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DETERMINATION OF GENETIC VARIATION IN OPIOID-RESISTANT HOSPICE PATIENTS USING A PHARMACOGENOMICS APPROACH

Daniel Bianculli

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by

Daniel Bianculli

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DANIEL BIANCULLI

DR. MARC GILLESPIE

ABSTRACT

DETERMINATION OF GENETIC VARIATION IN OPIOID-RESISTANT HOSPICE PATIENTS USING A PHARMACOGENOMICS APPROACH

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Managing pain requires a delicate balance between the advantages of opioid analgesia and the possible adverse effects. This balance becomes even more difficult in hospice and palliative care environments where dosage regimens are frequently ambiguous. Some hospice patients experience opioid resistance, in which opioids no longer produce an analgesic effect on a patient.

Evidence-based analyses frequently omit this vulnerable population, resulting in a need for concrete procedures. The emergence of pharmacogenomic analysis and personalized treatment presents a solution to this problem by using each patient's genomic data to interpret opiate dosing.

This pharmacogenomic study aimed to single nucleotide polymorphisms (SNPs) influencing opioid response in 18 hospice patients aged 37-84 years (mean 63 years, 72% male). Participants had diagnoses including COPD, cancer, and HIV. Buccal swabs provided DNA for 50-gene panel genotyping. Medical records supplied demographic information, medication lists, chronic health conditions, morphine doses, pain scores, and palliative performance status data. Linear regression analysis identified single nucleotide polymorphisms (SNPs) significantly impacting outcomes.

According to genomic analysis, individuals with the non-TT genotype of the

CNR1 gene were found to require, on average, a dose of morphine that was 102.3 mg lower than the dose required by individuals with the TT genotype of the same gene ($p=0.031$). The SLCO1B1 genotype and intermediate activity phenotype reduced average pain ($p=0.046$). The COMT non-MET homozygous phenotype increased maximum pain versus MET homozygotes ($p=0.041$). The CYP2B6 genotype and G516T heterozygous/A785G homozygous phenotype decreased maximum pain ($p=0.050$). The CYP4F2 poor metabolizer phenotype increased palliative performance versus other CYP phenotypes ($p=0.028$).

These findings show that genetic variants can modulate opioid metabolism and pain response in hospice patients. This study marks an essential step towards the goal of personalized medicine in pain management at the end of life, suggesting that genotype- guided opioid dosing regimens could potentially lead to improved pain management.

However, the sample size of 18 of this pioneering pharmacogenomic study in hospice patients underscores the need for larger-scale studies to validate the predictive utility of our findings.

DEDICATION

This is dedicated to my mother Virginia, my father Angelo and my brother Michael.

Without them, none of this would be possible.

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CHAPTER 1. INTRODUCTION

1.1 Hospice and Palliative Care

As the population ages, the need for hospice and palliative care increases. The goal is to improve the quality of life for patients facing serious, chronic illnesses. Although often used interchangeably, there is a distinction between hospice and palliative care. In hospice care, the medical staff optimizes the quality of life and treatment is not conducted. In palliative care, treatment is continued along with quality-of-life improvements[1].

On the other hand, there are various scenarios where patients are placed under palliative care: a life-limiting illness though the patient is not terminally ill, a life-threatening illness with potential for recovery, or a chronic condition where a patient is being treated but will eventually die[2]. Examples of these illnesses include congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD).

Investigation into these populations is generally lacking, with most research focused on caregiving, access to care, and social issues. Clinical research was a significant gap in this patient population[3]. Palliative and hospice patients are also rarely involved in clinical trials. This is because of the many conditions they face, the large number of medications taken, and the likelihood of dropping out of the study due to deterioration in their health and age-related changes to drug activity[4]. These combinations of factors contribute to the stigma that hospice and palliative care research is too difficult to conduct in an evidence-based manner. Most research instead focuses on aspects such as health professionals in the field, access to hospice care and sociological aspects[5] of end-of-life care (ex: Hispanic access to hospice care).

There is a crucial need to conduct more research on hospice and palliative care populations, as evidenced by the funding from the National Institutes of Health (NIH). In 2015, the NIH budget allocated only 0.2% for end-of-life care, with hospice research and palliative care sharing the same budget[6]. In the latest review of NIH funding for palliative care, the budget has only shown an increase in dementia and Alzheimer's studies at the federal level. This increase in funding does not represent growth in the research of palliative care itself[7].

Because of this, fundamental gaps in our knowledge of hospice and palliative patients exist, especially in terms of evidence-based studies. More robust studies are needed to decrease treatment difficulty and provide the greatest quality of care and optimal outcomes.

1.2 Pain Management

Pain is a substantial burden in patient care, significantly impacting a patient's quality of life. Managing pain becomes much more complex and multifactorial in the palliative and hospice environment. Pain can result from the primary disease state and its treatments, such as surgery and narrow therapeutic index drugs[8]. There is also pain unrelated to the disease state, such as psychological factors, anxiety, depression, and anger[9]. All these factors make treatment difficult.

While there are many non-pharmacological and pharmacological methodologies for treating pain, opioids remain the front-line treatment. They are the most effective medications in terms of alleviating pain. Opioids come with their difficulties. Dosing opioids has always been problematic, with incredibly varied pain responses among patients[10]. Genetic variation among patients increases the likelihood of errors in dosing.

Currently, the FDA only recommends genomic testing for codeine and no other opioids[11]. The palliative and hospice patient population is far from typical. They are likely over 65, have multiple co- morbidities, and take multiple medications[12]. There is also the fact that palliative care patients stay much longer in the hospital than other patient populations[12], further increasing their vulnerability to dosing errors. In palliative care, errors in opioid prescribing are considered the primary cause of medical errors in the palliative care field[12]. The most common medication error involving opioids was underdosing. Underdosing accounted for about 66% of the dosing error for opioids[13].

The reasons for this are twofold: There is a lack of empirical-based research in palliative care[14], making concrete methodologies challenging to establish with so few studies. Next, there is a lack of knowledge about prescribing opioids among healthcare professionals, along with misconceptions, myths, and fears of addiction[15]. Clinicians, caregivers, and others in the palliative and hospice field would benefit from additional tools to prevent opioid errors.

1.3 Opioid Pharmacodynamics

The creation of pain relief by opioids occurs by binding to G-protein-coupled receptors. The primary location of G-protein-coupled receptors is in the brain and spinal cord. The critical receptors involved in opioid pharmacology are the μ, δ, and κ receptors. The therapeutic role of opioids in pain management is versatile. They exhibit varying degrees of activity as a full agonist, partial agonist, or antagonist across many receptor types[16].

These opioid receptors interact with G proteins and, as a result, can influence intracellular Ca^{2+} disposition and the phosphorylation of proteins[17]. Opioid receptors are

a type of G-protein-coupled receptor (Gi or Go), characterized by seven transmembrane regions. They close voltage-gated Ca^{2+} channels on pre-synaptic nerve terminals, which reduces neurotransmitter release.

Second, they induce the opening of K^+ channels, resulting in hyperpolarization and the subsequent inhibition of post-synaptic neurons[18]. Opioids have been observed to decrease neurotransmitter release through pre-synaptic action across a wide range of neurotransmitters, including glutamate (the primary excitatory amino acid released from nociceptive nerve terminals), acetylcholine, norepinephrine, serotonin, and substance P[19].

These effects are mediated by the opioid receptor family. These receptors consist of the μ (mu), κ (kappa), δ (delta), and ORL1 (Nociceptin/Orphanin FQ) subtypes[20]. The μ receptor is the most common and abundant of the opioid receptor family. The μ receptor plays a crucial role in supraspinal analgesia in addition euphoria. In the central nervous system (CNS), μ-opioid receptors are widely distributed[21]. In the periphery, μ-opioid receptors can be found but are less prevalent than in the CNS. They appear in tissues like the intestines, which cause constipation associated with opioid use, as opioid binding to these receptors decreases intestinal motility. Individual genetic variations can affect binding to μ receptors[22].

The opioid receptors in the CNS allow for the modulation of ascending and descending pain pathways. In the ascending pathways, which originate in the spinal cord and project to higher brain centers like the thalamus and cortex, opioids act by inhibiting neurotransmitter release from primary afferents. This diminishes the propagation of nociceptive signals[23]. Conversely, opioids also affect descending pain pathways, specifically those originating from supraspinal sites[24]. At these locations, opioids inhibit

neurons that would otherwise facilitate pain transmission[25]. This opioid-induced neuronal inhibition leads to the activation of descending inhibitory neurons. These neurons send axons to the spinal cord, inhibiting pain- transmitting neurons and amplifying opioid analgesia[26].

1.4 Opioid Pharmacokinetics

Clinicians commonly administer opioids through subcutaneous, intramuscular, or oral routes, and these methods ensure effective absorption[27]. The first- pass effect causes higher doses of some opioids, like morphine, when administered orally, compared to subcutaneous or intramuscular routes. Variability in the metabolism of oral opioids complicates accurate dosing. Codeine and oxycodone, less affected by first-pass metabolism, are better suited for oral administration[28]. Transdermal patches can deliver high-potency analgesics and relieve pain for several days[29]. When determining the optimal administration routes and doses, clinicians must consider the pharmacokinetic profile of each opioid. Once administered, all opioids bind to plasma proteins. The affinity for binding depends on the opioid in question. Despite this, the drugs rapidly leave the blood and localize in the tissues[28]. They are in the highest concentration in the brain, lungs, liver, kidneys, and spleen. Drug concentrations may be low in skeletal muscle, but because of its greater bulk, skeletal muscle serves as the main reservoir for opioids[28]. Accumulation in fatty tissues can be significant for certain opioids, especially after frequent high-dose opioid administration or a continuous infusion of highly lipophilic opioids that metabolize slowly, such as fentanyl[30].

Opioids undergo metabolism, resulting in the production of polar metabolites, most of which are glucuronides. The kidneys eventually eliminate these metabolites

through excretion. For example, the prototypical opioid morphine contains free hydroxyl groups. These hydroxyl groups are primarily conjugated to morphine-3-glucuronide (M3G), a compound with neuroexcitatory properties[31]. Around 10% of morphine is metabolized to morphine-6- glucuronide (M6G). M6G is an active metabolite with analgesic potency around four to six times that of morphine[32]. Despite this, these polar metabolites cannot cross the blood-brain barrier. Though, accumulating these metabolites may produce toxic effects in patients with renal failure or when exceptionally high doses are administered over a long period[33].

Codeine, oxycodone, and hydrocodone undergo metabolism in the liver by the P450 isozyme CYP2D6, producing metabolites of greater potency[34]. Codeine is demethylated to morphine, and hydrocodone is metabolized to hydromorphone. Though oxycodone metabolites are not generally responsible for the analgesic activity of the medication, they can accumulate in cases of renal failure[35].

Opioids are primarily eliminated through urine excretion. Small amounts of unchanged drugs may be detected. Once more, the danger is renal impairment, with metabolites having an increased effect, especially at high doses[36].

1.5 Opioid Resistance

A critical phenomenon that contributes to the difficulty in opioid prescribing is opioid resistance. Though opioid resistance is sometimes used interchangeably with tolerance, these terms differ. Opioid resistance differs from opioid tolerance in several ways. In opioid tolerance, tolerance develops from repeated opioid exposure over time[37]. Tolerance requires patients to take higher doses to achieve the same analgesic effect. The general mechanism is desensitization and downregulation of opioid receptors[38].

Opioid resistance is a phenomenon that is not solely attributed to prior or prolonged exposure to opioids. Opioid resistance manifests as an absence or markedly reduced efficacy of analgesic response even when the doses of opioids are escalated. This resistance can be categorized into innate and. There are multiple factors involved in opioid resistance. One such factor is epigenetic changes[39]. A recent definition of epigenetics is 'the structural adaptation of chromosomal regions to register, signal or perpetuate altered activity stages'[40]. Epigenetic changes are a bridge between genes and the environment.

In contrast to evolution, these changes can accumulate over a lifetime, allowing quick adaptability to changing environmental conditions[41].They can be caused by environmental stressors as well as aging. These changes can also occur due to disease, particularly in cancer.

Two primary types of epigenetic changes occur in individuals: DNA methylation and histone modification via acetylation or methylation[42]. DNA methylation happens when methyl groups bind to cytosines at CG-dinucleotides within CpG islands, primarily found in upstream gene promoter regions[43]. However, methylation can occur at any other loci throughout the genome. On the other hand, histone modifications alter chromatin conformation. Histone acetylation opens the chromatin, while deacetylation closes it. These mechanisms regulate gene expression throughout the chromosome[44].

In terms of opioids, epigenetic regulation, specifically the acetylation of lysine residues on histone proteins in spinal tissues, has modulated increased sensitivity to pain following surgical procedures and the development of reduced responsiveness to opioids, commonly referred to as tolerance[45]. The desensitization of opioid receptors occurs when

G-protein-coupled receptor kinases facilitate the phosphorylation of these receptors, followed by arrestin binding[46].

Another factor that is involved in opioid resistance is microRNAs (miRNA). These are non-coding, functional RNAs that play essential roles in gene regulation. These are typically 17 to 24 nucleotides long. miRNAs can bind to protein-coding messenger RNA (mRNA) to post-transcriptionally repress protein expression[47]. A large amount of expression of miRNA is found in the CNS. mRNA is responsible for neuronal development, including cell specificity and neuronal patterning[48]. miRNAs are also still expressed in adult neurons at high levels. There has also been evidence that miRNAs can down-regulate the mu opioid receptor (MOR) [49]. One method includes miRNA-16 can bind to the 3′-untranslated region (3′-UTR) of mRNA and weaken the translation of MOR mRNA[50].

In addition to epigenetic factors and miRNA, there are individual genetic variations. These gene variations can affect the pharmacodynamics and pharmacokinetics of opioids[51]. These genetic variations further complicate opioid dosing. Hospice and palliative care patients can face significantly high doses of opioids that should be toxic (or even lethal) but have no effect at all, analgesic or otherwise, due to opioid resistance[52].

1.6 Pharmacogenomics

Interindividual reactions to drugs, including opioids, falls under the study of Pharmacogenomics (PGx), a field focusing on the genome in drug response. This field seeks to understand how genes and genetic variation influence a patient's response to a particular drug. While considered relatively new, human geneticist Dr. Fredrich Vogel coined pharmacogenetics in 1959[53]. Initially, this term was used to describe gene-drug

interactions. With the advent of the human genome project in the 1990s, pharmacogenetics became PGx. That is the study of how drugs interact with the total genome to influence biological pathways and processes[54].

An individual's drug response is the phenotype of a pharmaceutical compound's two important features: pharmacokinetics or pharmacodynamics. This results in four possible drug phenotypic expressions: efficacy without toxicity, efficacy with toxicity, toxicity without efficacy, or neither effect nor toxicity[55]. The classic example of pharmacogenomic toxicity involves the gene Thiopurine methyltransferase (TMPT).

Thiopurines are first-line agents for myeloid leukemia and act as immunosuppressants. Genetic factors drive the metabolism of thiopurines and their efficacy[56]. Thiopurines are a classic example of PGx in clinical medicine since anyone taking these drugs must undergo genetic testing. Genetic testing is required because *TMPT* is involved in the methylation of thiopurines, a phase II metabolic process[57]. Alterations in the *TMPT* can have toxic and even lethal consequences. Those possessing a heterozygous *TMPT* variant exhibit reduced enzymatic activity for the metabolism of thiopurines and have a risk of increased toxic effects, which requires dose modulation[58]. However, those with the homozygous variant do not have enzymatic activity to metabolize thiopurines and require an alternative treatment regimen for efficient care.

Though *TMPT* is just one gene involved in thiopurine metabolism. Others like *NUDT15* can contribute to the metabolic pathway and influence the toxicity of thiopurines[59]. Their defined factors and severity make this a classic example of PGx in a clinical setting.

In PGx, various forms of genetic variations are investigated. These include Single

nucleotide polymorphisms (SNPs), structural variations from the addition or removal of base pairs (also known as indels), and substantial copy number variations that may lead to the complete absence or duplication of entire genes[60]. These variations in genetic disposition can play a role in gene expression by modifying the regulation of transcription and the splicing process and leading to changes in amino acids or truncation of protein-coding sequences[61].

1.7 Single Nucleotide Polymorphisms

SNPs are the most prevalent genetic variation in humans. The basis for human diversity is found in these single nucleotide variations dispersed across all species' genomes. SNPs occur in about one in every 300 nucleotide base pairs throughout the human genome. Approximately 10 million SNPs are present in the 3 billion nucleotides of the human genome[62]. These are base pair variations located at specific sites in human genes[63]. Millions of SNPs exist in every individual, which makes them great use for biomarkers[64]. Single nucleotide polymorphisms (SNPs) occur throughout the genome and can coexist within a genetic region. These SNPs are stable and do not change over time, making them ideal for consistent measurements[65]. Additionally, measuring SNPs is a simple process that utilizes PCR-based testing, eliminating the need for complex and expensive assays[66].

SNPs may be present in coding or non-coding genome regions, and some may not impact the gene product. Sometimes, SNP in a non-coding region influences the transcription of the gene. In other instances, even a SNP in the coding region may not affect protein structure or function[67].

Synonymous SNPs substitute one nucleotide for another that does not change the

amino acid[68]. Non-synonymous SNPs produce new encoded amino acids. The resulting altered proteins could possess different features. An example is a conformational alteration that may lead to a change in enzyme activities. In other cases, the function of the entire protein may be diminished[69]. Nevertheless, synonymous SNPs still cause disease states and altered gene function. Previously, these were considered 'silent' or 'neutral.' This line of thought is no longer the case. Silent SNPs can result in abnormal mRNA splicing as well as mRNA stability. These factors, like non-synonymous SNPs, affect protein expression and enzymatic activity[68].

Because of this, SNPs can alter the pharmacodynamics and pharmacokinetics of drugs, including opioids. If SNPs are present, this may account for interindividual differences in response to opioid treatment[70]. Genes containing SNPs may encode intracellular targets like transcription factors, drug transporters, receptors, or metabolic enzymes. The examples below represent a subset of the SNPs that may contribute to variability in opioid effects.

The gene for the human μ opioid receptor, OPRM1, is controlled by multiple promoters and comprises more than one hundred SNPs[71]. The 118A>G (which possesses the accession number in the SNP database [dbSNP] as rs1799971) has been the most studied variant in terms of pharmacogenomic research into opioid drugs[72]. This SNP is located in exon 1 of OPRM1 and substitutes adenine (A) for guanine (G). This results in an amino acid shift from asparagine to aspartic acid, leading to the receptor's loss of an N-glycosylation site[73]. The loss of this site has been linked to several clinical effects, such as increased side effects from opioid doses[74]. In patients with cancer pain, this genetic variation was associated with increases in morphine dose in order to achieve

proper pain management[75].

