providers understand these differences when managing pain treatment and also if possible that researchers adjust for these confounding variables.

Along with these cultural variations that influence expression, the perspective of the healthcare provider who determines treatment further compounds the management of pain. Many studies such as Cleeland et al (1994) have documented healthcare provider bias as leading to ethnic disparities in pain. A national study conducted by Cleeland et al (1994) found that in healthcare settings with a predominant minority population, such as those including Hispanics and African-Americans, as high as 62% of those patients were undertreated according to World Health Organization standards. They found compromise in treatment led to minority populations receiving inadequate amounts of analgesics for pain management (Cleeland et al 1994). Often inadequate analgesia starts with the prescription of opioids. Many studies have found that African-Americans are less likely to be prescribed opioids as compared to Caucasians for the same conditions. Tamayo-Sarver et al (2003) found that African-Americans were less likely to receive opioids for migraines and lower back pain in comparison with both Caucasians and Hispanics. However, there was no significant difference in prescription of opioids between race/ethnicity for patients with long bone fractures (Tamayo-Sarver et al 2003). Thus, these findings show that the provider's perspective is affected by numerous factors, such as severity and objectivity of illness. The literature hypothesizes that these discrepancies arise from prejudice on the behalf of the provider, miscommunication, and/or lack of trust between the provider and patient. Researchers are, however, finding that racial/ethnic affiliation may have stronger, more physiological connection than purely subjective opinions. Examining cortical areas that are activated in pain processing, the anterior cingulated cortex (ACC), Xu et al (2009)

found that people were more likely to show increases in ACC activity and hence empathy when they viewed painful stimuli applied to members of their own race/ethnicity. Interestingly, however, conscience opinions for others' pain ratings did not show a significant difference in judgment between those of similar or dissimilar race/ethnicity (Xu et al 2009). This suggests that providers' response to patients is both a combination of subjective opinions and objective, subconscious cortical processing.

The association and between race/ethnicity and genetic research is dynamic. Factors of race/ethnicity such as culture and even provider bias may confound the finding of a strictly genetic basis for outcomes like pain and opioid use. The evidence for differences in pain tolerance between races/ethnicities and the findings for OPRM1 suggest that there may be an underlying molecular or genetic component responsible for these discrepancies. The advances in genetic research may equalize medical treatment between all races/ethnicities as more objective findings support subjective patient reports. It is therefore necessary to include a diverse range of races/ethnicities in order to evaluate genotypic differences; however, experimental designs should include measures to limit the confounding results.

2.7 SUMMARY

This review shows both the subjective and objective nature of pain. Often a person's self reported pain response may vary with their race/ethnicity as this characteristic has an essential role in shaping culture and hence personality. Scientists, however, seek to quantify this subjectivity in order to appropriately and effectively treat pain. In understanding the mechanisms of pain and its detrimental effects such as the progression to chronic pain one can

develop treatments that reduce adverse outcomes. The implication for genetic research is therefore to better customize pain management for each individual patient. The OPRM1 gene is of particular interest because in binding both endogenous and exogenous opioids, it exerts a direct effect on pain modulation. The research has already shown those with the variant allele, G, have an altered response to endogenous and perhaps exogenous opioids. Therefore research into the SNPs at locations A118G and C17T may provide insight into how the receptor or ligand may be manipulated for pharmaceutical purposes. This study seeks to explore the genotypic frequencies of these two SNPs, A118G and C17T, in conjunction with outcomes such as pain levels, opioid use, and the related variable of race/ethnicity in order to provide support for the implementation of genetic testing within pain management.

3.0 CHAPTER 3: METHODS

3.1 DESIGN

This study was performed as a secondary analysis of a study designed as a prospective, comparative study that examined the variations in genotype for the mu opioid receptor, OPRM1 (The association between mu-receptor genotypes and postoperative pain response, American Association of Nurse Anesthetists Foundation, Dr. Richard Henker). These variations in genotype were examined for associations between the race/ethnicity of the subject, use of opioids, and pain response in isolated extremity orthopedic trauma patients.

All of the data for the parent study was collected by investigators with clinical privileges at Presbyterian Hospital-University of Pittsburgh Medical Center. Data collected for analysis included race/ethnicity, preoperative pain scores, post-anesthesia care unit (PACU) pain scores within the first 15 and 45 minutes in the PACU. Amount of opioid used was measured during the time in the operating room and the first 45 minutes in the PACU. Race/ethnicity was collected from patient report or the medical record. The pain response was measured using an 11-point verbal pain score, VPS. The type, amount of opioid as well as the time the opioid was given were obtained from the perioperative and PACU record. Saliva samples were also collected in the

post-PACU setting. The DNA for genotypic analyses was extracted from saliva preserved in Oragene DNA self collection kit from DNA Genotek corporation (Ottawa, ON, Canada).

3.2 SAMPLE

The inclusion criteria included the following: having single isolated extremity fracture and being between the ages of 18-70 years. Exclusion criteria included: receiving opioids within the past 6 months (not including the hospital admission for current injury), having a history of alcohol abuse, using alcohol within the last 24 hours (3 drinks or greater), having a history of mental illness, currently using phenothiazines, having a history of hepatic disease, having a history of renal disease, having an American Society of Anesthesiologist Physical Status rating greater than 3, and having a history of neurological trauma.

The decision to conduct the parent study in a relatively opioid naïve orthopedic trauma population (experienced opioid use on the current admission for isolated extremity orthopedic surgery) was done specifically to limit the effects of opioid tolerance that may affect opioid use subsequent to variations in the opioid receptors. The populations with isolated extremity fractures included subjects from a wide age span who were also usually younger and therefore less susceptible to co-morbidities that may distort opioid response. This population of orthopedic trauma also usually has a significant amount of pain and hence opioid use, which provides a substantial amount of data for analysis.

3.3 DATA COLLECTION

3.3.1 Recruitment

Recruitment for the parent study was conducted in the preoperative holding area and medical surgical units at Presbyterian Hospital-University of Pittsburgh Medical Center. After reviewing demographics, co-morbidities, physical status, laboratory values, preoperative medication use, and intraoperative, data from the chart and patient report, patients were enrolled who met the selection criteria and provided informed consent.

3.3.2 Anesthesia Management

Standard intraoperative anesthesia management included: Midazolam <4 mg; Propofol (≤3mg/kg) or Sodium thiopental 4-6mg/kg; Succinylcholine or Rocuronium; Fentanyl; hydromorphone; vasoactive medications as needed; and inhaled agents such as isoflurane, sevoflurane, or desflurane. No greater than 400mcg of phenylephrine and no greater than 25mg of ephedrine were used during surgery.

3.3.3 Pain Scale

The verbal pain response scale was implemented preoperatively and at 15 and 45 minutes in the PACU. The 11-point VPS was explained to patients prior to surgery, where a rating of 0 is absence of pain and 10 is the worst pain imaginable (Au et al 1994).

3.3.4 Opioids

The type, amount, and time of administration for opioids given were recorded from the perioperative, PACU, and post-PACU data. The amount of fentanyl administered was recorded and included with the opioid medications and also recorded in a separate category. Fentanyl was coded as total amount and as mcg/kg. In order to standardize the amount of opioid used, it was converted to mg of morphine and divided by kilograms of body weight.

3.3.5 Demographic Data

Gender, race/ethnicity, age, weight, height, body mass index, and mechanism of injury were obtained from the medical record and patient report. Race/ethnicity was recorded with the following NIH categories: Caucasian, Black or African-American, Asian or Pacific Islander, Hispanic, and Native American (NIH 2001). Co-morbidities, such as diabetes mellitus, hypertension, cardiac heart failure, myocardial infarction, and chronic obstructive pulmonary disease, past surgical history, smoking, and alcohol use were extracted from the medical record and also patient report. The physical status was assessed using the following American Society of Anesthesiologist scale, where 1 – a normal healthy patient, 2 – a patient with mild systemic disease and no functional limitations, 3 – a patient with moderate to severe systemic disease that results in some functional limitation, 4 – a patient with severe systemic disease that is a constant threat to life and functionally incapacitating, 5 – a moribund patient that is not expected to survive 24 hours with or without surgery, and 6 – a brain dead patient whose organs are being harvested. If the procedure was an emergency, the physical status rating was followed by "E".

3.3.6 Saliva Samples

Saliva samples for the parent study were collected with the use of the Oragene DNA self-collection kit. This collection kit contains a vial for saliva collection where upon closing of the vial the saliva mixes with a stabilization buffer. This saliva/buffer combination is stable at room temperature for prolonged periods of time. The DNA was then extracted by Dr. Yvette Conley's lab using the protocol and reagents supplied by the Orangene kit. This extraction process usually yields 100ug of DNA from one saliva sample. This quantity of DNA was more than required for genotypic analyses for this proposed project.

3.4 DATA MANAGEMENT

3.4.1 Genotype Data

The two variants of the mu opioid receptor gene that were genotyped and analyzed for this study are the A118G (rs1799971) and C17T (rs1799972). At the position 118 the AA is the homozygous wild type whereas the AG and GG are the genetic variants. At the position 17 the CC homozygous wild type while the CT and TT are the variant SNPs. The determination of these genotypes was accomplished with a sequencing reaction that was facilitated by the fact that only one sequencing reaction was needed due to A118G and C17T being within 101 base pairs of each other in exon 1. In order to identify the variants, primers were designed to flank the

variants and that were then amplified with polymerase chain reaction (PCR). These amplified PCR fragments were then cleaned using exoSAP reagents (US Biochemicals, Cleveland, OH) and sequenced using Big Dye Cycle Sequencing reagents (Applied Biosystems Inc, Foster City, CA). In order to isolate the desired variants, the products were electrophoresed using an AB1377 automated sequencer (Applied Biosystems, Inc). These data were then converted to viewable data and assigned genotypes using Sequencer software (Gene Codes Corporation, Ann Arbor, MI). All of the genotype data were stored in a secure data file within SPSS (SPSS 17.0, Chicago, IL) on a password protected computer.

3.4.2 Data Cleaning

All data were entered into SPSS for organization and analyses. Retrospective data were also collected for some variables such as opioid use as the data were not available for during prospective collection. All categorical data were assigned a number code. Spot checks were performed frequently throughout the data entry period to verify data were entered correctly into SPSS from the patient records.

3.5 ANALYSIS

3.5.1 Preliminary Analysis

Using exploratory data analysis, the data were initially screened to identify any data anomalies that may invalidate the planned parametric analysis. Age, fracture type, opioid use, and pain

scores were evaluated using exploratory analyses. Descriptive statistics were computed including frequency counts and percentages, measures of central tendency (means, medians, modes) and measures of dispersion [standard deviations (SD), interquartile ranges (IQR), minimums (Min), maximums (Max)]. Descriptive and exploratory data analyses were performed for the total sample and within categories of the genotypes (A118G, C17T) and race/ethnicity.

The variables of interest for this study were the variant genotypes of the opioid mu receptor (OPRM1), A118G (rs1799971) and C17T (rs1799972), and also race/ethnicity, which was considered a related variable to both the OPRM1 genotypes and the outcome variables of pain and opioid use. The level of statistical significance for two-sided hypothesis testing was set .05, and 95% confidence intervals (CI) where computed for point estimates. In order to evaluate the consistency of the genotypes, Hardy-Weinberg Equilibrium (HWE) analysis was performed. The A118G sample was found to be within HWE (X^2=1.54; df=1; p=.215). The C17T sample, however, was not found to be within HWE (X^2=5.43; df=1; p=.020). This therefore indicated that significant results for C17T sample would not be relevant to the C17T population.