Uridine diphosphate glucuronosyltransferase family 2 member B7 (UGT2B7) is a metabolic enzyme involved in the glucuronidation of compounds, such as fatty acids[76]. The central location where UGT2B7 is expressed is in the endoplasmic reticulum of hepatocytes. However, UGT2B7 can also be found in the gastrointestinal tract, kidney, pancreas, and brain[77]. UGT2B7 metabolizes morphine into M3G (90%) and M6G (10%). Polymorphisms of *UGT2B7* have been implicated in influencing interindividual variability in morphine dosing. One study reported that the *UGT2B7*802T* allele experienced extended and more significant analgesic effects than those with the 802C allele. This may indicate that the 802T allele has reduced glucuronidation activity[78].

Somewhat related to UGT2B7 is the organic cation transporter isoform 1 (OCT1). The *SLC22A1* gene, which belongs to the solute carrier family 22, encodes the influx transporter called OCT1. *SLC22A1* is expressed in several tissues, predominant in the liver[79]. UGT2B7 metabolize*s* morphine in the liver and has a high affinity for OCT 1, so gene variants affecting *SLC22A1* may impact the metabolism of morphine[80]. The most frequently studied polymorphisms of the *SLC22A1* gene relate to a loss of function in the OCT1 transporter. In one study, carriers of the loss-of-function *OCT1* polymorphism possessed significantly higher plasma concentrations of morphine than those that did not carry the variant[81]. In children, the clearance of morphine was significantly lower in homozygote carriers of *OCT1* loss-of-function variants[82].

The previous examples demonstrate how SNPs in critical genes can influence opioid pharmacokinetics and pharmacodynamics. Through research, we can gain a greater understanding of genetic polymorphisms and the interaction between drugs. However,

the desired end-point for PGx is to apply it to patient care. In this way, PGx can be a valuable tool for hospice patients, their caregivers, and healthcare providers in opioid treatment.

1.8 Pharmacogenomics in Practice

The first hurdle to put clinical guidelines for opiates, opioid resistance, or any other drug is the link between genotype and phenotype. In PGx, accurate genotype-phenotype associations are critical to transitioning into clinical practice. The genotype is defined as the genetic variant of the gene of interest. Conversely, the phenotypes are typically linked to drug responses. These can include drug-induced toxicity, the efficacy of the drug, the concentration of the drug in the bloodstream, or the drug's impact on other biological markers within the body[83].

Pharmacogenomic tests in clinical laboratories are currently designed to detect a different genotype. Once this variant is detected, the variant is used to assign alleles. Then, informed assumptions are made about haplotypes. A haplotype is a set of alleles at different loci on a single chromosome[84]. Haplotypes are typically inferred because most analytic methods can identify a variant genotype; they cannot ascertain each variant's chromosome position or locus. Instead of general testing, specialized testing is utilized to pinpoint the exact location where genetic variations are likely to occur[85]. The alleles are then assembled as a diplotype (a maternal and paternal allele), and the phenotype is predicted based on previous studies[86]. This can be rather complex in certain instances.

The genotype-phenotype relationship is clearly established for life-threatening or toxic variants. There is a gene called human leukocyte antigen (HLA) that has different variants. Some of these variants can cause dangerous reactions to certain drugs. Testing is

required to determine if a person has at least one of these variant alleles. *HLA-B*57:01* is a specific variant that is known to increase the risk of hypersensitivity to the drug abacavir. Only one allele is needed for a possibly life-threatening immune-mediated reaction to the drug[87].

Outside of these variants, risk becomes difficult to pinpoint. Furthermore, for synonymous SNPs, the phenotype may be complicated. Since synonymous SNPs do not directly change the produced gene product, their effect may lie elsewhere. To find genotype/phenotype relationships, research, literature, and clinical studies are needed. This also presents its own problems since there are variabilities in studies on defining a pharmacogenomic phenotype and agreeing on what that phenotype represents[88]. Also, the wide variety of tests and methodologies has led to genotype-phenotype associations failing to be replicated. Clinical tests require standardization due to variations in nomenclature.

PharmGKB (http://www.pharmgkb.org) and the Pharmacogenomics Research Network (PGRN) collaborated to form the Clinical Pharmacogenetics Implementation Consortium (CPIC) in 2009. The CPIC provides guidelines for pharmacogenomic results in accurate prescribing decisions for certain drugs[89]. The CPIC launched a project intending to standardize terminology across PGx. The project involved reaching a consensus among experts in pharmacogenetics. The objective was, whenever feasible, to agree on standardized terms that could be applied universally across genotypes to define both an allele's functional status and the inferred phenotypes based on the combined effects of both alleles[89]. This has allowed for accurate translation into clinical practice.

In addition to the standardization of results allowing for accurate prescribing

information, the nomenclature has also been standardized. PGx uses the Human Genome Variation Society (HGVS) nomenclature. This naming system clarifies the genomic position of the variant as well as alterations to the DNA, RNA, and protein sequences[90].

In addition, there is the use of the star-allele system. This allows for the categorization of sequence variations easily. A *1 typically signifies the reference allele. As new variations are discovered, they are sequentially assigned numbered star alleles[55]. An example of the naming system in practice is the *CYP2D6*4* allele and one of its variants, c.1847 G > A (rs3892097). In this case, *4 is the abbreviation for known *CYP2D6* alleles containing different variants. The location of the DNA change is represented by c.1847, the specific nucleotide shift would be G > A, and the variant name and unique location in the genome is represented by rs3892097.

1.9 Rationale

The purpose of this study partially stems from the gap in research concerning the PGx factors that influence the efficacy of opioid therapy in hospice patients. Opioids are the cornerstone of managing severe pain. However, their effectiveness varies widely among individuals. The effectiveness of opioid therapy is due partly to genetic variations. This study aims to discern PGx differences between hospice patients and opioid therapy.

Utilizing PGx will examine whether there are associations between SNPs in this population concerning opioid resistance. This study will be done by cross-referencing patient data with molecular genetic information. By doing so, this study seeks to discover unique SNPs that differ from previous studies and see if there is a relationship between SNPs and patient data. These research questions resulted in three hypotheses found below.

1.10 Hypotheses

1. Molecular genetics data from hospice patients can be associated with patient medical information to identify potential SNPs influencing response to opioid medication.
2. Potential genes linked with resistance to opioid medication can be uncovered by correlating molecular genetics data with pain measurements, opioid responsiveness, and treatment response.
3. By identifying individual genetic variations that influence the response to opioid medication, clinicians can develop personalized pain-management plans, potentially leading to improved quality of life and reduced side effects for patients in hospice and palliative care.

CHAPTER 2. MATERIALS AND METHODS

2.1 Instruments Used

The study employed the Palliative Performance Scale (PPS) as an evaluative tool to gauge both the functional status and prognosis of patients receiving palliative care. Initially introduced by Anderson et al.[91], the PPS utilizes an 11-point scale ranging from 0% to 100% in increments of 10%. This scale examines four key parameters: ambulation, activity, evidence of disease, self-care, and level of consciousness (Figure 1). In this study, our focus was on patients having a PPS score of 30%. We chose this threshold because patients with this score. However, their functional impairment is significant, disease progression is evident, and they can still perform a limited number of daily activities with some assistance[91]. This score indicates relative functional stability, suggesting a prognosis that could extend for several weeks. We excluded patients with lower PPS scores from the study, as their functional status would be too unstable and their life expectancy too short to yield meaningful data.

A Numerical Rating Scale for Pain (NRS) was utilized to assess the average and maximum pain of study participants (Figure 2). The NRS is among the most common pain scale instruments used in hospice care[92]. This 11-point scale ranging from 0 (“no pain”) to 10 (“worst possible pain) has demonstrated clinical utility in assessing changes in chronic pain intensity[93]. Patients will be asked to grade their average and worst level of pain in the previous 24 hours (maximum pain) due to the primary diagnosis at the end of each visit.

The Numerical Opioid Side Effect (NOSE) Assessment Tool (Figure 3) was used to record common opioid side effects experienced by patients during the study[94]. The

NOSE records the severity of 12 opioid side-effects on an 11-point scale from 0 ("not at all") to 10 ("extremely"). Total NOSE scores can range from 0 to 120, with higher scores indicating a more significant side effect burden.

In order to create a standard among opioid doses, morphine milligram equivalents (MME) were used. Patients use different opioids and different opioid dosing regimens, so it was necessary to standardize them. These conversion factors have been established by the Centers for Disease Control and Prevention (CDC). For example, 10 mg of hydrocodone is equivalent to 10 mg of morphine, while 30 mg of oxycodone is equivalent to 20 mg of morphine[95]. By MME conversion, the total daily opioid doses for patients taking different opioid medications can be compared using a standardized metric (Figure 4).

2.2 Pharmacogenomic Analysis

Buccal swab samples were collected by Puritan DNA/RNA shield for sample collection and preservation from Puritan Medical Products (Guilford, ME). These were then sent to Admera Health (South Plainfield, NJ). Admera Health is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high-complexity clinical laboratory testing. Admera Health utilized its PGxOne™ Plus assay, a next-generation sequencing panel that analyzes 50 pharmacogenomically relevant genes and 211 variants of those genes. These genes were chosen based on their availability at Admera, the researchers in the study as well as the Clinical Pharmacogenomics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG), and the United States Food and Drug Administration (FDA) work group guidance.The report from Admera is comprised of three sections (Figures 5 and 6). The

first section reviews the pharmacogenomic implications for the patient's existing medications and their conditions. The conditions coded by the International Classification of Diseases, 10th Revision (ICD-10). The clinician provided the information about the patient's disease state and medication. Gene-drug interactions are organized alphabetically by drug name and categorized based on the potential impact on prescribing: consider alternatives, use with caution, and adjust dose/monitoring.
The second section summarizes pharmacogenomic information for approximately 100 commonly used medications across therapeutic areas. Drugs are grouped by class and mechanism of action. Guidance is provided on gene-drug effects and recommended prescribing considerations. The final section is pure pharmacogenomic information. The clinical results page displays the patient's gene, genotype as well as phenotype. The full gene list, along with phenotypes and genotypes, that were analyzed is available in Appendices 1.

2.3 Ethics and Regulatory

This study was submitted to the Institutional Review Board (IRB) under protocol #18113-01. This study adhered to ethical principles outlined in the Declaration of Helsinki and complied with the guidelines for Good Clinical Practice from the International Conference on Harmonization (ICH/GCP). All applicable regulatory requirements related to human subject research will be followed.

All patients were provided with written informed consent prior to study participation following ethical principles. Principal investigators ensured patients were given comprehensive oral and written information about the study purpose, possible risks and benefits, and their rights as participants. Patients had opportunities to ask questions

and time to consider the information before providing signed and dated informed consent agreeing to take part. Principal investigators maintained all original signed consent forms per site procedures. The approved consent materials included descriptions of any incentives or provisions for patients potentially harmed by the study. Patients were notified that they could voluntarily discontinue their participation at any point. Informed consent procedures protected patient rights and adhered to ethical guidelines for human subject research.

In terms of risk, the main factor is the loss of privacy and confidentiality. Patient data will be used with the utmost care. Confidentiality was ensured through several measures. All computers required individual logins and full disk encryption, maintained by information technology (IT) staff. Servers storing study data were secured with routine backup procedures. Physical hard copies were stored in locked filing cabinets in secured rooms, only accessible to study personnel. Patient data was anonymized by using a unique numeric code for each patient. The code key was not stored on any computer or information network but in secure rooms. No individually identifiable information was contained in patient codes.

Due to the study population's expected deterioration, serious adverse events may occur (SAEs). These were defined as unexpected adverse outcomes such as hospitalizations, disability, or death that are not part of normal disease progression. Any SAEs assessed by clinicians potentially related to study procedures would be reported as Suspected Unexpected Serious Adverse Reactions (SUSARs) to oversight bodies. All adverse events were monitored, including undesirable symptoms or deteriorating conditions, regardless of causality. All unexpected and related adverse events were

documented in the participant's records and reviewed following research guidelines.

2.4 Study Design

Patients and clinicians were recruited from a single healthcare system to eliminate variability within healthcare systems and protocols.

Clinicians were eligible to be recruited for the study if they were physicians or nurse practitioners involved in practicing hospice and palliative care and were responsible for making and implementing decisions about opioid therapy for pain. Clinicians were identified by the investigators and were provided IRB-approved signed informed consent. The clinicians recruited for the study helped to identify potential candidates. The inclusion criteria for patients are indicated in Table 1, and the exclusion criteria are detailed in Table 2. There was no exclusion criteria for clinicians. They were recruited only if they met the inclusion criteria.

Research personnel themselves explained the study to patients who consented to receive information. Patients who consented to the study had three study visits. The first visit was a baseline visit. At the patient's baseline visit, eligibility and inclusion criteria were established. Patients identified as appropriate for the study and agreed to participate signed Informed Consent forms. Signing of consent forms was done prior to the completion of any study procedures. During the baseline visit, patients enrolled in the study completed a basic questionnaire and provided a cheek swab for pharmacogenomic analysis. Other data included recording the PPS score, NRS scale measurements, and a review of medications (opioid and non-opioid) were all recorded. The cheek swabs were shipped to Admera Health to undergo a PGxOne™ Plus assay.

The turn-around for the PGxOne™ Plus assay was available for review by the

investigator's clinical pharmacist 72 h after receiving a patient sample. The PGxOne™ report was sent to the patient's treatment team with suggested recommendations (based on pharmacogenomic analysis) between 24 – 48 h after review by the investigator's clinical pharmacist.

Visit two occurred between 5 and 14 days and was conducted either by phone or by an in-person clinic visit. Changes to medications were solely at the clinician's discretion and reviewed once more. During this evaluation, another NRS scale was given. The PPS score was only given once as recruitment criteria. Any adverse events that were present were collected and reviewed.

Visit 3 occurred on day 28, within a margin of +/- 7 days. This visit was to collect follow-up questions and re-evaluate pain therapy. Clinicians in charge of the patients could intervene or make any modifications during the study. A final NRS scale and a concluding NOSE scale were given to the patient. Medications and adverse events were given a final review. Patients were also given follow-up questionnaires about their emotional state and feelings, and pain therapy was re-evaluated. This final visit would conclude study participation.

2.5 Database Construction

In order to properly statistically analyze the data, all patient information was compiled in a database using Excel 2022 (Microsoft Software, Redmond, WA). This database was constructed using compiled anonymized data for 18 patients receiving hospice and palliative care. Variables extracted from medical records and rating instruments included: demographics (gender, age, ethnicity), clinical status (Palliative Performance Scale (PPS) score, primary/comorbid diagnoses, substance abuse disorders), and medications (opioids, non-opioid analgesics, other medications and morphine equivalent doses). Average and maximum pain were based on NRS measurements

provided by the patients during the three visits. In addition to patient records, pharmacogenomic data was compiled from each patient report provided by Admera Health.

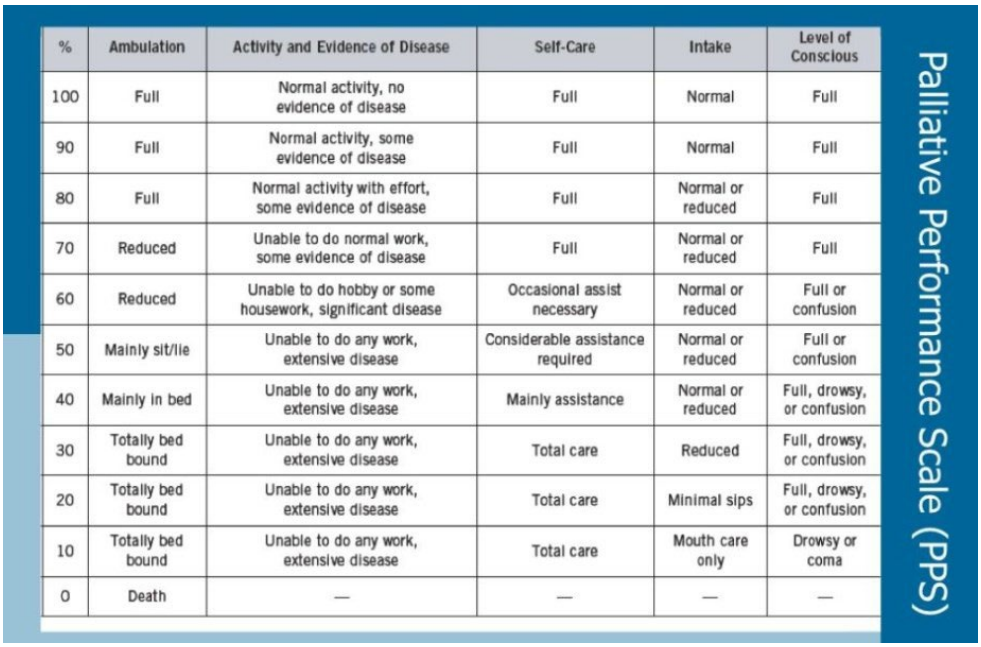
Categorical variables were coded numerically in order to facilitate statistical analysis. For example, gender was coded as 1 = male and 2 = female. In total, about 61 variables across the categories of demographics, clinical status, medications, symptoms, pharmacogenomics, and other related factors. These variables were carefully selected to provide a comprehensive overview of the factors that might influence the efficacy of opioids. In addition to the compiled database, separate data sheets were created for each of the 50 genes analyzed over each of the 18 patients for a more straightforward analysis.

2.6 Statistical Analysis

Minitab® 20.4 (Pennsylvania State University, PA) was used to analyze the patient database statistically. The primary statistical model used in order to analyze the database was the General Linear Model (GLM). This is because the dataset is a combination of multiple types of variables. The model contains continuous variables (e.g., age, PPS scores) and coded categorical factors (e.g., disease codes, gender). This model also provides more statistical power to detect effects than ANOVA models[96]. The GLM is also well-suited to handle group sizes that are not balanced, such as different variables having an uneven number of patients. The model also allows testing for interactions between variables (such as if the effect of a particular genotype differs by ethnicity). The F-value in the GLM evaluates which predictors are significantly associated with a variable. This allows the GLM to assess whether different predictors on a particular variable are statistically significant. This is paramount for determining which predictors are essential for understanding pain levels. Calculations of the regression equations used can be found in (Appendices 1).

Individual predictors with p-values < 0.05 were considered statistically significant. Tukey's HSD post hoc test was utilized when the GLM detected significant effects. F-value indicates the ratio of model improvement over error, with higher values indicating more variance

explained by model predictors. F-values exceeding critical values based on the degrees of freedom provide evidence of an overall significant model beyond chance.