3.5.2 Primary Analysis

The preliminary analysis of genotypic frequencies for C17T and A118G, revealed one subject for each of the homozygous variants, TT and GG. Realizing that the literature reports more significant differences between the wild-type and variant genotypes than between the variants themselves, the homozygous and heterozygous variants for A118G and C17T were collapsed (AG/GG and CT/TT). The decision to collapse the genotypes resulted in higher cell counts and therefore a more robust statistical analysis. It was also felt some races/ethnicities should be excluded or collapsed for racial/ethnic analyses as initial analyses revealed low cell

counts. Cross-tabulations analysis for all races/ethnicities (analyzed with the collapsed variant genotype categories) revealed that 75-83% of cell counts were below the minimum expected cell size of 5 for a valid analysis. The American Indian and Asian categories were dropped from analyses as they only had one subject each with genotypic data. There also were three subjects who had claimed race unknown and so they were excluded from the racial/ethnic analyses. Lastly, there were four subjects with genotypic data for the Hispanic race/ethnicity, yet noticing their inclusion still resulted in 67% of cell counts being below the minimum expected count of 5 and that they had indicated Hispanic and Caucasian during the screening process their genotypic data were collapsed with the Caucasian race/ethnicity. This process of excluding and collapsing racial/ethnic categories resulted in analysis being performed for two racial/ethnic categories, Caucasian and African-American, and only 25% (1 cell) of the cell counts were below the expected count. Understanding, however, that cell counts for some categories were still sparse (as many as 75% below 10) it was decided to use the Fisher exact test, instead of the Pearson Chi-Square test of independence, to investigate distributional differences in race/ethnicity between genotypic categories.

The continuous dependent variables, pain score and opioid use, were evaluated for normality of distribution in order to determine if parametric or non-parametric tests should be used. To assess for normality, the Kolmogorov-Smirnov test was evaluated for group sample sizes above 50 and the Shapiro-Wilk test was evaluated for group samples sizes under 50. A p-value below the significance level of .05 for the Kolmogorov-Smirnov or Shapiro-Wilk tests or either group indicated that the distribution was not normally distributed and therefore were evaluated with non-parametric significance testing. The A118G distribution for pain scores for both AA ($p=.162$) and AG/GG ($p=.447$) genotypes showed that only the preoperative pain scores

were approximately normally distributed and therefore tested with both the two sample t-test (parametric) and Mann-Whitney U-test (non-parametric). The PACU pain scores at 15 minutes for both AA ($p<.001$) and AG ($p=.016$) genotypes and at 45 minutes for AA ($p=.038$) and AG/GG ($p=.026$) genotypes were not normally distributed and therefore evaluated with only the Mann-Whitney U-test. Similar to A118G, the distribution of pain scores for C17T showed that the preoperative scores for both CC ($p=.200$) and CT/TT ($p=.258$) genotypes were normally distributed and therefore evaluated with the two sample t-test and with the Mann-Whitney U-test. The opioid use for A118G showed that the data for the wild-type, AA, were not normally distributed for OR opioid use ($p=.004$), OR opioid use by weight ($p=.042$), PACU opioid use within the first 45 minutes ($p<.001$), and also PACU opioid use within the first 45 minutes by weight ($p<.001$). The opioid use for AG/GG, however, was normally distributed for OR opioid use ($p=.436$), PACU opioid use within the first 45 minutes ($p=.167$), and also PACU opioid use within the first 45 minutes distributed by weight ($p=.135$), but not for OR opioid use by weight ($p=.008$). Despite the findings of normal distributions for AG/GG, only the Mann-Whitney U-test was performed as the two genotype groups, AA and AG/GG, were being compared against each other. The opioid use for the CC genotype was not normally distributed amongst any of the categories, OR opioid use ($p<.001$), OR opioid use by weight ($p<.001$), PACU opioid use with the first 45 minutes ($p=<.001$), and PACU opioid use within the first 45 minutes by weight ($p<.001$). The CT/TT genotypes were normally distributed for each category, OR opioid use ($p=.912$), OR opioid use by weight ($p=.559$), PACU opioid use with the first 45 minutes ($p=.410$), and PACU opioid use within the first 45 minutes by weight ($p=.938$). Therefore because the opioid amounts for CC genotype were not normally distributed, C17T genotypes were compared with the Mann-Whitney U-test.

4.0 CHAPTER 4: RESULTS

4.1 CHARACTERISTICS OF SUBJECTS

Characteristics of subjects are reported in Table 1. The sample consisted of 83 subjects, 18-70 years of age (M=38.6; SD=13.04) with mostly lower extremity fractures (ankle/tibia/fibula) (See Table 2). The racial/ethnic makeup of the sample consisted of 64 Caucasians, 10 African-Americans, 4 Hispanics, 1 Native American, 1 Asian, and 3 of unknown race/ethnicity. The mean operating room (OR) opioid use for all subjects was 34.563 (SD=19.378). The mean by weight distribution was 0.414 (SD=0.248). The mean post-anesthesia care unit (PACU) opioid use after 45 minutes was 6.745 (SD=5.981) and by weight distribution the mean was 0.085 (SD=0.076). The mean preoperative pain score was 4.64 (SD=3.02). The mean PACU pain score at 15 minutes was 6.53 (SD=3.45). The mean PACU pain score at 45 minutes was 6.50 (SD=2.90).