%	Ambulation	Activity and Evidence of Disease	Self-Care	Intake	Level of Conscious
100	Full	Normal activity, no evidence of disease	Full	Normal	Full
90	Full	Normal activity, some evidence of disease	Full	Normal	Full
80	Full	Normal activity with effort, some evidence of disease	Full	Normal or reduced	Full
70	Reduced	Unable to do normal work, some evidence of disease	Full	Normal or reduced	Full
60	Reduced	Unable to do hobby or some housework, significant disease	Occasional assist necessary	Normal or reduced	Full or confusion
50	Mainly sit/lie	Unable to do any work, extensive disease	Considerable assistance required	Normal or reduced	Full or confusion
40	Mainly in bed	Unable to do any work, extensive disease	Mainly assistance	Normal or reduced	Full, drowsy, or confusion
30	Totally bed bound	Unable to do any work, extensive disease	Total care	Reduced	Full, drowsy, or confusion
20	Totally bed bound	Unable to do any work, extensive disease	Total care	Minimal sips	Full, drowsy, or confusion
10	Totally bed bound	Unable to do any work, extensive disease	Total care	Mouth care only	Drowsy or coma
0	Death	—	—	—	—

Figure 1: Palliative Performance Scale. The 11-point Palliative Performance Scale (PPS) used to assess patient functional status and prognosis at baseline and throughout the study. Patients were required to have a PPS of at least 30% for study inclusion[97].

Patients will be asked to grade their average and worst level of pain in the previous 24 hours due to the primary diagnosis at the end of each day.

"Using a scale of "zero" to "ten", where zero equals no pain and ten equals the worst pain you can imagine, how would you rate the severity of pain, on average during the past 24 hours?

0_____1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None *Worst Possible Pain*

"Using a scale of "zero" to "ten", where zero equals no pain and ten equals the worst pain you can imagine, how would you rate the severity of your pain at its worst during the past 24 hours?"

0_____1_____2_____3_____4_____5_____6_____7_____8_____9_____10

None *Worst Possible Pain*

Figure 2: **Numerical Rating Scale for Pain.** The NRS was used to assess the pain of patients during visitations[93]

	Not Present										As Bad as You Can Imagine
	0	1	2	3	4	5	6	7	8	9	10
1. Nausea, vomiting, and/or lack of appetite	O	O	O	O	O	O	O	O	O	O	O
2. Fatigue, sleepiness, trouble concentrating, hallucinations, and/or drowsiness/somnolence	O	O	O	O	O	O	O	O	O	O	O
3. Constipation	O	O	O	O	O	O	O	O	O	O	O
4. Itching	O	O	O	O	O	O	O	O	O	O	O
5. Decreased sexual desire/function an/or diminished libido	O	O	O	O	O	O	O	O	O	O	O
6. Dry mouth	O	O	O	O	O	O	O	O	O	O	O
7. Abdominal pain or discomfort/cramping or bloating	O	O	O	O	O	O	O	O	O	O	O
8. Sweating	O	O	O	O	O	O	O	O	O	O	O
9. Headache and/or dizziness	O	O	O	O	O	O	O	O	O	O	O
10. Urinary retention	O	O	O	O	O	O	O	O	O	O	O

Figure 3: Numerical Opioid Side Effect Scale. Patients completed the NOSE during required visits, rating the severity of the following side effects: nausea, vomiting, drowsiness, tiredness, dizziness, itching, constipation, dry mouth, headache, sleep problems, sweating, and confusion[94].

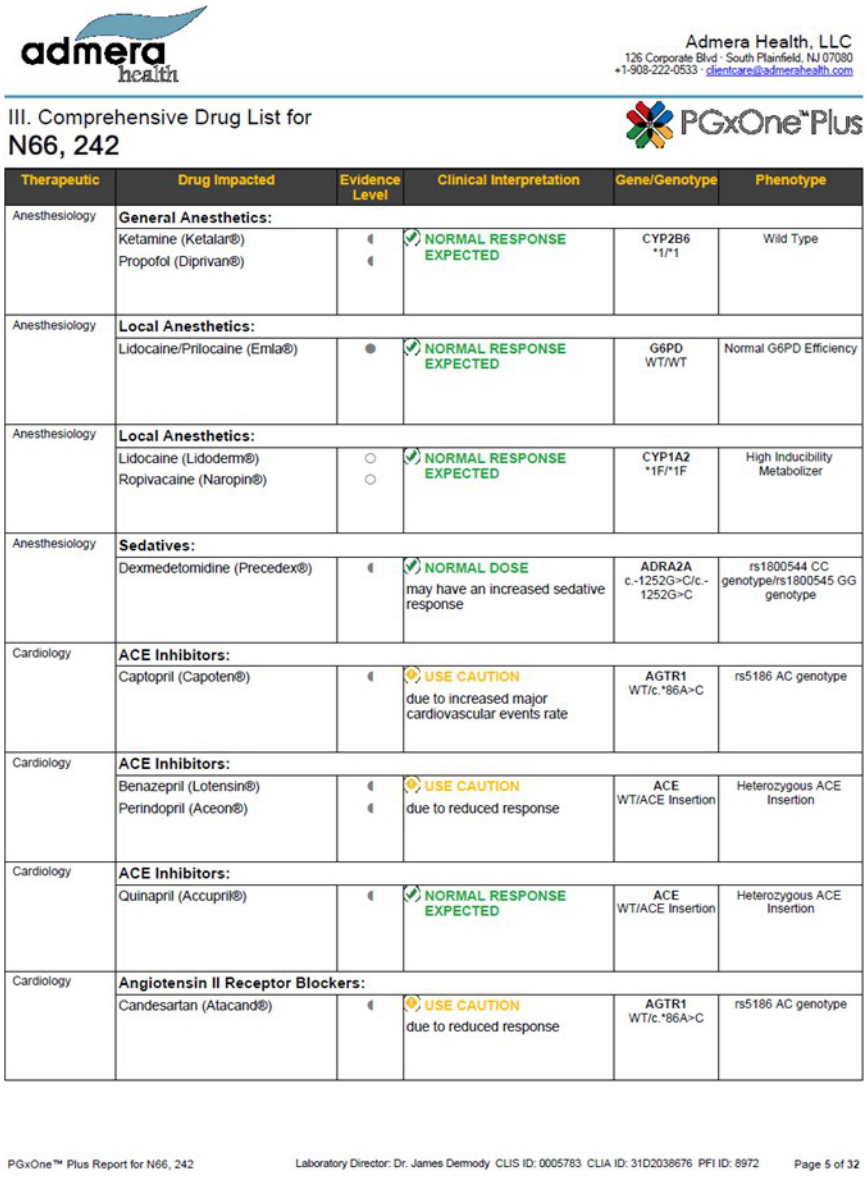
Calculating Morphine Milligram Equivalents (MME)*			
Opioid	**Conversion Factor** (convert to MMEs)	**Duration (hours)**	**Dose Equivalent Morphine Sulfate (30mg)**
Codeine	0.15	4-6	200 mg
Fentanyl (MCG/hr)	2.4		12.5 mcg/hr**
Hydrocodone	1	3-6	30 mg
Hydromorphone	4	4-5	7.5 mg
Morphine	1	3-6	30 mg
Oxycodone	1.5	4-6	20 mg
Oxymorphone	3	3-6	10 mg
Methadone†			
1-20 mg/d	4		7.5 mg
21-40 mg/d	8		3.75 mg
41-60 mg/d	10		3 mg
≥61 mg/d	12		2.5 mg

**The dose conversions listed above are an estimate and cannot account for an individual patient's genetics and pharmacokinetics.*

***Fentanyl is dosed in mcg/hr instead of mg/day, and absorption is affected by heat and other factors.*

†Methadone conversion factors increase with increasing dose.

Figure 4: Dose Conversion Table for MME. This is the conversion methodology used to convert various opioid doses into MME. It is in accordance with the CDC guidelines of 2016[95].



admera health

Admera Health, LLC
126 Corporate Blvd · South Plainfield, NJ 07080
+1-908-222-0533 · clientcare@admerahealth.com

III. Comprehensive Drug List for
N66, 242

PGxOne™ Plus

Therapeutic	Drug Impacted	Evidence Level	Clinical Interpretation	Gene/Genotype	Phenotype
Anesthesiology	**General Anesthetics:**				
	Ketamine (Ketalar®) Propofol (Diprivan®)	◖ ◖	✓ NORMAL RESPONSE EXPECTED	CYP2B6 *1/*1	Wild Type
Anesthesiology	**Local Anesthetics:**				
	Lidocaine/Prilocaine (Emla®)	●	✓ NORMAL RESPONSE EXPECTED	G6PD WT/WT	Normal G6PD Efficiency
Anesthesiology	**Local Anesthetics:**				
	Lidocaine (Lidoderm®) Ropivacaine (Naropin®)	○ ○	✓ NORMAL RESPONSE EXPECTED	CYP1A2 *1F/*1F	High Inducibility Metabolizer
Anesthesiology	**Sedatives:**				
	Dexmedetomidine (Precedex®)	◖	✓ NORMAL DOSE may have an increased sedative response	ADRA2A c.-1252G>C/c.-1252G>C	rs1800544 CC genotype/rs1800545 GG genotype
Cardiology	**ACE Inhibitors:**				
	Captopril (Capoten®)	◖	! USE CAUTION due to increased major cardiovascular events rate	AGTR1 WT/c.*86A>C	rs5186 AC genotype
Cardiology	**ACE Inhibitors:**				
	Benazepril (Lotensin®) Perindopril (Aceon®)	◖ ◖	! USE CAUTION due to reduced response	ACE WT/ACE Insertion	Heterozygous ACE Insertion
Cardiology	**ACE Inhibitors:**				
	Quinapril (Accupril®)	◖	✓ NORMAL RESPONSE EXPECTED	ACE WT/ACE Insertion	Heterozygous ACE Insertion
Cardiology	**Angiotensin II Receptor Blockers:**				
	Candesartan (Atacand®)	◖	! USE CAUTION due to reduced response	AGTR1 WT/c.*86A>C	rs5186 AC genotype

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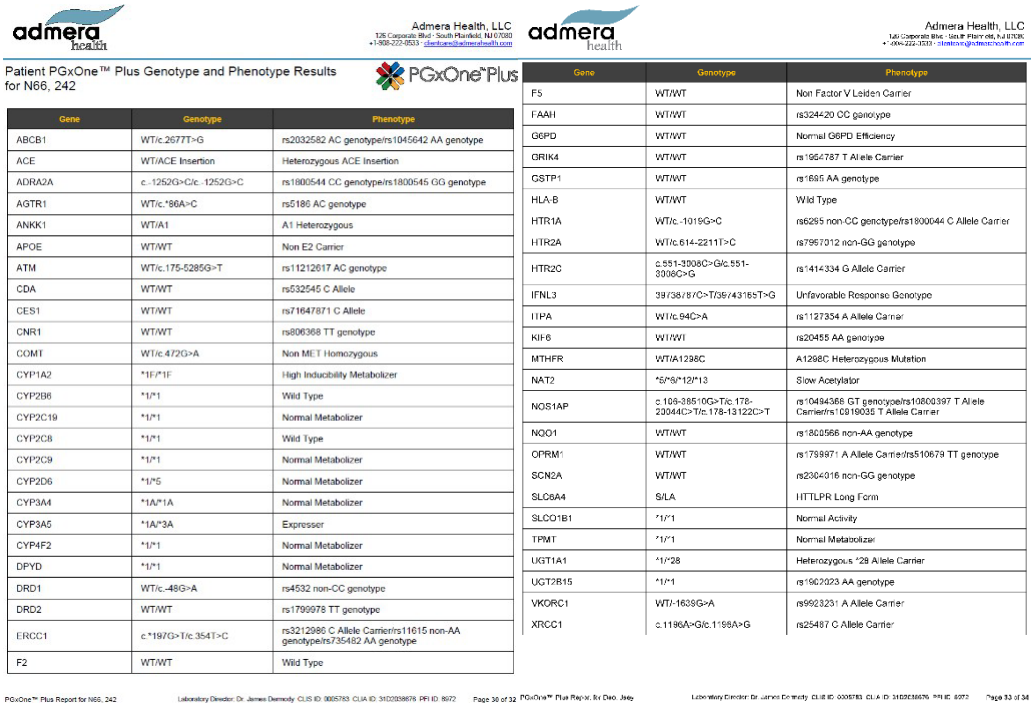
Figure 5: Patient Pharmacogenomic Report. An example of a patient's pharmacogenomic report. The patient's name is coded as a number to protect their privacy.



Admera Health, LLC
125 Corporate Blvd · South Plainfield, NJ 07080
+1-908-222-0533 · clientcare@admerahealth.com

Patient PGxOne™ Plus Genotype and Phenotype Results for N66, 242

PGxOne™ Plus

Gene	Genotype	Phenotype
ABCB1	WT/c.2677T>G	rs2032582 AC genotype/rs1045642 AA genotype
ACE	WT/ACE Insertion	Heterozygous ACE Insertion
ADRA2A	c.-1252G>C/c.-1252G>C	rs1800544 CC genotype/rs1800545 GG genotype
AGTR1	WT/c.*86A>C	rs5186 AC genotype
ANKK1	WT/A1	A1 Heterozygous
APOE	WT/WT	Non E2 Carrier
ATM	WT/c.175-5285G>T	rs11212617 AC genotype
CDA	WT/WT	rs532545 C Allele
CES1	WT/WT	rs71647871 C Allele
CNR1	WT/WT	rs806368 TT genotype
COMT	WT/c.472G>A	Non MET Homozygous
CYP1A2	*1F/*1F	High Inducibility Metabolizer
CYP2B6	*1/*1	Wild Type
CYP2C19	*1/*1	Normal Metabolizer
CYP2C8	*1/*1	Wild Type
CYP2C9	*1/*1	Normal Metabolizer
CYP2D6	*1/*5	Normal Metabolizer
CYP3A4	*1A/*1A	Normal Metabolizer
CYP3A5	*1A/*3A	Expresser
CYP4F2	*1/*1	Normal Metabolizer
DPYD	*1/*1	Normal Metabolizer
DRD1	WT/c.-48G>A	rs4532 non-CC genotype
DRD2	WT/WT	rs1799978 TT genotype
ERCC1	c.*197G>T/c.354T>C	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 AA genotype
F2	WT/WT	Wild Type

Gene	Genotype	Phenotype
F5	WT/WT	Non Factor V Leiden Carrier
FAAH	WT/WT	rs324420 CC genotype
G6PD	WT/WT	Normal G6PD Efficiency
GRIK4	WT/WT	rs1954787 T Allele Carrier
GSTP1	WT/WT	rs1695 AA genotype
HLA-B	WT/WT	Wild Type
HTR1A	WT/c.-1019G>C	rs6295 non-CC genotype/rs1800044 C Allele Carrier
HTR2A	WT/c.614-2211T>C	rs7997012 non-GG genotype
HTR2C	c.551-3008C>G/c.551-3008C>G	rs1414334 G Allele Carrier
IFNL3	39738787C>T/39743165T>G	Unfavorable Response Genotype
ITPA	WT/c.94C>A	rs1127354 A Allele Carrier
KIF6	WT/WT	rs20455 AA genotype
MTHFR	WT/A1298C	A1298C Heterozygous Mutation
NAT2	*5/*5/*12/*13	Slow Acetylator
NOS1AP	c.159-35510G>T/c.178-20044C>T/c.178-13122C>T	rs10494366 GT genotype/rs10800397 T Allele Carrier/rs7919035 T Allele Carrier
NQO1	WT/WT	rs1800566 non-AA genotype
OPRM1	WT/WT	rs1799971 A Allele Carrier/rs510679 TT genotype
SCN2A	WT/WT	rs2304016 non-GG genotype
SLC6A4	S/LA	HTTLPR Long Form
SLCO1B1	*1/*1	Normal Activity
TPMT	*1/*1	Normal Metabolizer
UGT1A1	*1/*28	Heterozygous *28 Allele Carrier
UGT2B15	*1/*1	rs1902023 AA genotype
VKORC1	WT/-1639G>A	rs9923231 A Allele Carrier
XRCC1	c.1196A>G/c.1196A>G	rs25487 C Allele Carrier

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PGxOne™ Plus Report, Rx Dep. Joby Laboratory Director: Dr. James Dermody CLIS ID: 0005783 CLIA ID: 31D2038676 PFI ID: 8972 Page 33 of 34

Figure 6: Patient Report for All Genes. An example of a patient's pharmacogenomic report. The patient's name is coded as a number to protect their privacy. It shows their genotype and phenotype combination for target genes,

Table 1: Inclusion Criteria

Criteria	Description
Age	18 years of age or older
PPS	PPS ≥ 30% (see Appendix I)
Residence	Reside in the community or the inpatient residence
Serious chronic illness	Have >1 serious chronic illness
Life-expectancy	Have an assessed life-expectancy of at least 4 weeks
History of pain	Have a history of pain
Opioid medication	Have been taking prescribed opioid medication with a regimen that provides a minimum dose of 20 mg morphine or equivalent per day in scheduled doses for at least one week prior to recruitment
Opioid therapy	Is expected to continue opioid therapy for the duration of study participation
Informed consent	Able to read, understand, and provide Informed Consent to participate

Table 2: Patient Exclusion Criteria

Criteria	Description
Pregnancy/nursing	Pregnant, trying to conceive, or nursing. Women of childbearing potential must use reliable contraception.
PPS score	< 30%
Investigational agents	Taken any within the past 30 days
Substance use	Current illicit substance use
Site Staff	Immediate family or staff unless IRB approved
Medical conditions	Any that could prohibit study completion per investigator

CHAPTER 3. RESULTS

3.1 Demographic Data

Our patient sample was composed of 18 patients, with 38.89% (n=7) female and 61.11% (n=11) male participants (Table 3). In the present study, the patient pool comprised a diverse ethnic background, including 27.78% African American (n=5), 5.56% Asian (n=1), 27.78% Hispanic (n=5), and 38.89% Caucasian (n=7) hospice and palliative care patients. The age of the participants spanned a wide range, from 37 to 84 years, with a modal age of 64 years, constituting 27.78% of the sample (n=5).

Furthermore, addiction and substance abuse factors were also evaluated: 55.56% of the participants were smokers (n=10), and 38.89% had a documented history of substance abuse disorder. Our study involved patients diagnosed with multiple chronic and terminal conditions. These included alcoholic liver cirrhosis, chronic obstructive pulmonary disease (COPD), several conditions co-morbid with HIV, end-stage congestive heart failure (CHF), fibrosis of the lung, and multiple types of cancer (Table 4). The most common medical condition among the participants was COPD at 27.78% (n=5).

Patients recruited for the study were on at least one opioid, which included morphine, oxycodone, tramadol, fentanyl, hydromorphone, and methadone. In order to equalize the amounts of morphine used per patient, a calculated morphine dose was used based on CDC guidelines. The calculated morphine doses showed a wide variation across the sample, from 20 mg at 5.56% (n=1) up to 540 mg at 11.11% (n=2), indicating the differential opioid response and tolerance among the participants (Table 5).

3.2 Descriptive Statistics

For this study, descriptive statistics were analyzed for basic demographics, the main

variables (average pain, maximum pain, calculated morphine dose, and PPS%), opioid side effects, and drug interactions. These were conducted in addition to regression analysis and the GLM.