Table 1. Descriptive Statistics for the Characteristics of Subjects

Characteristics	n	Mean	Min-Max	SD	Median	IQR
Age (years)	82	38.60	18-70	13.04	38.00	18.00
OR opioids	80	34.989	5-108	19.277	33.000	19.580
OR opioids/weight	80	0.414	0-1.460	0.248	0.360	0.261
PACU opioids	80	6.745	0-26.660	5.981	6.300	10.000
PACU opioids/weight	80	0.085	0-0.275	0.076	0.085	0.135
Preoperative Pain Score	77	4.64	0-10	3.02	5.00	5.00
PACU Pain Score at 15 min	82	6.53	0-10	3.45	6.70	5.0
PACU Pain Score at 45 min	81	6.50	0-10	2.90	7.00	4.00

*SD=Standard deviation, IQR=Interquartile range

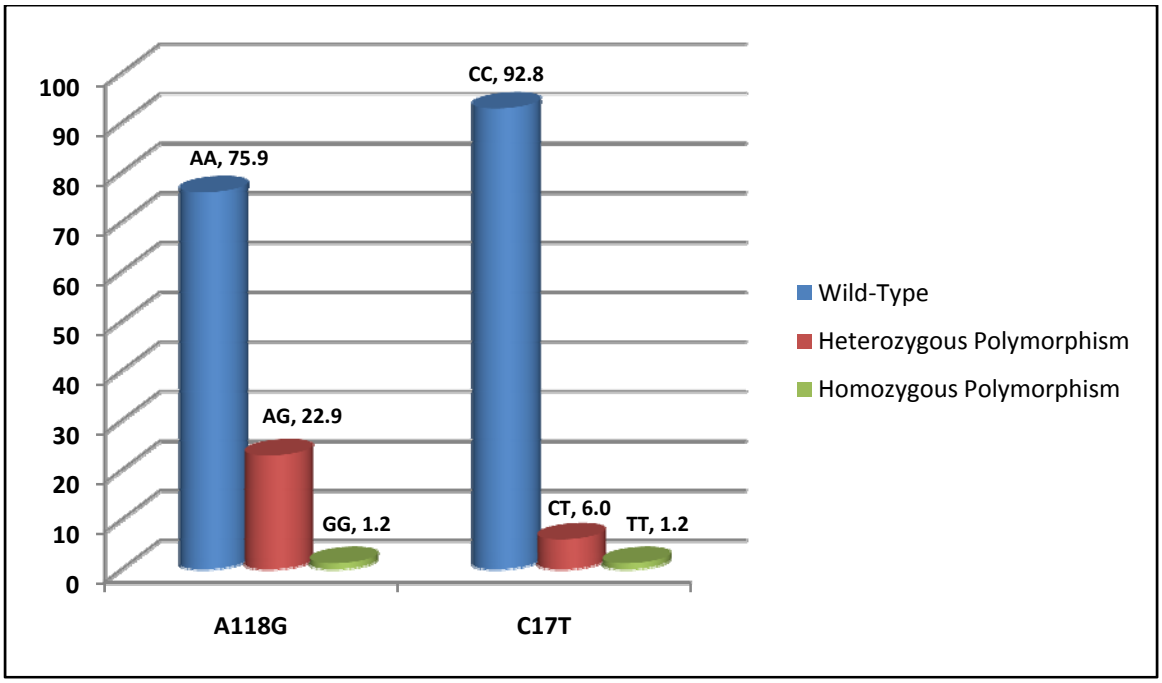
Table 2. Fracture Type

Fracture Type	n	%
Ankle	26	31.3
Femur	10	12.0
Tibial plateau	16	19.3
Tib Fib	25	30.1
Acetabular	1	1.2
Humerus	1	1.2
Radial	1	1.2
Hip	1	1.2
Ulnar	1	1.2
Total	**83**	100

4.2 DISTRIBUTION OF A118G AND C17T GENOTYPES AND ALLELES

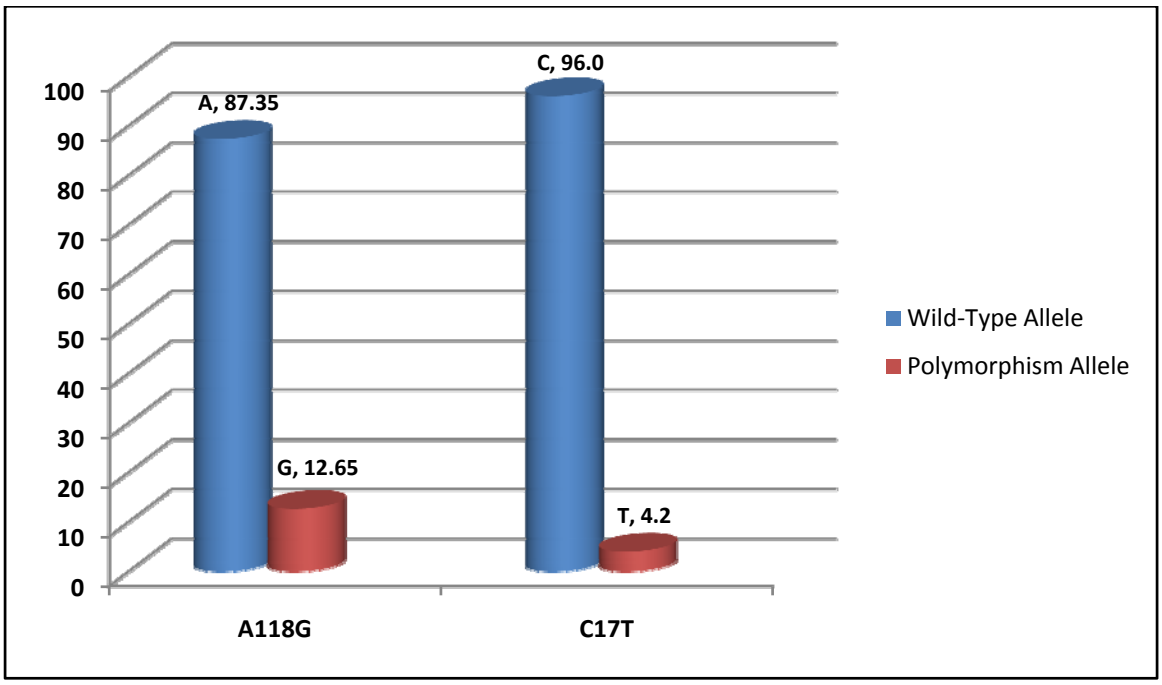
The first specific aim was to examine the distributions of genotypes and alleles within the total sample. The distributions of A118G and C17T genotypes for the entire sample are reported in Figure 1. The distribution of A118G and C17T alleles for the entire sample are reported in Figure 2. For A118G, the variant allele had a frequency of about 12.65% (95% CI= 7.59, 17.71). For C17T, the variant allele had a frequency of 4.2% (95% CI= 1.66, 7.28). The genotypic distribution for A118G was as follows: 75.9% (n=63) for AA (95% CI= 66.7, 75.9), 22.9% (n=19) for AG (95% CI= 13.85, 31.93), and 1.2% (n=1) for GG (95% CI= -1.14, 3.54). The genotypic distribution for C17T was as follows: 92.8% (n=77) for CC (95% CI= 87.20, 98.34), 6.0% (n=5) for CT (95% CI= 0.90, 11.14), and 1.2% (n=1) for TT (95% CI= -1.14, 3.54).