3.2.1 Descriptive Statistics of Demographics

Gender was coded with 1 and 2 (1= male, 2= female). The mean was 1.389 with a standard error (SE) of 0.118. The standard deviation (SD) of 0.502 indicates a near-even distribution of male and female participants in the study. The age range of patients was 37 to 84 years, with a mean age of 63 years (SE=3.02), a median age of 64 years, and an SD of 12.79 years. Most of the study participants were older adults, with the age distribution being slightly negatively skewed, indicating a relatively uniform age distribution with fewer outliers. Ethnicity was coded from 1 to 4 (1 = Caucasian, 2 = Hispanic, 3 = African American, 4 = Asian). While skewness and kurtosis were calculated (0.43 and -0.96, respectively), these measures are generally more interpretable for continuous variables and may not provide meaningful insights into the distribution of categorical variables such as ethnicity.

Due to the patients having multiple chronic conditions, a primary disease state was assigned to each of the 18 study participants based on the organ affected or whether it was cancer. They were coded as follows: 1 = disease of the lung, 2 = cancer (any type), 3= disease of the heart, and 4 = liver disease. Cancer was indicated as the primary disease if any conditions were co-morbid with cancer. The mean value for the primary disease code was close to cancer at 1.889 (SE=0.227). Given that each disease was coded from 1 to 4, this mean value indicates that most patients fell within the first two categories (lung disease and cancer). The distribution of diseases was slightly skewed to the right at 1.13,

suggesting that a more significant proportion of patients in the sample had diagnoses coded lower (i.e., lung disease and cancer). However, there were still substantial numbers of patients with heart disease and Liver Disease, as shown by the range and the median value of 2 (which lies between Cancer and Heart Disease). The kurtosis value of 0.83 indicates a slightly more peaked and heavy-tailed distribution than a normal distribution. This value suggests that there are more frequent extreme deviations from the mean in our dataset, which could imply a higher likelihood of observing certain diseases over others in our sample.

3.2.2 Descriptive Statistics of Main Variables

The mean Palliative Performance Scale (PPS) score was 42.22 (SE = 1.73), with scores ranging from 30 to 60 (Table 6). This wide range underscores the heterogeneity in patient conditions within hospice and palliative care settings. The distribution of these scores was slightly right-skewed (skewness = 0.63), suggesting a tendency towards PPS scores. Furthermore, the mean square successive difference (MSSD) of 35.29 indicates substantial variability in patient conditions throughout the study.

Regarding pain management, the mean average pain score was 5.83 (SE = 0.513), with a standard deviation of 2.176. This moderate spread in pain scores across the study population implies varying levels of pain intensity among participants. Moreover, the mean for maximum reported pain scores were significantly higher at 7.66 (SE = 0.577), highlighting that patients often experience acute episodes of severe pain that exceed their average levels.

In our sample, MME was 187.7, with a considerable standard deviation of 194.8, emphasizing the high variability in dosing among participants. This was further

highlighted by the broad range of MMEs, which ranged from 20 to 540 mg. The skewness of 1.9 and a significant mean square successive difference (MSSD) of 45,216.6 indicate a tendency toward higher dosages, with several outliers and significant fluctuations between different MME values.

3.2.3 Descriptive Statistics for Opioid Side Effects

The evaluation of opioid side effects was measured using the NOSE scale for patients (Table 7). Contrary to conventional wisdom that nausea is a common adverse effect of opioid use, our findings suggest otherwise. Most patients reported no nausea; the mean score was relatively low at 2.167. The data exhibited positive skewness and a high level of kurtosis, indicating that extreme nausea cases were rare but existed.

Fatigue, with a mean score of 2.778, appeared less of a problem for a substantial portion of the patient sample, as evidenced by a median and mode of zero. The negative kurtosis suggests that extreme fatigue is even less common, corroborating the general low-to-moderate impact of this side effect.

However, constipation and dry mouth were the most severe side effects. The former had a high mean score of 4.222 with considerable variance, while the latter's mean score was an even higher 4.778. Both side effects showed significant inter-individual variability, stressing the need for personalized intervention strategies.

Itching and abdominal pain were generally moderate but displayed high inter-individual variability, as evidenced by their standard deviations. In contrast, sweating was the least frequently reported side effect, with the highest score only reaching 5 out of 10 on the NOSE scale. This suggests that sweating may be of lesser concern in this patient population.

Headaches and urinary retention presented with wide dispersion from their means, suggesting that while these side effects are not universally experienced, they can be severe when they do occur.

The standard deviations and skewness of side-effect profiles point to high inter-individual variability, while the mean scores indicate which side effects are most pressing on a population level. This suggests that while nausea and fatigue may not be as significant as traditionally believed, attention should be given to constipation and dry mouth, among others, for targeted interventions.

3.2.4 Descriptive Statistics for Medication Interactions

In addition to side effects, demographics, and main variables, drug-drug interactions and drug-opioid interactions were analyzed (Table 8). On average, there were less than 1 (0.889) possible drug interactions per participant, with a standard deviation of 1.132, indicating variability in this measure. MSSD was 1.353, demonstrating the variability of possible drug interactions across subjects or over time.

Further, the average number of severe drug interactions was significantly lower at 0.278 per participant. The skewness of 1.08 and a negative kurtosis of -0.94 suggest that most participants had no severe drug interactions. The average number of drugs that did not interact was around 2.667 per participant, with a SD of 1.328.

This showed a considerable spread. There was a slight negative skewness and kurtosis, indicating the distribution was slightly skewed to the left, indicating fewer extreme values. The average number of drug interactions affection opioids was around 1 per participant. A high skewness of 1.86 and high kurtosis of 4.29 show that many study participants had few interactions.

Lastly, the study's total number of drugs per patient averaged more than 3 per participant. While this may seem small, consideration must be taken that the majority of patients were for end-of-life-care, which prioritizes quality of life and treatment is generally ceased. There was an SD of 1.689, which shows a large variability in this measurement. For patients, the total number of drugs presented with a low skewness of 0.2 and kurtosis of 0.42, suggesting a symmetrical distribution similar to a normal distribution.

3.3 Non-Genomic Statistical Analysis

The four main variables of interest (average pain, maximum pain, MME, and PPS%) were compared to demographic variables. Ethnicity, gender, substance abuse, smoking, and disease state were not significant to any of the four main variables. However, age was correlated with morphine dose (Table 9). A simple linear regression was conducted to discover the relationship between age and the MME (Figure 7). This indicates that the calculated MME decreases by approximately 8.20 mg for each additional year of age.

This finding is statistically significant but accounts for only 25% of the dose variability when adjusted for model complexity, thereby indicating that while age is a notable factor in morphine dosing, it does not fully account for the variation in dosing. Age significantly predicted morphine dose ($F(1,16) = 6.54$, $p = 0.021$). Age explained 29.01% of the variance in MME dosing (R-squared = .29). The adjusted R-squared of .246 indicates that approximately 25% of the variance in dose can be accounted for by age after adjusting for model complexity.

In terms of opioid-related side effects, demographics were analyzed, and it was found that gender, ethnicity, and age had several significant associations (Table 10). The table

includes effects of factors like gender, ethnicity, and age on various side effects such as constipation, dry mouth, abdominal pain, urinary retention, sweating, and itching.

The statistical analysis of patient data revealed intricate relationships between demographic variables and the morphine dose and its associated side effects. While variables like ethnicity, gender, substance abuse, smoking, and disease state showed no significant impact on morphine dose, they manifest associations with various side effects.

Gender significantly influenced the incidence of constipation, dry mouth, abdominal pain, and urinary retention, accounting for approximately 30-36% of their variability. The association between gender and these side effects raises the possibility that the pharmacodynamics of morphine may vary between genders, suggesting a need for gender-specific guidelines in managing these side effects.

Age demonstrated a strong relationship between the incidence of itching and sweating. This relationship explained more than 90% of their variability. It is uncommon to find high R-squared values in clinical studies. While it can indicate a strong correlation, it may also indicate overfitting of the model.

Unexpectedly, the MME had no significant influence over any of the expected side effects. The lack of association is notable because conventional wisdom would make one expect higher doses to have more severe or frequent side effects (Table 11). The lack of association between MME and side effects suggests other variables influence them, such as PGx related to opioid resistance or pharmacokinetic factors.

3.4 Genomic Statistical Analysis

Of the 50 genes analyzed, 24 demonstrated statistically significant associations across key variables (average pain, maximum pain, PPS%, and MME) and opioid side effects.

These associations did not extend to age, drug interactions, or gender. The genomic influence was primarily on pain perception and opioid response. However, one gene, IFNL3, emerged as significant concerning ethnicity (Table 12).

The GLM for the African American population was particularly strong, accounting for half the observed variance (48.08% R-sq) in the Favorable and Unfavorable response genotypes. The model was statistically significant ($F(1,6)=14.81$, $p= 0.001$). The positive coefficient for the Unfavorable Response Genotype implies that African Americans are likelier to exhibit this genotype.

In contrast, the model was statistically significant for the Caucasian population, but the lower percentage of the variance (23.44% R-sq), with an F-value of 4.90 and a p-value of 0.042. The coefficient for the unfavorable response genotype was negative, suggesting Caucasians are less likely to possess this phenotype.

It is worth noting that these findings align with previous research on ethnic distribution of the IFNL3 genotype[98]. This lends additional weight to our results and underscores the necessity for targeted, ethnicity-based approaches in PGx studies.

3.4.1 Average Pain

Regarding average pain, statistical significance was discovered across four genes: *AGTR1, HTR2A, SLCO1B1, and UGT2B15* (Table 13).

For *AGTR1*, we examined two variables: genotype (wild type WT/c.*86A>C and WT/WT) and phenotype (rs5186 AA genotype and rs5186 AC genotype). The GLM significantly affected genotype and phenotype ($F(1,16)=8.96$, $p=0.009$). These models explained about 36% of the variability in average pain levels with R-squared adj = 31.89%. Those with the WT/c.*86A>C and the SNP rs5186 AC reported higher average

pain levels than the WT/WT group.

In *HTR2A*, our GLM with three genotypes (c.614-2211T>C/c.614-2211T>C, WT/c.614-2211T>C, WT/WT) showed a significant effect of genotype on average pain ($F(2,15)=4.24$, $p=0.035$). This model accounted for about 28% of the variability in average pain levels. This results in the genotype WT/c.614-2211T>C being associated with a decrease in average pain compared to the wild type.

For *SLCO1B1*, the model demonstrated a significant effect of both genotype (*1/*1, *1/*5) and phenotype (Intermediate Activity, Normal Activity) on average pain levels ($F(1,16)=4.69$, $p=0.046$). The models explained that about 18% of the variability in average pain levels through an adjusted R-squared was 17.82%. In terms of genotype, this indicates that individuals with the *1/*1 genotype have a slight increase in pain over those with the *1/*5 genotype. For phenotype, it means that the intermediate activity phenotype will have a slightly lower average pain score when compared to the normal activity phenotype.

Lastly, in *UGT2B15*, we detected a significant effect of genotype (*1/*1, *1/*2,*2/*2) ($F(2,15)=5.19$, $p=0.019$) and phenotype (rs1902023 AA genotype, rs1902023 non- AA genotype) ($F(1,16)=9.52$, $p=0.007$) on average pain levels. These models explained about 33% and 33.39% of the variability in pain, respectively, through the adjusted R-squared. This means that the presence of the *1/*1 genotype is associated with an increase in average pain. In contrast, the presence of the *1/*2 genotype is associated with a decrease in average pain, and the 2*/2* genotype is associated with a slight decrease in pain. For phenotype, individuals with the AA genotype have a higher than average pain score, while individuals with the non-AA genotype have a lower

average pain score. The effect of these genes on average pain is summarized in Table 14.

3.4.2 Maximum Pain

For maximum pain, there was a significant association between several genes. These include: *COMT*, *CYP1A2*, *CYP2B6*, *DRD1*, *FAAH*, *MTHFR* and *SLCO1B1* (Table 15). For COMT, the GLM revealed significance in maximum pain on phenotype ($F(1,16) = 4.96$, $p = 0.041$). This model explained around 23.67% of the variance in maximum pain due to its R-squared value. Non-MET homozygous individuals had higher maximum pain scores by around 2.79 points on average.

Regarding *CYP1A2*, the GLM demonstrated that genotype significantly affected maximum pain ($F(4,13) = 8.37$, $p = 0.001$), with R-squared showing 72.03% of the variance on maximum pain. The different genotypes had varying influences on pain, with the *1A/*1F, *1C/*1F/*1F, and *1F/*1F genotypes increasing pain scores. In this case, the *1A allele represents the wildtype allele. *1C and *1F are variant alleles that can affect pharmacodynamics. Regarding phenotype, significance was found ($F(1, 16) = 5.19$, $p = 0.037$), with the model explaining 24.51% of the variance in maximum pain. The normal metabolizer group reported lower maximum pain than the high inducibility metabolizer group.

CYP2B6 was analyzed both on the level of genotype and phenotype. There was a significant difference in the max pain scores across the four genotypes ($F(3,14)=3.36$, $p=0.050$). The regression model explained 41.83% of the variance in the max pain scores, with an adjusted R-squared of 29.37%. For specific genotypes, patients with the A785G/A785G/G516T genotype experienced significantly less pain compared to those with the *1/*1 genotype. Conversely, those with the A785G/A785G/G516T/G516T

genotype experienced more pain than the Wild Type. In phenotypic analysis, there was a significant difference in max pain scores across phenotypes ($F(3,14)=3.36$, $p=0.050$). The GLM accounted for 41.83% of the variance in max pain scores based on the R-squared value. The G516T Heterozygous/A785G Homozygous genotype scored 4 points lower on maximum pain scores. The G516T Homozygous/A785G Homozygous variable had on average 3 points higher on maximum pain than the wild type. Conversely, individuals with the 'G516T Homozygous/A785G Homozygous' genotype had on average 3 points higher maximum pain scores compared to individuals with the Wild Type.

DRD1 showed significance in both genotype and phenotype concerning the maximum pain score. For genotype, it was significant ($F(2,15)=4.48$, $p=0.030$), with the model accounting for 37.37% of variance based on the R-squared. Compared to the c.-48G>A/c.-48G>A genotype, the WT/WT genotype showed lower maximum pain scores. In terms of phenotype, it significantly affected maximum pain ($F(1,16) = 8.00$, $p = 0.012$). The results indicated that the phenotype accounted for approximately 33.33% of the variation in max pain, with an adjusted R-squared value of 29.17%. The rs4532 non-CC genotype was associated with significantly higher maximum pain than the rs4532 CC genotype.

The *FAAH* genotype on maximum pain scores was significant ($F(2,15)=4.12$, $p=0.037$), with the model accounting for 35.48% of variance based on the R-squared. Compared to the c.385C>A/c.385C>A genotype, the WT/c.385C>A and WT/WT genotypes showed higher maximum pain overall. The phenotype was also significant ($F(2,15)=4.12$, $p=0.037$). The rs324420 CA and CC genotypes had higher maximum pain than the rs324420 AA genotype. The R-squared for the phenotypic analysis was the same

as for the genotype.

The GLM utilized for *MTHFR* indicated significance in both genotype and phenotype. In terms of genotype, there was significance found across the genotypes studied ($F(5,12)=4.33$, $p=0.017$). The model accounted for 64.35% of the variance, but that was lowered with an adjusted R-squared of 49.49%. Compared to the A1298C/A1298C genotype, the C677T/A1298C genotype was associated with lower max pain scores. No other genotypes differed significantly other than these. Regarding phenotype, the significance was the same as those in the genotype, along with the adjusted R-squared value. The C677T Heterozygous Mutation/A1298C Heterozygous Mutation phenotype was associated with lower maximum pain scores.

Lastly, the genotypes and phenotypes for the gene *SLCO1B1* were significant with maximum pain. Significance in the genotype was found ($F(1,16) = 5.64$, $p=0.030$). The model accounted for 26.08% of the variance in pain based on the R-squared value. Compared to the *1/*1 genotype, the *1/*5 genotype showed significantly lower maximum pain. The phenotype and the same R-squared value showed identical significance as the genotype model. The normal activity group reported higher maximum pain scores for phenotype than the intermediate activity group. The table summarizing genomic effects on maximum pain scores is in Table 16.

3.4.3 Morphine Milligram Equivalents

Three genes explicitly showed a relationship with MME dosages. These were *CDA*, *CNR1*, and *CYP1A2* (Table 17). All were analyzed through the GLM. *CDA* showed a statistically significant association between phenotype and the calculated morphine dosage ($F(1,16)=5.32$, $p=0.035$). The adjusted sum of squares (Adj SS) for the phenotype

was 160839, indicating that the *CDA* phenotype could explain a substantial proportion of the variance in morphine dosage. The model accounted for 24.94% of the variance in the calculated morphine dosage (R-squared=24.94%). Upon adjusting for the number of predictors, this value slightly decreased to 20.24% (R- squared adjusted=20.24%). The rs532545 C Allele was associated with an increase in the calculated morphine dose by 94.5 mg (p=0.035) relative to the rs532545 T Allele.

In *CNR1*, there was statistical significance between phenotypes ($F(1,16) = 5.61$, $p= 0.031$). The model accounted for approximately 25.98% of the variation in the calculated morphine based on the R-squared. The rs806368 non-TT genotype was associated with a decrease in the calculated morphine dosage by 102.3 mg ($p = 0.031$) compared to the rs806368 TT genotype.

Lastly, *CYP1A2* showed significance in the phenotype on the calculated morphine dose ($F(1,16) = 4.68$, $p = 0.046$). The model accounted for 22.61% of the variance in the calculated morphine dosage (R-squared = 22.61%). The high-inducibility metabolizer phenotype was associated with a 95.5-mg reduction in the calculated morphine dose ($p = 0.046$) compared to normal metabolizers. The genomic effects of these three genes on MME are detailed in Table 18.

3.4.4 Palliative Performance Scale

In terms of PPS, the GLM demonstrated that several genes showed significance in this area. These were *CYP4F2*, *HLA-B*, *NOS1AP*, and *UGT2B15* (Table 19). Only *CYP4F2* and *HLA*-B were significant in terms of both genotype and phenotype. Table 20 summarizes the association between genomic data and PPS% in its entirety.

For *CYP4F2*, in terms of genotype, a significant association was found between

them (F(2,15) = 4.56, p = 0.028). The R-squared showed that this model could explain 37.80% of the variance in PPS. Regarding specific genotypes, *1/*1 and *1/*3 genotypes associated with lower PPS% compared to the *3/*3 genotype. Regarding phenotype, the GLM also indicated significance (F(2,15) = 4.56, p = 0.028). Poor metabolizers showed a higher PPS% than those with other phenotypes.

In *HLA-B*, genotype, and phenotype exhibited the same significance (F(2,15) = 6.17, p = 0.011). The R-squared explains 45.12% of the variance in the GLM. However, the model was not robust enough to indicate specific phenotypes and genotypes and their association with PPS%. *NOS1AP* was the most complex model, with seven phenotypes. It was significant overall (F(6,11) = 3.73, p = 0.028), with the R-squared value explaining 67.07% of the variance in PPS%. Despite the many phenotypes analyzed, the only one significantly associated with PPS% was the rs10494366 TT genotype/rs10800397 T Allele Carrier/rs10919035 T Allele Carrier.