Figure 1. Distributions of A118G and C17T Genotypes in the Total Sample



* Labels above columns represent genotypes and percentage of genotypes

Figure 2. Distributions of A118G & C17T Alleles in the Total Sample



* Labels above columns represent alleles and percentage of alleles

4.3 DISTRIBUTIONS OF A118G AND C17T GENOTYPES AND ALLELES BY RACE/ETHNICITY

The second specific aim was to examine the distributions of the genotypes and alleles by race/ethnicity. The frequencies and percentages of genotypes and alleles by race are reported in Tables 3 and 4. Contingency table analyses using Fisher exact test showed no differences in the racial/ethnic distribution between A118G categories [OR=1.22; 95% CI= (0.23, 6.40); p=1.00]. For C17T, however, African-Americans had a greater odds of having the CT or TT genotypes relative to Caucasians [OR=63.00; 95% CI= (6.12, 648.94); p<.001].

Table 3. Distributions of A118G Genotypes and Alleles by Race/Ethnicity

	Genotype [% (n)]			Alleles [% (n)]	
	Wild Type	Polymorphism			
Race/ethnicity	**AA**	**AG**	**GG**	**A**	**G**
Caucasian	76.6 (n=49)	21.9 (n=14)	1.6 (n=1)	87.5(n=112)	12.5 (n=16)
African-American	80.0 (n=8)	20.0 (n=2)	0 (n=0)	90 (n=18)	10 (n=2)
Total	77.0 (n=57)	21.6 (n=16)	1.35 (n=1)	87.8(n=130)	12.2 (n=18)

Table 4. Distribution of C17T Genotypes and Alleles by Race/Ethnicity

	Genotype [% (n)]			Alleles [% (n)]	
	Wild Type	Polymorphism			
Race/ethnicity	**CC**	**CT**	**TT**	**C**	**T**
Caucasian	98.4 (n=63)	1.6 (n=1)	0 (n=0)	99.2 (n=137)	0.8 (n=1)
African-American	50.0 (n=5)	40.0 (n=4)	10.0 (n=1)	70 (n=14)	30 (n=6)
Total	91.9 (n=68)	6.76 (n=5)	1.35 (n=1)	95.3 (n=141)	4.7 (n=7)

4.4 DISTRIBUTION OF PAIN SCORES BY A118G AND C17T GENOTYPES

The third specific aim was to examine the differences in the distribution of pain scores by genotype. Table 5 summarizes the pain scores by genotype. There was no significant difference in levels of self-reported levels of pain found between the wild-type and variant genotypes for A118G. For C17T, however, a significant difference between the CC and CT/TT genotypes was

found for PACU pain within the first 15 minutes (p=.039). The subjects with CT/TT genotypes were found to have a lower median and mean values for pain reported within the first 15 minutes in the PACU in comparison to those subjects with the wild-type, CC, genotype.

Table 5. Descriptive and Comparative Statistics for Pain Scores by A118G and C17T Genotypes

Genotypes/Outcomes	Mean	Median	Min-Max	SD	IQR	Test Stat t-test & Mann-Whitney U-test
AA						
Preoperative Pain	4.78	5.00	0.00-10.00	3.12	5.00	[(t=.793; p=.430)(U=494.00; p=.441)]
PACU Pain 15	6.81	8.00	0.00-10.00	3.38	5.20	(U=498.00; p=.185)
PACU Pain 45	6.40	7.00	0.00-10.00	3.00	4.00	(U=540.00; p=.587)
AG/GG						
Preoperative Pain	4.21	4.00	0.00-9.00	2.70	4.00	
PACU Pain 15	5.68	7.00	0.00-10.00	3.64	7.00	
PACU Pain 45	6.84	8.00	2.00-10.00	2.63	4.00	
CC						
Preoperative Pain	4.52	5.00	0.00-10.00	2.92	5.00	[(t=-1.099; p=.275)(U=159.00;
PACU Pain 15	6.85	8.00	0.00-10.00	3.20	5.00	p=.293)]
PACU Pain 45	6.44	7.00	0.00-10.00	2.84	4.00	(U=116.00; p=.039)
CT/TT						(U=159.00; p=.240)
Preoperative Pain	6.00	7.50	0.00-10.00	4.05	8.00	
PACU Pain 15	2.83	0.00	0.00-10.00	4.49	7.8	
PACU Pain 45	7.33	8.50	0.00-10.00	3.78	4.80	

*SD=Standard deviation; IQR=Interquartile range

4.5 DISTRIBUTION OF OPIOID USE BY A118G AND C17T GENOTYPES

The fourth specific aim was to examine the differences in the distribution of opioid use during OR time and the first 45 minutes in the PACU by genotype. Table 6 describes opioid use during OR time and the first 45 minutes in the PACU for each genotype. Although the PACU opioid use for CC genotype (M=6.45; SD=5.63) and the variant CT/TT (M=10.43; SD=9.22) genotypes appeared quite different based on descriptive statistics, there were no significant differences found in opioid use during OR time and the first 45 minutes in the PACU between the wild-type and variant genotypes for A118G or C17T.

Table 6. Descriptive and Comparative Statistics for Opioid use by A118G and C17T Genotypes

Genotypes/Outcomes	Mean	Median	Min-Max	SD	IQR	Test Stat Mann-Whitney U-test
AA						
OR Opioid use	34.538 mg	32.000 mg	8.000-108.000 mg	19.216 mg	18.500 mg	(U=569.00; p=.586)
OR Opioid use by wt	0.398 mg/kg	0.361 mg/kg	0.000-1.460 mg/kg	0.234 mg/kg	0.249 mg/kg	(U=561.00; p=.596)
PACU Opioid use	6.433 mg	6.000 mg	0.000-26.660 mg	5.970 mg	10.000 mg	(U=499.00; p=.362)
PACU Opioid use by wt	0.079 mg/kg	0.079 mg/kg	0.000-0.275 mg/kg	0.074 mg/kg	0.125 mg/kg	(U=475.50; p=.238)
AG/GG						
OR Opioid use	36.439 mg	35.000 mg	5.000-85.000 mg	19.719 mg	26.000 mg	
OR Opioid use by wt	0.463 mg/kg	0.360 mg/kg	0.074-1.350 mg/kg	0.295 mg/kg	0.423 mg/kg	
PACU Opioid use	7.745 mg	7.333 mg	0.000-18.600 mg	6.069 mg	10.700 mg	
PACU Opioid use by wt	0.103 mg/kg	0.094 mg/kg	0.000-0.255 mg/kg	0.082 mg/kg	0.161 mg/kg	
CC						
OR Opioid use	35.119 mg	33.000 mg	5.000-108.000 mg	19.343 mg	16.750 mg	(U=227.00; p=.989)
OR Opioid use by wt	0.415 mg/kg	0.360 mg/kg	0.073-1.460 mg/kg	0.252 mg/kg	0.241 mg/kg	(U=210.00; p=.796)
PACU Opioid use	6.446 mg	6.000 mg	0.000-20.000 mg	5.629 mg	10.000 mg	(U=160.00; p=.262)
PACU Opioid use by wt	0.082 mg/kg	0.082 mg/kg	0.000-0.267 mg/kg	0.075 mg/kg	0.134 mg/kg	(U=165.50; p=.308)
CT/TT						
OR Opioid use	33.388 mg	31.500 mg	10.000-63.330 mg	19.375 mg	32.83 mg	
OR Opioid use by wt	0.393 mg/kg	0.450 mg/kg	0.000-0.653 mg/kg	0.241 mg/kg	0.380 mg/kg	
PACU Opioid use	10.431 mg	10.300 mg	0.000-26.660 mg	9.223 mg	13.168 mg	
PACU Opioid use by wt	0.120 mg/kg	0.115 mg/kg	0.000-0.275 mg/kg	0.096 mg/kg	0.159 mg/kg	

*SD=Standard deviation; IQR=Interquartile range

5.0 CHAPTER 5: DISCUSSION

A polymorphism is considered a minor allele found at least in 1% of the population (NCI n.d.). Therefore the primary importance of this study was to explore the allele and genotype frequencies and analyzing the genotypic frequencies for the SNPs, A118G and C17T, found on the OPRM1 gene. These frequencies were then compared to those found in other studies in order to infer the genotypic variation in the overall population. It is known that the A118G and C17T SNPs have been known to vary between races, and so another focus of this study was to compare the genotypic frequencies between races/ethnicities. This analysis was performed in order to provide greater insight in the complex nature of pain that often varies with factors such as culture and personality. Subsequent aims such as analyzing opioid use and pain score by genotype provided further insight into the implication of genetics on pain and its response.

5.1 VARIATION OF A118G AND C17T GENOTYPES WITHIN SAMPLE

Many of the polymorphisms of interest in the OPRM1 gene occur within the exon coding region as these changes lead to amino acid substitutions and hence may affect protein function. The A to G SNP at position 118 which changes asparagine to aspartic acid has been documented to result in a higher affinity for endogenous opioids, yet either a lower or no difference in binding

of exogenous opioids (Bond et al 1998). Therefore understanding the distribution of genotypes and alleles in this sample would allow us to better make connections to other outcomes such as opioid use and pain score. For the overall sample consisting of 83 subjects the allelic frequency of the variant G allele was 12.65% . This is similar to the frequencies found by National Center for Biotechnological Information (NCBI), which reports 13.0% (NCBI-rs17999711 1998). Similarly, this population consisted of 72 subjects of Caucasian and African-American race/ethnicity. According to LaForge et al (2000) who conducted a meta-analysis, the G allele was found to have a frequency ranging from 9% to 32%. Ones study's results from NCBI's database for A118G genotypes reported 71.3% with AA, 23% with AG, and 5.7% with GG (NCBI-rs17999711 1998). These genotypic frequencies correspond to the frequencies found in this study, 75.9% for AA, 22.9% for AG, and 1.2% (1 subject) for GG.