Lastly, the *UGT2B15* genotype was significantly associated with PPS% (F(2,15)= 6.64, p = 0.009), explaining 46.95% of the variance based on the R-squared. The *1/*2 genotype was associated with a significantly higher PPS% compared to the *1/*1 genotype.

Table 3: Patient Demographics

Gender	Count	Percentage	Age	Count	Percentage	Ethnicity	Count	Percentage
F	7	38.89%	37	1	5.56%	African American	5	27.78%
M	11	61.11%	46	1	5.56%	Asian	1	5.56%
			50	2	11.11%	Hispanic	5	27.78%
			54	1	5.56%	Caucasian	7	38.89%
			55	1	5.56%			
			60	1	5.56%			
			64	5	27.78%			
			68	1	5.56%			
			75	1	5.56%			
			77	1	5.56%			
			78	1	5.56%			
			80	1	5.56%			
			84	1	5.56%			
N =	18	100%	N =	18	100%	N =	18	100%

Table 4: Chronic Conditions in Cohort

Original Condition	Count	Percent
Alcoholic Liver Cirrhosis	1	5.56%
Cervical Cancer	1	5.56%
Colon Cancer	2	11.11%
COPD	5	27.78%
COPD/HIV	1	5.56%
End stage CHF	1	5.56%
Hepatitis C/Fibrosis of Lung	1	5.56%
Hepatitis C/HIV	1	5.56%
Lung Cancer	2	11.11%
Metastatic Adenocarcinoma of the Tongue	1	5.56%
Pancreatic Cancer	1	5.56%
Prostate Cancer	1	5.56%
N =	18	100%

Table 5: Morphine Milligram Equivalents

MME (mg)	Patients	Percent
20	1	5.56%
30	3	16.67%
37.5	1	5.56%
60	1	5.56%
75	1	5.56%
91	1	5.56%
115	1	5.56%
120	2	11.11%
135	1	5.56%
180	1	5.56%
225	1	5.56%
505	1	5.56%
525	1	5.56%
540	2	11.11%
N=	18	100%

Morphine milligram equivalents were calculated using Figure 4

Table 6: Descriptive Statistics – Main Variables

Variable	Mean	SEM	StDev	Var	SS	Min	Max	Med	Range	Mode	MSSD
PPS%	42.22	1.73	7.32	53.59	33000	30	60	40	30	40	35.29
Av.Pain	5.83	0.51	2.18	4.74	693	2	10	6	8	5, 6, 7	4.94
Max pain	7.67	0.58	2.45	6	1160	2	10	8	8	8	6.94
MME	187.70	45.90	194.80	37942	1279	20	540	117.5	520	30	45217

PPS% = palliative performance scale as percentage, MME = morphine milligram equivalents in mg, SEM = standard error of the mean, StDev = standard deviation, var = variance, SS = sum of squares, Med = median, MSSD = mean of the squared successive differences

Table 7: Descriptive Statistics – Opioid Side Effects

Variable	Mean	SEM	StDev	Var	SS	Min	Max	Med	Rng	Mode	MSSD
Nausea	2.17	0.72	3.07	9.44	245	0	10	1	10	0	12.97
Fatigue	2.78	0.65	2.76	7.60	268	0	9	2	9	0	8.21
Constipation	4.22	0.87	3.69	13.60	552	0	10	3.5	10	1	11.06
Itching	2.00	0.76	3.22	10.35	248	0	10	1	10	0	9.12
Dry mouth	4.78	0.95	4.04	16.30	688	0	10	5	10	0	11.59
Abd. Pain	3.39	0.88	3.74	14.02	445	0	10	2	10	0	9.82
Sweating	1.11	0.38	1.61	2.58	66	0	5	1	5	0	2.53
Headache	2.94	0.84	3.57	12.76	373	0	10	1	10	0	15.59
Urine Retention	3.06	0.81	3.44	11.82	369	0	10	1	10	0	7.44

SEM = standard error of the mean, StDev = standard deviation, var = variance, SS = sum of squares, Med = median, Rng=Range, MSSD = mean of the squared successive differences

Table 8: Descriptive Statistics – Medication Interactions

Variable	Mean	SEM	StDev	Var	SS	Min	Max	Rng	Mode	MSSD
Possible Interactions	0.89	0.27	1.13	1.28	36	0	4	4	0	1.35
Serious Interactions	0.28	0.11	0.46	0.21	5	0	1	1	0	0.27
Non-Interacting Drugs	2.67	0.31	1.33	1.77	158	0	5	5	3	1.56
Interactions with Opioids	1.11	0.30	1.28	1.63	50	0	5	5	1	1.74
Total Number of Drugs	3.17	0.40	1.69	2.85	229	0	7	7	4	2.06

SEM = standard error of the mean, StDev = standard deviation, Var = variance, Rng = range, SS = sum of squares, MSSD = mean of the squared successive differences

Table 9: Regression Analysis – Age vs MME

Source	DF	SS	MS	F	P
Regression	1	187145	187145	6.74	0.021
Error	16	457866	28617		
Total	17	645012			

DF = degrees of freedom, SS = sum of squares, MS = mean square, F = f-value, P = p value

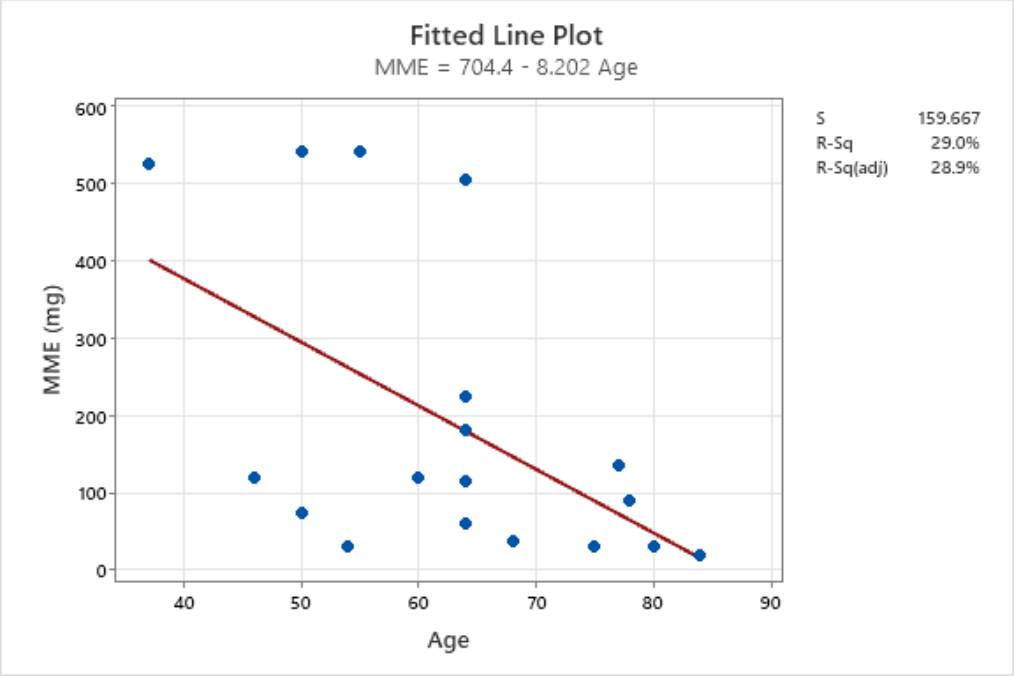

Figure 7: Fitted Line Plot – Age vs. MME. The graph here shows the regression fitted line, which shows the negative relation between Age and Calculated Morphine Dose. The adjusted R-squared is 24.6% mean that this percent of variation in the dependent variable (Cal. Morphine Dose) is explained by the independent variable (Age)

Table 10: Significant Morphine Side Effects Based on Demographic Information

Variable	Factor	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
Constipation	Gender	1	79.53	79.53	8.39	0.011	3.08	34.41%	30.31%
Dry mouth	Gender	1	89.40	89.40	7.62	0.014	3.43	32.26%	28.03%
Abd.pain	Gender	1	69.78	69.78	6.63	0.020	3.25	29.29%	24.87%
Urine Retention	Gender	1	72.50	72.50	9.03	0.008	2.83	36.08%	32.09%
Dry mouth	Ethnicity	3	139.90	46.62	4.75	0.017	3.13	50.47%	39.85%
Sweating	Age	12	42.08	3.51	10.31	0.009	0.58	96.12%	86.80%
Itching	Age	12	162.30	13.53	4.94	0.045	1.66	92.22%	73.53%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 11: MME vs. Opioid Side Effects

Variable	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
Dry mouth	13	227.94	17.53	1.43	0.40	3.51	82.26%	24.59%
Abd. pain	13	164.28	12.64	0.68	0.73	4.30	68.94%	0.00%
Sweating	13	26.61	2.05	0.48	0.86	2.07	60.79%	0.00%
Nausea	13	107.33	8.26	0.62	0.77	3.65	66.87%	0.00%
Fatigue	13	93.94	7.23	0.82	0.65	2.97	72.76%	0.00%
Constipation	13	180.61	13.89	1.10	0.51	3.55	78.15%	7.13%

MME = morphine milligram equivalents, DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 12: IFNL3 – Ethnicity and Phenotype

Ethnicity	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
Caucasian	1	1.00	1.00	4.90	0.042	0.45	23.44%	18.66%
African American	1	1.74	1.74	14.81	0.001	0.34	48.08%	44.83%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 13: Average Pain vs. Genomic Data

Gene	Variable	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
AGTR1	Genotype	1	28.90	28.90	8.96	0.009	1.80	35.90%	31.89%
AGTR1	Phenotype	1	28.90	28.90	8.96	0.009	1.80	35.90%	31.89%
HTR2A	Genotype	2	29.07	14.53	4.24	0.035	1.85	36.11%	27.59%
SLCO1B1	Genotype	1	18.24	18.24	4.69	0.046	1.97	22.66%	17.82%
SLCO1B1	Phenotype	1	18.24	18.24	4.69	0.046	1.97	22.66%	17.82%
UGT2B15	Genotype	2	32.92	16.46	5.19	0.019	1.78	40.89%	33.01%
UGT2B15	Phenotype	1	30.04	30.04	9.52	0.007	1.78	37.31%	33.39%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 14: Average Pain Genomic Summary

Gene	Factor	Polymorphism	Effect	P
AGTR	Genotype	*WT/c.86A>C	+1.70	0.009
	Phenotype	rs5186 AA genotype	-1.70	0.009
HTR2A	Genotype	WT/c.614-2211T>C	-2.01	0.011
SLCO1B1	Genotype	*1/*1	+1.03	0.046
	Phenotype	Intermediate Activity	-1.03	0.046
UGT2B15	Genotype	*1/*1	+2.03	0.019
		*1/*2	-1.47	0.019
	Phenotype	rs1902023 AA genotype	+1.55	0.007

The effect refers to the outcome derived from the statistical analysis and its impact on the baseline pain score. A negative result signifies a reduction in pain level due to the specific polymorphism, whereas a positive value indicates an elevation in pain score. The P-value shows the statistical significance of each effect.

Table 15: Max Pain vs. Genomic Data

Gene	Factor	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
COMT	Phenotype	1	24.14	24.14	4.96	0.041	2.21	23.67%	18.90%
CYP1A2	Genotype	4	73.47	18.37	8.37	0.001	1.48	72.03%	63.42%
CYP1A2	Phenotype	1	25.00	25.00	5.19	0.037	2.19	24.51%	19.79%
CYP2B6	Genotype	3	42.67	14.22	3.36	0.050	2.06	41.83%	29.37%
CYP2B6	Phenotype	3	42.67	14.22	3.36	0.050	2.06	41.83%	29.37%
DRD1	Genotype	2	38.12	19.06	4.48	0.030	2.06	37.37%	29.02%
DRD1	Phenotype	1	34.00	34.00	8.00	0.012	2.06	33.33%	29.17%
FAAH	Genotype	2	36.19	18.09	4.12	0.037	2.09	35.48%	26.87%
FAAH	Phenotype	2	36.19	18.09	4.12	0.037	2.09	35.48%	26.87%
MTHFR	Genotype	5	65.63	13.13	4.33	0.017	1.74	64.35%	49.49%
MTHFR	Phenotype	5	65.63	13.13	4.33	0.017	1.74	64.35%	49.49%
SLCO1B1	Genotype	1	26.60	26.60	5.64	0.030	2.17	26.08%	21.46%
SLCO1B1	Phenotype	1	26.60	26.60	5.64	0.030	2.17	26.08%	21.46%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 16: Max Pain Genomic Summary

Gene	Factor	Polymorphism	Effect	P
COMT	Phenotype	MET Homozygous	-1.39	0.041
CYP1A2	Genotype	*1A/*1A	-2.75	0.004
		*1C/*1F/*1F	+1.65	0.027
	Phenotype	High Inducibility Metabolizer	+1.25	0.037
CYP2B6	Genotype	A785G/A785G/G516T	-4.00	0.026
		A785G/A785G/G516T/G516T	+3.00	0.029
	Phenotype	G516T Heterozygous/A785G Homozygous	-4.00	0.026
		G516T Homozygous/A785G Homozygous	+3.00	0.029
DRD1	Genotype	WT/c.-48G>A	+2.57	0.013
	Phenotype	rs4532 CC genotype	-3.00	0.012
FAAH	Genotype	c.385C>A/c.385C>A	-3.96	0.015
		WT/c.385C>A	+2.34	0.016
	Phenotype	rs324420 AA genotype	-3.96	0.015
		rs324420 CA genotype	+2.34	0.016
MTHFR	Genotype	C677T/A1298C	-4.99	0.001
	Phenotype	C677T Heterozygous Mutation/ A1298C Heterozygous Mutation	-4.99	0.001
SLCO1B	Genotype	*1/*1	+1.25	0.030
	Phenotype	Intermediate Activity	-1.25	0.030

The effect refers to the outcome derived from the statistical analysis and its impact on the baseline pain score. A negative result signifies a reduction in pain level due to the specific polymorphism, whereas a positive value indicates an elevation in pain score. The P-value shows the statistical significance of each effect.

Table 17: Morphine Milligram Equivalents vs. Genomic Data

Gene	Factor	DF	Adj SS	Adj MS	F	P	S	R^2	R^2 (Adj)
CDA	Phenotype	1	162040	160839	5.32	0.035	173.96	24.94%	20.24%
CNR1	Phenotype	1	160839	167554	5.61	0.031	172.46	25.98%	21.35%
CYP1A2	Phenotype	1	162040	145860	4.68	0.046	176.63	22.61%	17.78%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R^2 (Adj) = adjusted R^2

Table 18: Morphine Milligram Equivalents Genomic Summary

Gene	Factor	Polymorphism	Effect (mg)	P
CDA	Phenotype	rs532545 C Allele	+94.50	0.035
CNR1	Phenotype	rs806368 non-TT genotype	-102.30	0.031
CYP1A2	Phenotype	High Inducibility Metabolizer	-95.48	0.046

The effect refers to the outcome derived from the statistical analysis and its impact on the calculated morphine dose. A negative result signifies an association with lower morphine levels due to the specific polymorphism, whereas a positive value indicates an elevation in the need for morphine. These are in milligram equivalents. The P-value shows the statistical significance of each effect.

Table 19: Palliative Performance Scale vs. Genomic Data

Gene	Factor	DF	Adj SS	Adj MS	F	P	S	R^2	R^2(Adj)
CYP4F2	Genotype	2	344.4	172.22	4.56	0.028	6.15	37.80%	29.51%
CYP4F2	Phenotype	2	344.4	172.22	4.56	0.028	6.15	37.80%	29.51%
HLA-B	Genotype	2	411.1	205.56	6.17	0.011	5.77	45.12%	37.80%
HLA-B	Phenotype	2	411.1	205.56	6.17	0.011	5.77	45.12%	37.80%
NOS1AP	Phenotype	6	611.1	101.85	3.73	0.028	5.22	67.07%	49.11%
UGT2B15	Genotype	2	427.8	213.89	6.64	0.009	5.68	46.95%	39.88%

DF = degrees of freedom, Adj SS = adjusted sum of squares, Adj MS = adjusted mean square, F = f-value, P = p value, S = root mean square error, R2 (Adj) = adjusted R2

Table 20: PPS% Genomic Summary

Gene	Factor	Polymorphism	Effect	P
CYP4F2	Genotype	*1/*1	-5.56	0.045
		*1/*3	-7.22	0.021
	Phenotype	Normal Metabolizer	-5.56	0.045
		Intermediate Metabolizer	-7.22	0.021
HLA-B	Genotype	WT/*5801	+8.33	0.016
	Phenotype	HLA-B*5801 Allele Carrier	+8.33	0.016
NOS1AP	Genotype	c.106-38510G>T/c.106-38510G>T/ c.178-20044C>T/c.178-13122C>T	+16.83	0.007
		c.178-20044C>T/c.178-13122C>T	-9.83	0.011
	Phenotype	rs10494366 GG genotype/ rs10800397 T Allele Carrier/ rs10919035 T Allele Carrier	-10.24	0.006
UGT2B15	Genotype	*1/*2	+6.11	0.004

The effect refers to the outcome derived from the statistical analysis and its impact on the PPS%. A negative result signifies a functional decline due to the specific polymorphism, whereas a positive value indicates an elevation function. These are in percentages. The P- value shows the statistical significance of each effect.

CHAPTER 4. DISCCUSION

This study has made significant strides in understanding the intricate relationship between genetic variations and the efficacy of opioid therapy in the context of hospice and palliative care. Specifically, we identified several single nucleotide polymorphisms (SNPs) and corresponding genes that may influence an individual's response to opioids, including those potentially linked to opioid resistance. These findings are especially noteworthy given the limited application of pharmacogenomics (PGx) techniques in hospice settings.

The potential clinical implications of these results are manifold. For example, identifying genetic markers associated with opioid resistance could enable personalized treatment strategies, reducing the trial-and-error approach commonly employed in pain management for hospice patients. This is a pressing need because this patient population often struggles with complex, severe pain resistant to standard opioid therapies.

4.1 Implications of Non-Genomic Data

Initially, non-genomic data was statistically analyzed. The only demographic factor correlated with the four significant variables (Average Pain, Maximum Pain, MME, and PSS%) was age and MME. Age was a predictive factor for morphine dosing. Age negatively impacted morphine dosage with a coefficient of -8.20 ($p=0.021$). The linear regression for age implies that for each year of age, the calculated morphine dosage decreases by 8.20 mg, holding all other factors constant. While it may seem counterintuitive, there is a fear of addiction among long-term hospice and palliative care patients on opioid therapy[99]. A possible explanation for the gradual tapering of opioid therapy based on age could be addiction-related worries. Another possible explanation is

that hospice and palliative care patients may be unable to communicate their pain management needs as their disease progresses and they age[100].

Opioid-related side effects in terms of demographics were also analyzed. We found that gender significantly influences the severity of specific opioid side effects—specifically constipation, dry mouth, abdominal pain, and urinary retention. Two side effects, itching and sweating, were linked to age. Older patients experienced a greater degree of itching and sweating than younger patients. However, we cannot definitively say that opioids caused these factors in these patients, as the MME did not significantly impact any opioid side effects documented by the NOSE.

The variation in side effects in males and females could be related to sex differences, but there are too many confounding variables for this to be a definitive explanation. Patients might have secondary conditions we were not privy to that influence these results. These conditions might be related to secondary diseases, post-operative symptoms, or a condition that has gone undiagnosed. These might also be the long-term effects of substance abuse or drugs they were previously on. These reasons are purely speculative, and the data afford no definitive explanation.

Our results are in line with previous research regarding itching and age. The high prevalence of these symptoms in aging patients is due to the physiology of aging skin: poor hydration, impaired barrier function of the skin, and alterations in kidney function[101]. For sweating, the causes could be varied. These can be a symptom of pain or other medications patients are taking. Sweating can also result from psychological conditions such as depression and anxiety[102].

In the genomic analysis, we found 24 out of the 50 analyzed genes to be

significant. Regarding demographics, only one gene, interferon lambda 3 (IFNL3), showed significance concerning ethnicity. This gene's SNP is crucial in predicting the treatment of hepatitis C with interferon and ribavirin and plays a vital role in antiviral host defense[103]. An important point to note is that the versions of *IFNL3* differ based on ethnicity. The favorable variant is most common among Caucasians and least common among African Americans. A large majority of African Americans possess the non-favorable variant[104]. Our results follow this, with 80% of Caucasians possessing the favorable genotype and 100% of African Americans possessing the non-favorable genotype. These statistics align with previous research and demonstrate the validity of our methodology.

4.2 Main Variables and Significant Relationships

We based the four main variables in this study on our hypotheses and PGx relationships. They were average pain, maximum pain, MME, and PPS%. These were the primary variables due to their relationship with opioids, pain, and clinical outcomes.

Along with maximum pain, our analyses found that several genes also influenced a patient's average pain, which we defined as the typical pain over a 24-hour period. These were *AGTR1*, *HTR2A*, *SLCO1B1* and *UGT2B15*. We found a significant correlation between the angiotensin II receptor type I (AGTR1) phenotype, genotype, and maximum pain. AGTR1 is accountable for synthesizing a protein identified as the angiotensin II receptor type 1 (AT1 receptor). This protein plays a crucial role in the renin-angiotensin system, which is pivotal for regulating blood pressure and the equilibrium of fluids and electrolytes within the organism[104].

In terms of genotype, individuals with the heterozygous WT (WT/c.*86A>C)

genotype experienced higher average pain compared to those with the homozygous WT (WT/WT) genotype. For phenotype, results indicate higher average pain for the rs5186 AC variant than the AA variant. Most of the PGx research on AGTR1 is based on the cardiovascular system, and no robust evidence is related to pain or opioids. However, AGTR1 polymorphisms are responsible for increased inflammation[105]. These polymorphisms may explain why patients experience more significant pain with the genotype and phenotype variants.

The serotonin receptor 2A (HTR2A) demonstrated significance in genotype but not phenotype in relation to average pain. *HTR2A* encodes for one of the receptors for serotonin. Animal models have shown that the HTR2A receptor mediates pain processing in the spinal cord by regulating glutamatergic activity[106]. Our results showed that *HTR2A* polymorphisms had higher post-operative analgesic requirements[107]. Our model was not robust enough to establish and could not determine if the *HTR2A* genotype increased or decreased average pain. However, due to the significance of the association and previous literature associating *HTR2A* with pain, this gene would benefit from PGx studies related to analgesics. More focused research would further strengthen the association between *HTR2A* and pain.

The following gene that was significant for average pain was lute carrier organic anion transporter family member 1B1 (SLCO1B1). SLCO1B produces a protein called organic anion transporting polypeptide, 1B1. This protein is found in hepatocytes and transports compounds from the blood into the liver to be cleared[108]. *SLCO1B* was significant in both genotype and phenotype. Specifically, the *SLCO1B1*1* genotype is associated with higher average pain scores than other genotypes. Currently, there are no

PGx relationships with analgesics. *SLCO1B1* and genotype. One reason for this might be that other genotypes have reduced function and this may increase the systemic circulation of some opioids since SLCO1B1 functions in the liver. In terms of phenotype, those with the intermediate activity phenotype experienced lower-than- average pain. As there are no PGx relationships between analgesia and *SLCO1B1*, it is difficult to determine how these relationships affect one another. The low explanation of variance within the model indicates other factors at play for both the genotype and phenotype.

Lastly, UDP glucuronosyltransferase family 2 member B15 (UGT2B15) had both genotype and phenotype have a significant association with average pain. The *UGT2B15* gene is responsible for producing an enzyme involved in glucuronidation. This process, called a phase II metabolic reaction, converts lipophilic molecules into water-soluble substances that can be easily excreted. The UGT2B15 enzyme is primarily found in the liver, which plays a critical role in the metabolism of various substrates, including therapeutic drugs like benzodiazepines[109].

In terms of genotype, based on the regression equation, individuals with the *1/*1 genotype reported the highest average pain, followed by the *2/*2 genotype and then the *1/*2 genotype. Most studies involving UGT2B15 relate to the metabolism of anxiolytics. In a study with lorazepam, clearance of the drug was lower in the *2/*2 genotype than in the *1/*1 genotype [110]. Since UGT2B15 is produced primarily in the liver and performs glucuronidation, it may be related to opioid metabolism. A possible reason for the *1/*1 genotype experiencing more pain than the homozygous and heterozygous genotypes is that opioids and their metabolites are cleared faster from their body than the *2/*2. This polymorphism would alter the amount of drugs a patient would

need. However, this is only a possible explanation based on a different drug class. More research is needed to investigate this relationship.

For phenotype, the rs1902023 AA variant significantly contributes to pain in individuals with this variant than without it. This corresponds with the above results as the *1/*1 is the genotype for the rs1902023 AA phenotype. Much like genotype, there is little literature on rs1902023 AA and pain. The closest evidence was that those with the AA genotype are associated with a more significant metabolism of acetaminophen. However, that study was in healthy patients. Increased clearance of acetaminophen may be contributing to the pain score. More research is needed to ascertain this relationship[109].

Maximum pain was the worst pain the patient experienced in the past 24 hours. The genes significantly associated with maximum pain were *COMT*, *CYP1A2*, *CYP2B6*, *DRD1*, *FAAH*, *MTHFR*, and *SLCO1B1*. Out of all the genes in the study, only *SLCO1B1* was involved in both maximum and average pain. Catechol-O-methyltransferase (COMT) is a prominent gene in pharmacogenomics. Genetic alterations within *COMT* have been associated with mental health conditions such as schizophrenia, pain perception mediated by opioid receptors, and the development of breast cancer.

The COMT enzyme facilitates the transmission of a methyl group from S-adenosyl-l-methionine (SAM) to a hydroxyl group on the catechol substrate. This reaction requires the presence of magnesium ions[111]. We found a positive association between *COMT*'s phenotypes and pain. Specifically, we predict that individuals with the MET Homozygous phenotype will have a maximum pain score 1.393 points lower than Non-MET Homozygous individuals. This finding follows throughout the literature. A polymorphism replaces a methionine (Met) with a valine (Val) at codon 158 in COMT.

We have observed that subjects with a Met/Met genotype exhibit low COMT activity[112]. This polymorphism has led to an increase in pain response. Many studies have confirmed that this homozygous genotype is associated with increased pain from fibromyalgia to the transition from acute to chronic lower back pain[113].

Regarding opioids, patients with the homozygous Met substitution had a higher pain sensitivity and more extended amounts of chronic pain than other polymorphisms. Researchers believe this mechanism impacts enkephalin, an endogenous opioid capable of reciprocally regulating MOP expression[114]. Based on the literature, we have observed that individuals with a homozygous Non-Methionine polymorphism may experience increased pain[115].

CYP1A2 is part of the cytochrome P-450 (CYP) enzyme family that plays a vital role in drug metabolism. Various CYP enzymes are responsible for the catalysis of 70-80% of all phase I reactions. However, the CYP1A2 isoform is only responsible for metabolizing 5% of drugs in the CYP family of enzymes[116].

CYP1A2 is not associated with opioids and is most commonly associated with antipsychotics and methylxanthines such as theophylline and caffeine. In our study, both genotype and phenotype affected maximum pain. Regarding genotype, we associated the *1A/*1F, *1C/*1F/*1F, and *1F/*1F genotypes (which are all classified as the high inducibility metabolizer phenotype) with increasing pain scores. We consider *CYP1A2*1A* to be the principal or 'wild-type' genotype. We associate the *1F genotype with the 'ultrarapid metabolizer'[117]. While not associated with opioids, it is associated with a faster caffeine metabolism. This genotype is also associated with a faster metabolism of clozapine[118]. The *1C allele is associated with decreased enzymatic

activity[51]. In our study, the *1F allele was present in all the groups with increasing pain scores. This polymorphism concerning pain scores is a new finding, as there have not been any associations with opioids or analgesics in the literature. Our group identified the ultra- rapid metabolizer allele in individuals who reported increased pain. Ultra-rapid metabolizers may suggest a pharmacogenetic reaction that requires further investigation. Adding to the evidence that ultra-rapid metabolizers in *CYP1A2* contribute to maximum pain, we found that the normal metabolizer group reported lower maximum pain than the high inducibility metabolizer group. This finding reinforces the link between individuals with a high metabolism and increased pain levels.

Another member of the CYP family was involved in increased pain, which was cytochrome P450 family 2 subfamily B member 6 (CYP2B6). CYP2B6 is one of the most highly polymorphic human members of the CYP family, with over 100 different SNPs[119]. *CYP2B6* makes up about 3-6% of the total CYP in the liver and processes numerous drugs, including bupropion, ketamine, and propofol. It also can de-toxify and bioactivate several procarcinogens and environmental toxicants[120]. *CYP2B6* was significant in both terms of genotype and phenotype.

Regarding genotype, two were relevant: A785G/A785G/G516T for association with less pain than WT and A785G/A785G/G516T/G516T with more pain than WT. These CYP2B6*6 SNPs 785A>G (rs2279343) and 516G>T (rs3745274) were associated with high methadone clearance, a lower plasma concentration, and a lower concentration to dosage ratio of (S)-methadone[121]. Therefore, it makes sense that a patient that is homozygous for both of these polymorphisms clears opioids faster, leading to a need for more pain relief.

These factors align with the with phenotype: G516T Heterozygous/A785G Homozygous exhibits lower pain than G516T Homozygous/A785G Homozygous. Due to these findings, we must conduct more research to confirm this association conclusively. However, it does mark an indicator for possible clinical use.

Dopamine Receptor D1 (DRD1) is the most abundant dopamine receptor in the CNS. DRD1 is a G-protein coupled receptor, which stimulates adenylyl cyclase. *DRD1* also activates cyclic-AMP protein kinases[122]. For our study, in terms of maximum pain, both genotype and phenotype were significant in average pain.

Regarding genotype, the maximum pain level of those homozygous for the c.-48G>A polymorphism was greater than that of the WT/WT. Furthermore, those heterozygous with the wild-type, WT/c.-48G>A, also experienced a higher maximum pain level.

Regarding phenotype, analysis was between the rs4532 CC genotype and the non-CC genotype. We observed a significant association between the *DRD1* phenotype and maximum pain, explaining approximately 33.33% of the variance in pain perception.

Individuals with the rs4532 CC genotype reported lower maximum pain levels than those with the non-CC phenotype. However, our cohort contained only one patient with the rs4532 CC phenotype, corresponding to the homozygous wild-type genotype.

In terms of opioids and other drugs, *DRD1* is an integral part of reward-related processes, especially those in drug reward and conditioning[123]. *DRD1* is implicated in addiction to drugs as well as engaging in high-risk behavior[124]. The SNP rs4532 was studied in chronically prescribed opioid patients in pain clinics[125]. The study focused on the Genetic Addiction Risk Test (GARS), which is clinically proven to be able to predict

vulnerability to pain using PGx[125]. The *DRD1* rs4532 allele was the most frequently observed risk allele in the study cohort, with a presence in 87.60% of the participants.

The prevalence of rs4532 made it the most prevalent allele among those tested[125]. In one study, there was an association with the SNP rs4532 in Han Chinese addicts from the rapidity of the first use of an opioid to addiction[126]. Another study found that rs4532 could be associated with less pleasurable opioid responses after dependence[127]. The lack of a pleasurable response can explain why maximum pain scores were higher in those with this SNP since euphoria can mask pain. The high pain scores with this SNP could be because opioids in these patients do not provide the complete analgesic response, leading to more pain. PGx analysis could undoubtedly help these patients and modulate opioid dosing based on these findings. However, further investigation is needed to determine an exact treatment protocol and the reasoning for the higher pain scores.

Next, we found that the genotype and phenotype of Fatty-acid amide hydrolase 1 (FAAH) significantly influenced maximum pain. FAAH is vital in mammals, as it produces an enzyme that breaks down a group of signaling lipids known as fatty acid amides. These are naturally occurring within the body. Notably, these lipids include anandamide, an endogenous cannabinoid, and oleamide, which induces sleep. For genotype, the c.385C>A/c.385C>A polymorphism reported, on average less pain than those with the WT/WT genotype (WT). On the other hand, individuals with the WT/c.385C>A genotype experienced more pain than the WT/WT genotype. It makes logical sense that someone with the homozygous polymorphism will experience more intense pain relief than someone with the WT or heterozygous polymorphism. The c.385C>A substitution results in around a 50% cellular expression loss of FAAH due to

the reduced stability of the mutant protein [128]. The mutant protein could result in reduced metabolism of endogenous endocannabinoids, such as anandamide. The endocannabinoid system is known to modulate pain[129], and with these SNPs inactivating a large amount of FAAH, this could, in turn, increase pain relief. FAAH inhibitors also reduced anxiety in mice[130], a psychological component of pain.

In terms of phenotype, the results follow identically. The rs324420 CA and CC genotypes had higher maximum pain than the rs324420 AA variant. The results we discovered in the genotypic expression are consistent with this information. The homozygous AA variant lessens the metabolism of endogenous cannabinoids and boosts endocannabinoid activity.

Another gene related to max pain is Methylenetetrahydrofolate reductase (MTHFR). This gene is responsible for producing an enzyme named methylenetetrahydrofolate reductase. This enzyme is essential in the metabolism of amino acids. It is crucial to note that MTHFR facilitates a chemical reaction that depends on the vitamin folate[131]. MTHFR assists in the conversion of folate into its primary form found in the blood. The gene also converts homocysteine to methionine[132].

Genotype and phenotype were associated with lower maximum pain scores: C677T/A1298C and C677T Heterozygous Mutation/A1298C Heterozygous Mutation genotype. This mutation reduces the activity of MTHFR by 35% in those who are heterozygous. This results in blood loss of folate and amino acid conversion[133]. Currently, with this variant, no relevant PGx relationship would explain a drop in pain. There have been studies with conflicting results on whether variants in *MTHFR* produce headaches or migraines[134]. Recent findings indicate that *MTHFR* variants occur more

frequently in opioid users than in the general population[135]. The literature does not establish any connections between *MTHFR* function and opioids. Without research, it is difficult to speculate what these pain scores represent inside our population. Further research is necessary into these *MTHFR* variants and associated pain in hospice and palliative care.

Lastly, SLCO1B1 was significant for maximum pain, as it was for average pain. Both the genotype and phenotype were the same in terms of results. The *SLCO1B1*1* genotype is associated with higher maximum pain scores, and the Intermediate metabolizer phenotype was associated with lower maximum pain scores. The model's r-squared was still low. For clinical data, there is evidence that variants with lower methotrexate clearance[136]. The clearance of opioids might be affected as it is a hepatic enzyme, but we need to conduct more research to confirm this.

We found three genes significantly associated with MME: *CDA*, *CNR1*, and *CYP1A2*. The *CDA* gene's primary role is the recycling of free pyrimidines, salvaging them. CDA is one of several deaminases responsible for maintaining the cellular pyrimidine pool. The recycling activity of CDA within the pyrimidine salvage pathway is essential for DNA and RNA synthesis. The gene itself plays a role in the metabolism of the cancer drug cytarabine[137]. CDA can deaminate cytidine and deoxycytidine-based therapies. CDA overexpression is associated with lower chemotherapeutic side effects and resistance to chemotherapeutic treatment[138]. There is no literature on the relationship between *CDA* and morphine metabolism. However, our findings indicate that the rs532545 C allele was associated with a substantial increase in the calculated morphine dosage by 94.5 mg ($p=0.035$) compared to the rs532545 T allele. This finding highlights

the potential impact of genetic variation in the *CDA* gene on opioid dose requirements. This particular allele may alter the function or expression of the *CDA* enzyme, leading to changes in morphine metabolism or response. Our model presents predictive power (R-squared predictive=5.00%) but is relatively low. There are still significant variations in morphine dosing that we have yet to consider. We need further research to strengthen the association between the rs532545 C allele and increased morphine dosage, as we have not yet explained why this is the case.

The gene *CNR1* is part of the endocannabinoid system in the central nervous system. Like opioid receptors, cannabinoid receptors are part of the guanine-nucleotide binding protein (G-protein) receptor family[139]. Significant cross-talk exists in the endogenous cannabinoid and opioid systems[140]. Due to this, there is evidence that *CNR1* plays a role in the addiction process, including opioid addiction[141]. Our study observed a statistically significant relationship between the *CNR1* phenotypes and the calculated morphine dose ($F(1,16)=5.61$, $p=0.031$). The results indicate that alterations in CNR1 genetics, particularly in rs806368 non-TT and TT genotypes, could impact the morphine needed. The non-TT genotype was associated with a decrease in the calculated morphine dosage by 102.3 mg ($p=0.031$) relative to the TT genotype. People who do not have the TT genotype may need lower doses of morphine to achieve the same pain-relieving effects. Recent studies have shown that both receptor types can produce antinociception, drowsiness, hypotension, motor depression, and drug reward/reinforcement when used agonists[142]. A possible mechanism may be interaction with the endocannabinoid system, increasing analgesic effects and thus decreasing the need for larger doses of opioids.

CYP1A2 is part of the cytochrome P-450 (CYP) enzyme family essential to drug

metabolism. Various CYP enzymes are responsible for the catalysis of 70-80% of all phase I reactions. However, the CYP1A2 isoform is only responsible for metabolizing 5% of drugs in the CYP family of enzymes[116]. CYP1A2 is not associated with opioids and is most commonly associated with antipsychotics and methylxanthines such as theophylline and caffeine. Our results associated the high inducibility metabolizer phenotype with a 95.5 mg reduction in the calculated morphine dose ($p = 0.046$) compared to normal metabolizers. While this may seem contradictory, several opioid drugs are pro-drugs. A few include oxycodone, codeine, and hydrocodone[143]. Calculating morphine equivalents, we combine these drugs into a singular value. Those with the high inducibility metabolizer phenotype may convert opioid pro-drugs into drugs quicker, increasing their analgesic effect and thus negating the need for higher doses of opioids.