The C to T SNP at position 17 has not been documented to result in a specific mechanistic change such as that seen in A118G, but Bond et al (1998) did find a higher frequency of the variant T allele amongst those who were opioid-dependent. This study did not evaluate substance abuse such as opioid-dependence, but understanding the frequency of the variant allele and genotypes within the sample may provide further insight into the prevalence C17T being linked to substance abuse. The allelic frequency for this sample was 4% for the T allele. These results were compared to those found in NCBI database for the T allele, 4.2%-7% (NCBI-rs17999712 1998). LaForge et al (2000) reported a range of 1 to 16% for the T allele. The allelic frequencies for this study reflect the genotypic frequencies reported in the literature with 92.8% having CC, 6.0% having CT, and 1.2% (1 subject) having TT. Similarly NCBI's genotype frequencies were as follows: 89.0% for CC, 7.0% for CT, and 4.0% for TT (NCBI-rs17999712 1998). However because C17T was not in HWE further these analyses could not be

projected to the population. These variations from HWE in genotypic frequency may result from the differences in sample species, size, geographic location, and also the methods used to detect the genotypes.

5.2 VARIATION OF A118G AND C17T GENOTYPES BY RACE/ETHNICITY

The literature has reported that the single nucleotide polymorphisms found on the OPRM1 receptor at positions A118G and C17T vary between race/ethnicity. In the analyses we examined the Caucasian and African-American races/ethnicities as they were the races/ethnicities that composed the majority of the sample. In analyzing the frequency of the G allele, this study found it was present in 12.5% of Caucasians and 10% of African-Americans. This is comparable to the NCBI database, which reports the G allele in frequencies between 11.9%-16.7% for the Europeans samples and 0.8%-4.2% for African samples (NCBI-rs17999711 1998). The genotype frequency for Caucasians in this study was 76.6% for AA, 21.9% for AG, and 1.2% for GG. For African-Americans 80% were found to have AA, whereas 20% had AG and none were found to have the subsequent variant genotype, GG. Again this is comparable to the NCBI database as 70%-79.2% of Caucasians/Europeans have the AA genotype, 16.7%-29% have the AG gentoype, and 1.1%-4.2% had the GG genotype (NCBI-rs17999711 1998). For Africans-Americans the database reports 91.9%-100% as having the AA genotype, 0% to 4.3% having the AG gentoype, and none of their African samples had the GG genotype (NCBI-rs17999711 1998). Analyses of these data did not find any significant differences in these distributions between race/ethnicity. As Gelernter et al (1999) illustrates the G allele, at 49%

within the Japanese sample, is found predominately in Asian populations. Therefore the data may have shown significance if Asians composed a higher proportion of the sample.

The variant allele, T, for the C17T SNP has been reported to have a higher frequency within the African/African-American population. The results verified this as the T allele was found in 30% of the African-American sample. Similarly the genotypic distribution for African-Americans was proportionally more diverse than that found for Caucasians as 50% of African-Americans had the CC genotype, 40% had the CT genotype, and 10% had the TT genotype. Conversely the T allele was found in 1% of Caucasians and 98% had CC genotype, 2% had CT genotype, and none exhibited the variant TT genotype. Based on the Fisher exact test African-Americans were found to be significantly more likely to exhibit either the CT or TT variant genotypes ($p<.001$). This result is comparable to LaForge et al (2000) who found the T allele and CT/TT genotypes highest within African-Americans and Bond et al who found the CT/TT genotypes significantly higher within those opioid-dependent African-Americans. This finding may show a genetic link to substance abuse, which is prevalent amongst the African-American population. As the findings of 30% for the T allele is higher than that found in most studies, further sampling of the African-American population within this geographic area might reveal significant correlations between the variant T allele and CT/TT genotypes and addiction.

5.3 PAIN SCORES BY GENOTYPES

In order to analyze whether the SNPs in A118G and C17T altered pain perception, pain scores were analyzed between the wild-type and variant genotypes. Using the verbal pain scale, VPS, for preoperative and PACU pain within 15 and 45 minutes, this study did not find a significant

difference in self reported pain levels between the wild-type genotype for A118G and the variant genotypes, AG or GG. This is in contrast to other studies such as Fillingim et al (2005) that have measured pressure pain sensitivity and found a significantly higher pain threshold for those with the variant allele, G. Perhaps adjusting the method of data collection for pain such as correlating the VPS with neuronal imaging such as that used by Coghill et al (2003) will result in more significant and robust quantitative results. In analyzing the difference in pain responses between the C17T genotypes, a significant difference of was found for lower pain scores amongst those with the CT and TT genotypes (p=.039). Three out of the six subjects with the CT/TT genotype reported a rating of 0/10 for pain within 15 minutes after admittance to the PACU. This is in comparison to the entire sample that reported a mean PACU pain score of 6.53 within 15 minutes. This finding of significantly lower pain responses after orthopedic trauma surgery for subjects with the variant CT/TT genotypes has not been documented in other studies. This finding may show that the T allele confers added resistance against immediate post-operative pain.

5.4 OPIOID USE BY GENOTYPES

It was believed that analyzing opioid use by genotype would offer insight into the differences between the wild-type and variant genotypes for A118G and C17T in binding exogenous opioids. As the variant allele G increases the affinity of binding endogenous opioids, many studies have examined the effects of this allele and variant genotypes, AG/GG, on binding of exogenous opioids. The study analyzed OR opioid use and PACU opioid use within the first 45 minutes, taking into account body weight in determining opioid distribution within the body.

The results for A118G did not show a significant difference in opioid use between the wild-type AA and variant AG genotype. Previous studies, however, have found a correlation between the G allele and opioid use. Reyes-Gibby et al (2007) found that those subjects with the GG genotype had a significantly higher need for opioids in relation to cancer pain than those with the AA or AG genotypes. Lotsch et al (2006) also found that the G allele had a decreased affinity for morphine and its metabolite, morphine-6-glucuronide. Bond et al (1998), however, did not find a difference between opioid use in those with or without the A118G SNP. These discrepancies are highlighted by Reyes-Gibby et al (2007), who also showed that the GG genotype had the lowest pain scores indicating that there may not be a distinct correlation between pain and opioid use.

Similarly, the results for C17T did not show significance in opioid use between genotypes. There, however, was a large difference in PACU opioid use based upon descriptive statistics between CC and CT/TT genotypes with the CT/TT using an average of 10.43mg of morphine equivalents as compared to 6.45mg for the CC genotype (p=.262). These findings do not support the literature which has found higher proportions of opioid use amongst those with the CT/TT genotypes and also linked the variant genotypes to opioid-dependence and other forms of substance abuse.

5.5 SUMMARY

The most crucial finding of this study were that the C17T variant genotypes, CT and TT, were found significantly higher within the African-American sample as compared to the Caucasian sample. This finding supports the literature that SNPs in the OPRM1 gene vary between

race/ethnicity. The most pertinent question then is what are the effects of variant OPRM1 genotypes for A118G and C17T on pain and opioid use? This study did not add conclusive evidence that pain and opioid use have associations with variations in the A118G and C17T genotype. Even though we did find that PACU pain scores within 15 minutes were significantly lower amongst those with the CT and TT genotypes, this finding was not consistent with the other assessment points of pain, which were higher for the CT/TT group preoperatively and in the PACU within 45 minutes. This study also did not find a significant amount of opioid use between wild-type and variants genotypes in both A118G and C17T. This is somewhat surprising as the literature has found those with G allele for A118G require increased amounts of exogenous opioids as the binding affinity is for the mutant mu receptor is decreased. This decreased binding affinity is perhaps explained by the amino acid substitution of asparagine for aspartic acid. As aspartic acid is a charged amino acid, it may destabilize interactions between the binding site on the mu opioid receptor and morphine. The literature has not found any conclusive evidence that binding affinity is changed with the C17T substitution and that may be validated by the change in amino acids (alanine for valine) resulting in similar hydrophobic interactions (Matthews et al 2003).

Overall the findings were somewhat contradictory to those found in the literature as we found a nonsignificant need for opioids amongst those with the C17T variant allele, T, for the PACU period. Synthesizing these results it may be concluded that those with the C17T variant genotypes, CT and TT, may have OPRM1 receptors than bind exogenous opioids with a stronger affinity, yet for shorter time frame. This decrease in binding time may be a result of difference in metabolism of morphine's active metabolite, morphine-6-glucuronide. Rakvag et al (2005) and Reyes-Gibby et al (2007) both found that morphine consumption is influenced by the

enzymatic activity of catechol-O-methyltransferase, COMT. Looking at COMT within cancer patients Rakvag et al (2005) found that those with the Val/Val genotype required more amounts of morphine than those with the Met/Met genotype. Reyes-Gibby et al (2007), however, found a correlation between COMT and OPRM1 for the A118G genotypes. They also found that those with the Met/Met genotype for COMT and AA for A118G required the lowest amounts of opioids in comparison to those without both of these genotypes (Reyes-Gibby 2007). This therefore suggest that there may be an association between dissimilar genes and pain modulation and further research between C17T and other genes such COMT may show this correlation. The needed increase in opioids within the C17T sample with the variant genotypes may also suggest that there is an increased rate of tolerance of morphine and this may be linked to the increased opioid dependence seen in those with the CT and TT genotypes. Of course cultural components may also add confounding variables that distort the genetic explanations.

5.6 LIMITATIONS

The C17T sample was not found to be within HWE. This may be a result of sample demographics. Those with orthopedic trauma injuries usually exhibit a higher level of risk taking and this may have a genetic component that correlates pain perception with the propensity for risk-taking behavior. In fact a study by Bart et al (2005) researched the correlation between OPRM1 and other genes related to risk-taking behavior. These have primarily been performed in the A118G genotype and further exploration between risk-taking genes and the C17T genotype may show significant correlations.

The results of this study were affected by numerous factors within the methods plan. Small sample size was most likely due to recruitment occurring at one site and also within a specific population of isolated extremity fractures with few co-morbidities and no history of opioid use. Orthopedic trauma injuries are common amongst those with risk taking behavior and hence this population may have a high incidence of substance abuse. Expanding the sample size to other sites and recruiting those subjects with prior opioid use and co-morbidities may expand the number of subjects. These subjects, however, would receive their own separate categories for analyses as these factors may alter the pharmacodynamics of opioids. The expansion beyond the current geographic location would also add racial/ethnic diversity to the sample.

Isolating race/ethnicity was another limitation of this study. Subjects were recruited by racial categories instead of ethnicity, and this excludes some diversity that may found amongst the distribution of alleles and genotypes. For example Gelernter et al (1999) found that 17% of Ethiopians exhibited the G allele as compared to 3% of African-Americans. In this study the two groups would have been collapsed into one. Separating subjects by ethnicity may produce more relevant results.

A further limitation was the way in which some of the results were collected such as pain scores and opioid use. Both had compromises in accuracy as they were taken from both the medical record and patient report. These two methods do not always agree as there may be discrepancies between the patient report and the healthcare provider recording the data. An increased number of recruiters would help ensure that most of the information was taken directly from patient report or from the recent medical record and any discrepancies could be clarified with the patient and healthcare provider.

5.7 CONCLUSIONS

This study evaluated the frequency and effect of the variant genotypes of A118G and C17T for OPRM1. The findings of this study showed that the genetic variability in the C17T SNP is found most frequently in the African-American group. They are more likely to have the variant, T, allele and therefore the variant CT and TT genotypes. Those with CT/TT are also more likely to have a reduced amount of pain within the immediate PACU period, but show a need for increased opioid use. These findings of greater opioid use amongst those with the CT/TT genotype which is commonly found in African-Americans signifies that a genetic component may be affecting opioid dependence and other forms of substance abuse.

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