Lastly, PPS% was analyzed. Four genes were significantly associated with PPS%. These included *CYP4F2*, *HLA-B*, *NOS1AP*, and *UGT2B15*. Cytochrome P450 family 4 subfamily F member 2 (CYP4F2), situated in the endoplasmic reticulum, initiates the breakdown and neutralization of leukotriene B4[144]. This is a potent inflammation inducer. *CYP4F2* also plays a role in metabolizing fatty acids and vitamin E[145]. *1/*1 and *1/*3 genotypes were associated with lower PPS% compared to the *3/*3 genotype. Individuals with a *3/*3 genotype may require less warfarin than those with other genetic variations, as the former may provide a protective effect [146]. This genotype also appears to increase human exposure to vitamin K[147]. *CYP4F2*3/*3* may relay a protective effect against bleeds and the low therapeutic index drug of warfarin, which many palliative and hospice patients must take. This may lead to an increase in PPS%, associated with higher survival and outcomes. In terms of phenotype, "Intermediate Metabolizer" and "Normal

Metabolizer" are associated with lower PPS% compared to the "Poor Metabolizer" phenotype in our study. Because of this, normal and intermediate metabolizers have worse patient outcomes than poor ones. The result of normal and intermediate metabolizers having poorer outcomes may have to do with the significance of warfarin dosing and the fact that *CYP4F2* is related to increased vitamin K levels. There is also evidence that high levels of CYP4F2 promote lung cancer[148], which may further impact PPS%.

Major histocompatibility complex, class I, B (HLA-B) plays a critical role as it enables the immune system to differentiate between proteins produced by the body and those produced by external invaders like bacteria and viruses. *HLA-B* is responsible for creating a protein pivotal to the immune system's functioning[149]. Both the phenotype and genotype were associated with significance with PPS%. The WT/*5801 has a higher PPS% than that of the WT. Typically, the variant HLA-B*5801 is implicated in many adverse drug reactions, notably allopurinol[150]. The HLA-B*5801variant having better patient outcomes (increased PPS%) compared to the WT seems counterintuitive. The cohort under investigation also includes people with HIV, and this variant is responsible for hepatotoxicity during retroviral therapy[151]. Due to the many negative effects of the variant, it is unclear why there is an association with HLA-B*5801 and higher PPS%. Further investigation needs to be done, especially in the realm of this patient population with this variant. For phenotype, being an HLA-B5801 Allele Carrier also was associated with a higher PPS%, an identical result.

Our analysis showed that the Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP) gene is significantly associated with PPS%. The protein NOS1AP interacts with neuronal

nNOS in cytoplasm[152]. Specifically, rs10494366 GG genotype/rs10800397 T Allele Carrier/rs10919035 T Allele Carrier was associated with lower PPS%. This has to do with several factors. Individuals with a GG genotype at rs10494366 compared to individuals with the TT genotype at the same site, using glibenclamide, results in a lower efficacy of glucose reduction and higher mortality rates when using the same medication[153]. In addition, the SNP rs10800397 increased the incidence of drug-induced long QT syndrome[154]. Because of these factors, this variant likely results in lowered patient outcomes due to its effect on the heart and medications relating to it.

Lastly, UGT2B15 was associated with PPS% and was also associated with average pain. In this case, we associate the '*1/2' variant with a higher PPS% than other variants. One study associated the UGT2B151/*2 variant with poorer outcomes when patients received tamoxifen for breast cancer[155]. This could be responsible for the degenerating palliative performance that our model predicts.

4.3 Clinical Implications

This study has significant implications for clinical practice and has the potential to change the traditional one-size-fits-all approach to pain management. Integrating genomic analyses with clinical practice can lead to personalized pain management based on the patient's genetic makeup. Genomic analysis has the potential to improve the effectiveness of opioid dosing. The unique variations in each patient can be better visualized through a matrix plot (Figure 8) showing the extreme variations in each patient. The visualization of these baseline scores helps put the genomic data in perspective.

To assess the clinical implications of this study, we combined the PGx results across

the four variables: average pain, maximum pain, Morphine Milligram Equivalents (MME), and Palliative Performance Scale (PPS%). The additive genomic effects were to provide a general overview of the study and better enable clinical analysis.

Table 21 displays the overall genomic effect on average pain for each individual patient. Patient 1 demonstrated a negative overall effect on average pain of -2.73. Patient 1 possessed a negative score despite having a baseline average pain score of 4. These scores might suggest a potential resistance to pain, which may warrant a potential lower dose of opioids. Conversely, Patient 6 exhibited the highest recorded pain score of 10, along with the highest overall genomic effect on average pain, which was +6.31. This patient's high genomic score suggests a heightened sensitivity to pain, necessitating a more nuanced approach to pain management.

Regarding maximum pain, the differences in patients are equally revealing, depending on the sum of genomic effects (Table 22). Patient 16 presents with a baseline maximum pain score of 2 and possesses a significant genomic effect of -26.29 on maximum pain. The genomic effect was less pronounced on average pain at -4.74, but it still was the second-highest reduction in average pain for all patients. Patient 16 also presented with an average pain score of 2. These negative scores indicate a genetic predisposition towards pain resistance or pain tolerance and may require a less aggressive opioid dosing regimen. In contrast, patient 4 and patient 6 had genomic effects in the double digits, +14.83 and +13.18, respectively. They also both had maximal pain scores of 10. Patients 4 and 6 possessed high average pain scores as well.

Regarding significant differences between average pain and maximum pain and genomic effects, patient 11 was the most stark. For average pain, their overall genomic

effect was -4.15, and their average pain baseline was 3. For maximum pain, patient 11's genomic effect was +4.00, and their baseline maximum pain 10. These variations demonstrate the stark complexities of pain management. This patient might have a genomic effect that amplifies the feeling of acute or peak pain and might take more aggressive pain management to maintain comfort and function during these episodes.

MME also highlights the crucial observations genomically as well (Table 23). Patient 16, who had high pain resistance on both average pain and maximum pain, was also associated with a lower morphine dose of -102.3 mg. Their baseline morphine dose was also low at only 30 mg. In contrast, patient 8, with an overall genomic effect on morphine of +94.50 mg and a high opioid requirement of 540 mg, might have a genetic makeup that leads to rapid metabolism or reduced opioid sensitivity. Patient 8 might require even higher opioid doses outside the normal range. Patient 8's pain scores provide insight into the reasoning. Their average pain score was 7, and their maximum score was 8. This result challenges the idea of what is adequate in pain management. Similarly, patient 11 possessed a genomic effect of morphine as +94.50 mg, along with an overall MME of 525 mg. While this might be adequate for average pain since their baseline was a 3, it might require more aggressive treatment for incidents of acute pain since their maximal pain score was an 8.

The effect on morphine dose can also reveal those patients who are not getting adequate treatment. Ineffective treatment is especially stark in terms of patient 3. They have both high average and maximum pain, with scores of 8 and 10. Their morphine dose is 60 mg. While their overall genomic effect indicates they might require a lower morphine dose at -95.48 mg, they still possess high average and maximum pain scores.

The overall genomic effect on maximum pain in terms of patient 3 is pronounced, being +8.83. Despite requiring less morphine, this is counteracted by the susceptibility to high acute pain, as well as still possessing a high average pain score. This genomic information would allow for a better and more specified treatment regimen since the current amount of opioids is not enough to counter the effects of the pain they are experiencing.

While essentially different than pain, it is essential to look at a patient's functional status (Table 24). The PPS% of patient 1, with a baseline of 30% and a considerable negative genomic effect of -31.19%, might experience a more pronounced decline in their functional status. This insight could prompt earlier interventions tailored to the patient's needs to maintain the best possible quality of life. Comparatively, patient 17, who exhibits a significant positive genomic effect of +39.60% with a baseline of 60%, may maintain a better functional status than expected despite significant disease progression. Understanding such genomic influences can help anticipate the patient's trajectory, guiding decisions about resource allocation and the intensity of supportive care services. The variation in PPS% and genomic effects merits further study in this area outside of the context of opioid and pain associations.

This study's approach merits integrating genomic analysis into the clinical practice, as with this small sample size, there is substantial variability. The one-size-fits-all pain management approach is suboptimal. The incorporation of genomic data into clinical practice can highlight patient differences and offer insights as to how pain can be managed more effectively with less risk.

4.4 Limitations

While the study shows significance, it is only the first step, as we must refine methodologies. As mentioned in the introduction, hospice and palliative care are complicated to study. Our patient population is relatively small. Swabs were typically taken instead of blood samples due to the nature of the frailty of the patients and the constant administering of medications, leaving them with few extraction sites for blood. Drawing blood from this patient cohort requires a skilled phlebotomist.

In addition to that, it is difficult to have an opioid naïve control group in this population. Frequently, these patients are at the end of their lives and have been taking opioids for an extended period. This makes it challenging to compare epigenetic factors from a naïve population to one that has taken opioids for an extended period. There is a lack of a robust control group due to this.

There is a large signal-to-noise ratio. This is because are a vast number of factors that comprise PGx, and hospice and palliative patients may obscure results. This is because they have multiple chronic conditions, are on many medications, and may have experienced different treatment regimens.

In terms of medical records, they are challenging to track down before they enter the hospice and palliative care environment. Patients might have visited multiple physicians or received treatment in different hospitals, making locating records challenging. This might mean there are confounding variables that we cannot account for because we do not know of them. Similarly, with medical records, some opioid doses were given 'as needed,' which means whenever the patient wanted them. If we do not meticulously keep these records, it hampers our ability to calculate morphine dosages

accurately.

Combining genotypic and phenotypic results provides its limitations. While it is necessary to understand the interplay of genomic factors on pain and opioid dosing, several difficulties arise. The analysis treated all values equally when adding genotype and phenotype together. Equal treatment among genotype and phenotype is generally not the case, as one genotype or phenotype will have a more significant effect than another, influencing overall genomic scores. There is also the fact that there may be confounding variables influencing these scores beyond what we can see.

Lastly, while we did show significance in nearly half of our genes of interest, several more were just shy of being significant. This is because our sample size consisted of only 18 individuals. A more significant number of subjects would allow for even more robust evidence for the associations that we have discovered.

4.5 Conclusions and Future Research

In conclusion, this study provides new insights into the complex hospice and palliative care management field. We discovered that SNPs in multiple genes, such as *CYP1A2*, *HTR2A*, *CNR1*, and others, can significantly influence this patient cohort's average pain, maximum pain, MME, and PPS%. We also discovered novel genes not previously associated with opioid or pain response: *AGTR1*, *SLCO1B1*, and *UGT2B15*. These findings indicate a possible path to the genetic basis of pain and response to opioid medications. The associations we discovered between specific genotypes and phenotypes and morphine dosing suggest the potential for personalized opioid treatment plans based on a patient's genetic profile. Such as, variants in *CNR1* may require different doses for effective pain management. This further validates the use of PGx in the association

between opioids, pain, and patient outcomes.

Additional research is required to validate the clinical utility of these genomic profiles and establish strong, causative evidence-based linkages for opioid dosing regimens and pain management. Future studies could eliminate variables like numerous health conditions and focus on cancer since cancer induces epigenetic changes. A larger patient cohort would be valuable as well.

One developing area is PGx reporting phone applications that immediately show patients their results once the report is complete. With more evidence, these applications can also be included in the clinic, clearing up confusion and stigma with opioid prescribing. They could be linked to patient databases and change prescriptions in real time. These reports could also be broken down into simpler terms so caregivers can respond appropriately to accurate information and not be confused or afraid they will harm their loved ones with inaccurate dosing.

This study offers proof of concept to allow for a stepping stone to discovering these complex gene-phenotype and gene-drug interactions. Discovering these PGx markers through comprehensive study and analysis could lead to more precise and effective pain management. By implementing this approach, errors in opioid dosing and pain management will significantly decrease. Moreover, clinicians can access personalized patient profiles for accurate dosing. Further and most importantly, increasing the quality of life for those who need it the most.

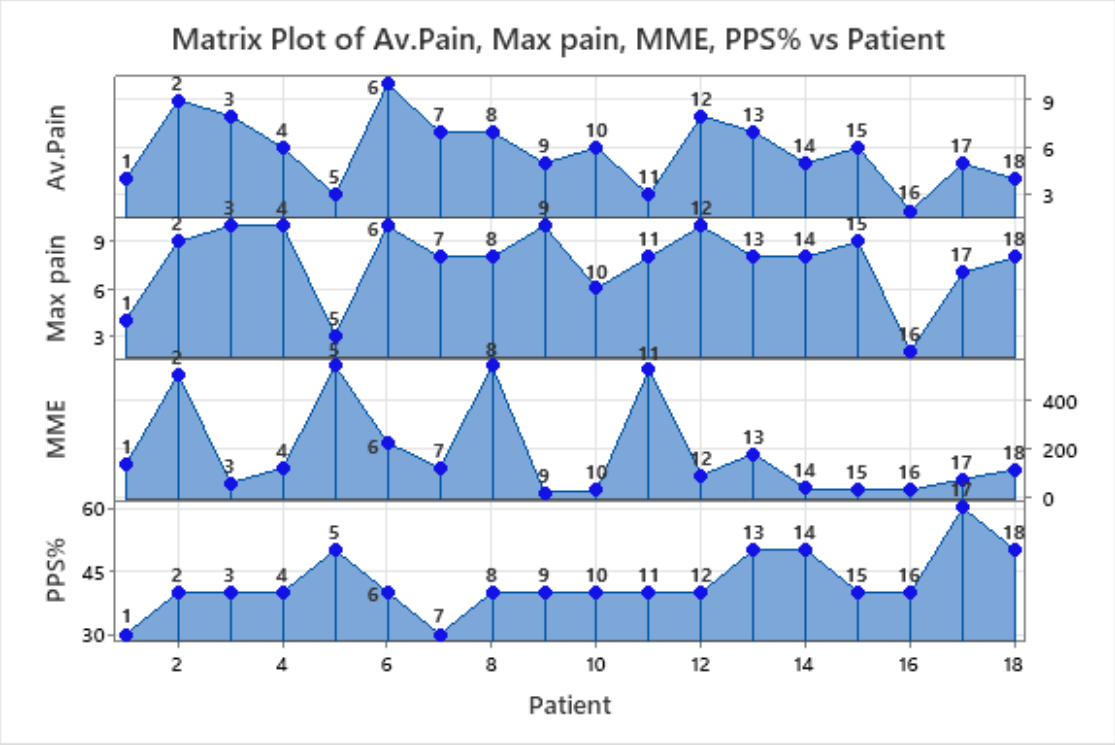

Figure 8: Matrix Plot for All Patients with All Variables. This matrix plot illustrates the distribution and relationship of average pain, maximum pain, Morphine Milligram Equivalent (MME), and Palliative Performance Scale (PPS%) across our patient cohort. Each row represents a variable, while the numbered dot represents an individual patient, identified by number. The x-axis on the chart refers to each patient, while the y-values indicate a specific patient.

Table 21: Overall Genomic Effect on Average Pain

Patient	Overall Effect	Av.Pain Baseline
1	-2.73	4
2	+4.25	9
3	-2.68	8
4	-0.67	6
5	-6.21	3
6	+6.31	10
7	-0.67	7
8	-0.67	7
9	-4.20	5
10	-2.14	6
11	-4.15	3
12	+2.91	8
13	+1.26	7
14	-2.14	5
15	+0.90	6
16	-4.74	2
17	-4.20	5
18	-6.21	4

This table represents the combined genomic effect on average pain across all genes, genotypes, and phenotypes. A positive score indicates a predisposition for higher pain levels, while a negative score indicates a predisposition to lower pain levels and pain tolerance.

Table 22: Overall Genomic Effect on Maximum Pain

Patient	Overall Effect	Max Pain Baseline
1	+4.68	4
2	+7.43	9
3	+8.83	10
4	+14.83	10
5	-23.37	3
6	+13.18	10
7	+4.15	8
8	+1.25	8
9	+7.43	10
10	+8.83	6
11	+4.00	8
12	+3.86	10
13	+5.93	8
14	+7.18	8
15	+2.50	9
16	-26.29	2
17	-2.64	7
18	+7.43	8

This table represents the combined genomic effect on maximum pain across all genes, genotypes, and phenotypes. A positive score indicates a predisposition for higher pain levels, while a negative score indicates a predisposition to lower pain levels and pain tolerance.

Table 23: Overall Genomic Effect on MME

Patient	Overall Effect	MME Baseline
1	-0.98	135
2	-0.98	505
3	-95.48	60
4	-95.48	120
5	+94.50	540
6	-95.48	225
7	-0.98	120
8	+94.50	540
9	-197.78	20
10	-0.98	30
11	+94.50	525
12	-95.48	91
13	0.00	180
14	-197.78	37.5
15	-103.28	30
16	-102.30	30
17	-102.30	75
18	-103.28	115

This table represents the combined genomic effect on morphine milligram equivalents across all genes, genotypes, and phenotypes. A positive score indicates an association with a higher morphine dose, while a negative score indicates a predisposition to lower morphine dose. All values are in milligrams.

Table 24: Overall Genomic Effect on PPS%

Patient	Overall Effect	PPS% Baseline
1	-31.19	30
2	-11.12	40
3	+2.39	40
4	-11.12	40
5	-5.01	50
6	-11.12	40
7	-31.19	30
8	-11.12	40
9	-8.33	40
10	-28.40	40
11	-5.01	40
12	-11.12	40
13	+11.65	50
14	-5.01	50
15	-14.44	40
16	-14.44	40
17	+39.60	60
18	-5.01	50

This table represents the combined genomic effect on PPS% across all genes, genotypes, and phenotypes. A positive score indicates an association with a higher percentage, while a negative score indicates a predisposition towards a lower percentage. All values are percentages.

APPENDIX 1. FULL GENE, GENOTYPE AND PHENOTYPE LIST

Gene	Genotype	Phenotype
ABCB1	c.3435T>C/c.3435T>C/c.2677T>G/c.2677T>G	rs2032582 CC genotype/rs1045642 GG genotype
	WT/c.2677T>G	rs2032582 AC genotype/rs1045642 AA genotype
	c.3435T>C/c.2677T>G	rs2032582 AC genotype/rs1045642 AG genotype
	WT/WT	rs2032582 AA genotype/rs1045642 AA genotype
	c.3435T>C/c.3435T>C/c.2677T>G	rs2032582 AC genotype/rs1045642 GG genotype
ACE	WT/WT	ACE Deletion
	WT/ACE Insertion	Heterozygous ACE Insertion
	ACE Insertion/ACE Insertion	Homozygous ACE Insertion
ADRA2A	WT/WT	rs1800544 GG genotype/rs1800545 GG genotype
	WT/c.-1252G>C	rs1800544 GC genotype/rs1800545 GG genotype
	c.-1252G>C/c.-1252G>C	rs1800544 CC genotype/rs1800545 GG genotype
	c.-1252G>C/c.-217G>A	rs1800544 GC genotype/rs1800545 GA genotype
AGTR1	WT/WT	rs5186 AA genotype
	WT/c.*86A>C	rs5186 AC genotype
ANKK1	WT/WT	Non A1 Carrier
	WT/A1	A1 Heterozygous
	A1/A1	A1 Homozygous
APOE	WT/WT	Non E2 Carrier
	WT/E2	E2 Carrier
ATM	WT/WT	rs11212617 CC genotype
	WT/c.175-5285G>T	rs11212617 AC genotype
	c.175-5285G>T/c.175-5285G>T	rs11212617 AA genotype
CDA	WT/WT	rs532545 C Allele
	WT/c.-451C>T	rs532545 T Allele
	c.-451C>T/c.-451C>T	rs532545 T Allele
CES1	WT/WT	rs71647871 C Allele
CNR1	WT/WT	rs806368 TT genotype
	WT/c.*3475A>G	rs806368 non-TT genotype
	c.*3475A>G/c.*3475A>G	rs806368 non-TT genotype
COMT	WT/WT	Non MET Homozygous
	WT/c.472G>A	Non MET Homozygous
	c.472G>A/c.472G>A	MET Homozygous
CYP1A2	*1C/*1C/*1F/*1F	High Inducibility Metabolizer
	*1F/*1F	High Inducibility Metabolizer
	*1C/*1F/*1F	High Inducibility Metabolizer
	*1A/*1A	Normal Metabolizer
	*1A/*1F	Normal Metabolizer
CYP2B6	*1/*1	Wild Type
	A785G/A785G/G516T/G516T	G516T Homozygous/A785G Homozygous
	A785G/A785G/G516T	G516T Heterozygous/A785G Homozygous
	A785G/G516T	G516T Heterozygous/A785G Heterozygous
CYP2C19	*1/*1	Normal Metabolizer
	*1/*2	Intermediate Metabolizer
	*2/*2	Poor Metabolizer
	*1/*17	Rapid Metabolizer
CYP2C8	*1/*1	Wild Type
	*1/*3	Allele 3 Carrier
CYP2C9	*1/*1	Normal Metabolizer
	*1/*2	Intermediate Metabolizer
	*1/*3	Intermediate Metabolizer

Gene	Genotype	Phenotype

CYP2D6	*1/*1xN	Ultrarapid Metabolizer
	*1xN/*2	Ultrarapid Metabolizer
	*1/*1	Normal Metabolizer
	*1/*2	Normal Metabolizer
	*1/*5	Normal Metabolizer
	*1/*9	Normal Metabolizer
	*1/*10	Normal Metabolizer
	*2/*2	Normal Metabolizer
	*2/*10	Normal Metabolizer
	*2/*35	Normal Metabolizer
	*4/*35	Normal Metabolizer
	*5/*41	Intermediate Metabolizer
	*41/*41	Normal Metabolizer
CYP3A4	*1A/*1A	Normal Metabolizer
	*1A/*1B	Intermediate Metabolizer
	*1B/*1B	Poor Metabolizer
CYP3A5	*1A/*1A	High Expresser
	*1A/*3A	Expresser
	*3A/*3A	Non Expresser
	*3A/*7	Non Expresser
CYP4F2	*1/*1	Normal Metabolizer
	*1/*3	Intermediate Metabolizer
	*3/*3	Poor Metabolizer
DPYD	*1/*1	Normal Metabolizer
	*5/*9A	Normal Metabolizer
	*1/*5	Normal Metabolizer
	*5/*5	Normal Metabolizer
	*1/*4	Normal Metabolizer
	*1/*9A	Normal Metabolizer
	*5/*9A/c.496A>G/IVS10-15T>C	Normal Metabolizer
	*9A/c.496A>G/IVS10-15T>C	Intermediate Metabolizer
	*6/IVS10-15T>C	Intermediate Metabolizer
	c.496A>G/IVS10-15T>C	Poor Metabolizer
DRD1	WT/WT	rs4532 CC genotype
	WT/c.-48G>A	rs4532 non-CC genotype
	c.-48G>A/c.-48G>A	rs4532 non-CC genotype
DRD2	WT/WT	rs1799978 TT genotype
	WT/c.-585A>G	rs1799978 C allele Carrier
ERCC1	c.354T>C/c.354T>C	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 AA genotype
	c.*197G>T/c.*197G>T/c.354T>C/c.354T>C	rs3212986 AA genotype/rs11615 non-AA genotype/rs735482 AA genotype
	c.354T>C/c.354T>C/c.*931T>G	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 non-AA genotype
	c.*197G>T/c.354T>C/c.354T>C	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 AA genotype
	c.*197G>T/c.354T>C	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 AA genotype
	c.354T>C/c.*931T>G	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 non-AA genotype
	c.*197G>T/c.354T>C/c.*931T>G	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 non-AA genotype
	c.*197G>T/c.354T>C/c.354T>C/c.*931T>G	rs3212986 C Allele Carrier/rs11615 non-AA genotype/rs735482 non-AA genotype
	WT/WT	rs3212986 C Allele Carrier/rs11615 AA genotype/rs735482 AA genotype
F2	WT/WT	Wild Type
F5	WT/WT	Non Factor V Leiden Carrier
	WT/c.1601G>A	Factor V Leiden Carrier
FAAH	WT/WT	rs324420 CC genotype
	WT/c.385C>A	rs324420 CA genotype
	c.385C>A/c.385C>A	rs324420 AA genotype
G6PD	WT/WT	Normal G6PD Efficiency
	A/A	G6PD Deficiency
GRIK4	WT/WT	rs1954787 T Allele Carrier
	WT/c.83-10039T>C	rs1954787 T Allele Carrier
GSTP1	WT/WT	rs1695 AA genotype
	WT/c.313A>G	rs1695 AG genotype
	c.313A>G/c.313A>G	rs1695 GG genotype
HLA-B	WT/WT	Wild Type
	WT/*5701	HLA-B*5701 Allele Carrier
	WT/*5801	HLA-B*5801 Allele Carrier

Gene	Genotype	Phenotype
HTR1A	WT/WT	rs6295 CC genotype/rs1800044 C Allele Carrier
	WT/c.-1019G>C	rs6295 non-CC genotype/rs1800044 C Allele Carrier
	c.-1019G>C/c.-1019G>C	rs6295 non-CC genotype/rs1800044 C Allele Carrier
HTR2A	WT/WT	rs7997012 non-GG genotype
	WT/c.614-2211T>C	rs7997012 non-GG genotype
	c.614-2211T>C/c.614-2211T>C	rs7997012 GG genotype
HTR2C	WT/WT	rs1414334 C Allele Carrier
	c.-759C>T/c.551-3008C>G/c.551-3008C>G	rs1414334 C Allele Carrier
	c.551-3008C>G/c.551-3008C>G	rs1414334 G Allele Carrier
	WT/c.551-3008C>G	rs1414334 C Allele Carrier
	c.-759C>T/c.-759C>T/ c.551-3008C>G/c.551-3008C>G	rs1414334 C Allele Carrier
IFNL3	WT/WT	Favorable Response Genotype
	WT/39738787C>T	Unfavorable Response Genotype
	39738787C>T/39743165T>G	Unfavorable Response Genotype
	39738787C>T/39738787C>T/ 39743165T>G/39743165T>G	Unfavorable Response Genotype
ITPA	WT/WT	Non-protective Wild Type
	WT/c.124+21A>C	rs7270101 C Allele Carrier
	WT/c.94C>A	rs1127354 A Allele Carrier
	c.94C>A/c.124+21A>C	rs1127354 A Allele Carrier/rs7270101 C Allele Carrier
KIF6	WT/WT	rs20455 AA genotype
	WT/c.2155T>C	rs20455 non-AA genotype
	c.2155T>C/c.2155T>C	rs20455 non-AA genotype
MTHFR	WT/WT	Wild Type
	WT/A1298C	A1298C Heterozygous Mutation
	WT/C677T	C677T Heterozygous Mutation
	A1298C/A1298C	A1298C Homozygous Mutation
	C677T/A1298C	C677T Heterozygous Mutation/A1298C Heterozygous Mutation
	C677T/C677T	C677T Homozygous Mutation
NAT2	*5/*6/*12/*13	Slow Acetylator
	*5/*5/*12/*12	Slow Acetylator
	*5/*5/*12	Slow Acetylator
	*6/*13	Intermediate Acetylator
	*5/*12/*12/*13	Intermediate Acetylator
	*4/*5	Intermediate Acetylator
	*5/*12	Intermediate Acetylator
	*5/*12/*12	Intermediate Acetylator
	*5/*7/*13	Slow Acetylator

Gene	Genotype	Phenotype
NOS1AP	c.178-20044C>T/c.178-13122C>T	rs10494366 GG genotype/rs10800397 T Allele Carrier/ rs10919035 T Allele Carrier
	c.106-38510G>T/c.178-20044C>T/ c.178-13122C>T	rs10494366 GT genotype/rs10800397 T Allele Carrier/ rs10919035 T Allele Carrier
	c.106-38510G>T/c.178-20044C>T/ c.178-20044C>T/c.178-13122C>T	rs10494366 GT genotype/rs10800397 T Allele Carrier/ rs10919035 T Allele Carrier
	WT/c.106-38510G>T	rs10494366 GT genotype/rs10800397 C Allele Carrier/ rs10919035 C Allele Carrier
	c.106-38510G>T/c.178-20044C>T	rs10494366 GT genotype/rs10800397 T Allele Carrier/ rs10919035 C Allele Carrier
	c.106-38510G>T/c.106-38510G>T	rs10494366 TT genotype/rs10800397 C Allele Carrier/ rs10919035 C Allele Carrier
	c.106-38510G>T/c.106-38510G>T/ c.178-20044C>T/c.178-13122C>T	rs10494366 TT genotype/rs10800397 T Allele Carrier/ rs10919035 T Allele Carrier
	WT/WT	rs10494366 GG genotype/rs10800397 C Allele Carrier/ rs10919035 C Allele Carrier
NQO1	WT/WT	rs1800566 non-AA genotype
	WT/c.559C>T	rs1800566 non-AA genotype
OPRM1	WT/WT	rs1799971 A Allele Carrier/rs510679 TT genotype
	WT/c.290+1050C>T	rs1799971 A Allele Carrier/rs510679 non-TT genotype
	WT/c.118A>G	rs1799971 G Allele Carrier/rs510679 TT genotype
	c.290+1050C>T/c.290+1050C>T	rs1799971 A Allele Carrier/rs510679 non-TT genotype
SCN2A	WT/WT	rs2304016 non-GG genotype
SLC6A4	S/S	HTTLPR Short Form
	S/LA	HTTLPR Long Form
	LA/LA	HTTLPR Long Form
	LA/LG	HTTLPR Long Form
	S/LG	HTTLPR Short Form
SLCO1B1	*1/*1	Normal Activity
	*1/*5	Intermediate Activity
TPMT	*1/*1	Normal Metabolizer
UGT1A1	*1/*1	Non *28 Allele Carrier
	*1/*28	Heterozygous *28 Allele Carrier
	*28/*28	Homozygous *28 Allele Carrier
UGT2B15	*1/*1	rs1902023 AA genotype
	*1/*2	rs1902023 non-AA genotype
	*2/*2	rs1902023 non-AA genotype
VKORC1	WT/WT	rs9923231 G Allele Carrier
	WT/-1639G>A	rs9923231 A Allele Carrier
	-1639G>A/-1639G>A	rs9923231 A Allele Carrier
XRCC1	WT/WT	rs25487 T Allele Carrier
	WT/-1639G>A	rs9923231 A Allele Carrier
	c.1196A>G/c.1196A>G	rs25487 C Allele Carrier

APPENDIX 2. REGRESSION EQUATIONS

Gene	Variable	Factor	Regression Equation
AGTR1	Avg. Pain	Genotype	= 6.967 + 1.700 Geneotype_WT/c.*86A>C - 1.700 Geneotype_WT/WT
		Phenotype	= 6.967 - 1.700 Phenotype_rs5186 AA genotype + 1.700 Phenotype_rs5186 AC genotype
HTR2A	Avg. Pain	Phenotype	= 5.788 + 0.412 Phenotype_rs7997012 GG genotype - 0.412 Phenotype_rs7997012 non-GG
SLCO1B1	Avg. Pain	Genotype	= 5.604 + 1.032 Geneotype_*1/*1 - 1.032 Geneotype_*1/*5
		Phenotype	= 5.604 - 1.032 Phenotype_Intermediate Activity + 1.032 Phenotype_Normal Activity
UGT2B15	Avg. Pain	Genotype	= 6.222 + 2.028 Geneotype_*1/*1 - 1.472 Geneotype_*1/*2 - 0.556 Geneotype_*2/*2
		Phenotype	= 6.696 + 1.554 Phenotype_rs1902023 AA genotype - 1.554 Phenotype_rs1902023 non-AA
COMT	Max Pain	Phenotype	= 6.893 - 1.393 Phenotype_MET Homozygous + 1.393 Phenotype_Non MET Homozygous
CYP1A2	Max Pain	Genotype	= 6.747 - 2.747 Geneotype_*1A/*1A + 1.253 Geneotype_*1A/*1F - 2.75 Geneotype_*1C/*1C/*1F/*1F + 1.653 Geneotype_*1C/*1F/*1F + 2.587 Geneotype_*1F/*1F
		Phenotype	= 7.250 + 1.250 Phenotype_High Inducibility Metabolizer - 1.250 Phenotype_Normal
CYP2B6	Max Pain	Genotype	= 7.000 + 1.333 Geneotype_*1/*1 - 4.00 Geneotype_A785G/A785G/G516T + 3.00 Geneotype_A785G/A785G/G516T/G516T - 0.333 Geneotype_A785G/G516T
		Phenotype	= 7.000 - 0.333 Phenotype_G516T Heterozygous/A785G Heterozygous - 4.00 Phenotype_G516T Heterozygous/A785G Homozygous + 3.00 Phenotype_G516T Homozygous/A785G Homozygous + 1.333 Phenotype_Wild Type
DRD1	Max Pain	Genotype	= 6.101 + 1.535 Geneotype_c.-48G>A/c.-48G>A + 2.566 Geneotype_WT/c.-48G>A- 4.10 Geneotype_WT/WT
		Phenotype	= 5.00 - 3.00 Phenotype_rs4532 CC genotype + 3.00 Phenotype_rs4532 non-CC genotype
FAAH	Max Pain	Genotype	= 5.957 - 3.96 Geneotype_c.385C>A/c.385C>A + 2.343 Geneotype_WT/c.385C>A + 1.614 Geneotype_WT/WT
		Phenotype	= 5.957 - 3.96 Phenotype_rs324420 AA genotype + 2.343 Phenotype_rs324420 CA genotype + 1.614 Phenotype_rs324420 CC genotype
MTHFR	Max Pain	Genotype	= 7.489 + 0.844 Geneotype_A1298C/A1298C - 4.99 Geneotype_C677T/A1298C + 1.01 Geneotype_C677T/C677T + 2.01 Geneotype_WT/A1298C + 1.011 Geneotype_WT/C677T+ 0.111 Geneotype_WT/WT
		Phenotype	= 7.489 + 2.01 Phenotype_A1298C Heterozygous Mutation + 0.844 Phenotype_A1298C Homozygous Mutation + 1.011 Phenotype_C677T Heterozygous Mutation - 4.99 Phenotype_C677T Heterozygous Mutation/A1298C Heterozygous Mutation + 1.01 Phenotype_C677T Homozygous Mutation + 0.111 Phenotype_Wild Type
SLCO1B1	Max Pain	Genotype	= 7.390 + 1.247 Geneotype_*1/*1 - 1.247 Geneotype_*1/*5
		Phenotype	= 7.390 - 1.247 Phenotype_Intermediate Activity + 1.247 Phenotype_Normal Activity
CDA	MME	Phenotype	= 187.7 + 94.5 Phenotype_rs532545 C Allele - 94.5 Phenotype_rs532545 T Allele
CNR1	MME	Phenotype	= 153.6 - 102.3 Phenotype_rs806368 non-TT genotype + 102.3 Phenotype_rs806368 TT genotype
CYP1A2	MME	Phenotype	= 219.5 - 95.5 Phenotype_High Inducibility Metabolizer + 95.5 Phenotype_Normal Metabolizer
CYP4F2	PPS%	Genotype	= 47.22 - 5.56 Geneotype_*1/*1 - 7.22 Geneotype_*1/*3 + 12.78 Geneotype_*3/*3
		Phenotype	= 47.22 - 7.22 Phenotype_Intermediate Metabolizer - 5.56 Phenotype_Normal Metabolizer + 12.78 Phenotype_Poor Metabolizer
HLA-B	PPS%	Genotype	= 46.67 - 1.67 Geneotype_WT/*5701 + 8.33 Geneotype_WT/*5801 - 6.67 Geneotype_WT/WT
		Phenotype	= 46.67 - 1.67 Phenotype_HLA-B*5701 Allele Carrier + 8.33 Phenotype_HLA-B*5801 Allele Carrier - 6.67 Phenotype_WT
NOS1AP	PPS%	Phenotype	= 43.57 - 3.57 Phenotype_rs10494366 GG genotype/rs10800397 C Allele Carrier/rs10919035 C Allele Carrier - 10.24 Phenotype_rs10494366 GG genotype/rs10800397 T Allele Carrier/rs10919035 T Allele Carrier + 1.43 Phenotype_rs10494366 GT genotype/rs10800397 C Allele Carrier/rs10919035 C Allele Carrier - 3.57 Phenotype_rs10494366 GT genotype/rs10800397 T Allele Carrier/rs10919035 C Allele Carrier - 1.90 Phenotype_rs10494366 GT genotype/rs10800397 T Allele Carrier/rs10919035 T Allele Carrier + 1.43 Phenotype_rs10494366 TT genotype/rs10800397 C Allele Carrier/rs10919035 C Allele Carrier + 16.43 Phenotype_rs10494366 TT genotype/rs10800397 T Allele Carrier/rs10919035 T Allele Carrier
UGT2B15	PPS%	Genotype	= 41.39 - 1.39 Geneotype_*1/*1 + 6.11 Geneotype_*1/*2 - 4.72 Geneotype_*2/*2

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Vita

Name	*Daniel Bianculli*
Baccalaureate Degree	*Bachelor of Science*, *Fordham University, New York* *Major: Psychology*
Date Graduated	*May, 2006*
Other Degrees and Certificates	*Master of Science,* *Long Island University, C.W. Post, Brookville,* *Major: Biology*
Date Graduated	*May, 2010*