Genetic Factors Associated With Pain Severity, Daily Opioid Dose Requirement, and Pain Response Among Advanced Cancer Patients Receiving Supportive Care

Genetic Factors associated with Pain Severity, Daily Opioid Dose Requirement, and Pain Response among Advanced Cancer Patients receiving Supportive Care

Short Title: Genetics, Cancer Pain and Response

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Abstract

Background: Current understanding of genetic factors associated with pain severity, and improvement of pain with opioids in advanced cancer patients (AC) is inadequate for delivery of personalized pain therapy(PPT). Therefore, the aim of this study was to determine the genetic factors associated with pain severity, daily opioid dose, and pain response in AC patients receiving supportive care.

Methods: In this prospective study, AC patients were eligible if they had cancer pain ≥4/10 on Edmonton Symptom Assessment Scale (ESAS) - Pain Item and needed opioid rotation for pain control by specialist at the outpatient supportive care center. Pain phenotype was assessed using logistic regression models and SKATO (Gene-block) analysis.

Results: 174/178 (98%) patient samples were analyzed. After adjustment for demographic and clinical variables, pain severity was negatively associated with intron variant alleles in OPRM1 rs9322446, P = 0.02; rs2270459, P=0.038; rs62052210, P= 0.038. Opioid daily dose was positively associated NFKBIA rs2233419 P=0.008, rs2233417 P=0.007, rs3138054 P=0.008, rs1050851, P= 0.015 ;ORPM1 rs9479759, P= 0.046, rs2003185, P= 0.047, rs636433, P= 0.044; COMT (rs9306234, P= 0.014, rs165728, P= 0.014, rs2020917, P= 0.036 , rs165728, P= 0.034); ARRB2 (rs1045280, P= 0.045); and pain response to opioids was negatively associated OPRM1 rs1319339 p=0.024, rs34427887 P=0.048, and COMT rs4646316 P=0.03, rs35478083 P=0.028 respectively. SKATO analysis showed association between pain severity and CXCL8 (P=0.0056), and STAT6 (P=0.0297) genes respectively, and pain response with IL-6 (P=0.00499).

Conclusions: This study identified that SNPs of OPRM1, COMT, NFKBIA, CXCL8, IL-6, STAT6,and ARRB2 genes were associated with pain severity, opioid daily dose, and pain response in AC receiving supportive care. Additional studies are needed to validate our findings for PPT.

Key message: This study shows unique SNPs of OPRM1, COMT, NFKBIA, CXCL8, IL-6, STAT6, and ARRB2 genes were associated with cancer pain severity, and pain response after supportive care consultation in advanced cancer patients. Additional studies are needed to validate our findings for personalized pain therapy.

Keywords: Cancer, Pain, Genetics, Single nucleotide Polymorphisms, Pain response, Supportive Care

Introduction

Seventy percent of advanced cancer patients report significant cancer pain. (1,2) Currently, opioids are the first line treatment for cancer pain. (3,4) However, the same systemic opioid drugs have been used for the last 60-280 years, and unfortunately many of these agents have been associated with significant toxicity and even mortality.(5) There is now additional concern of the opioid epidemic, which has revealed the need for a much more personalized and cautious approach in the assessment and management of patients with pain.(6) Prior studies found that the severity of pain, daily opioid dose, and pain response to opioid therapy is often variable.(7-9) Many factors contribute to the variability, and in fact many mechanisms remain unknown. Known factors include patient-related factors, and the factors related to the opioids themselves. (10) Prior studies by our team and other found that many patients have severe pain at their follow up visits despite opioid therapy. (5, 10, 11) Additionally, opioids can have debilitating side effects, and due to changing practices in the current opioid crisis, a vast proportion of advanced cancer patients receive suboptimal doses of opioids by the prescribers, particularly as there is a concern of non-medical opioid use when patients seek higher doses of opioids.(5) There have been studies attempting to find possible genetic markers, but these studies have not been comprehensive.(10) Genetic markers investigated have included drug-metabolizing enzymes, drug transporters, opioid receptors, cyclooxygenases, and genes encoding elements of the pathways involved in the perception and processing of nociceptive information, the modulation of the pharmacokinetics or pharmacodynamic effects of analgesics.(8,12,13) However, few studies have

generated the data necessary to draw conclusive evidence. Only a small number of well-designed prospective studies evaluated the genetic factors associated with cancer pain severity, daily opioid dose requirement, and pain response in patients with advanced cancer. Most studies had significant shortcomings in terms patient selection (phenotype), and sample size, and appropriate characterization of cancer pain. Additionally, up to now, delineation of contributions of individual genetic factors to pain severity, opioid daily dose, and pain response were hampered by the limitations of genotyping techniques, including techniques that allowed analysis of only some polymorphisms at a time. Even high throughput methods, like genome-wide association studies (GWAS) were limited by the ability to analyze only relatively common single nucleotide polymorphisms (SNPs); thus, the comprehensive analysis of the contribution of each genetic variant to the phenotype was not possible.

In this prospective study, our aim was to identify novel genetic factors that are associated with cancer pain severity (pain expression), daily opioid dose, and improvement of pain with opioids in advanced cancer patients receiving outpatient supportive care consultation. These genetic markers might point the way to novel therapeutic targets, risk factors, and provide a key to a more personalized pain management.

Methods

The institutional review board of The University of Texas M.D Anderson Cancer Center approved this protocol, and all participants were provided written informed consent as a condition of enrollment in the trial.

Participants

Patients were enrolled into the study if they met the following eligibility criteria: (a) a diagnosis of advanced cancer (defined as metastatic or recurrent incurable cancer) and seen at the MD Anderson Cancer Center outpatient supportive care clinic. (b) Patient should have a clinically significant pain i.e., ≥ 4/10 on a 0-10 Edmonton Symptom Assessment Scale (ESAS)-Pain item, wherein 0= no pain, 10=the worst possible pain, for the last 24 hours. (c) All patients should have nociceptive or mixed type of cancer pain requiring opioid rotation. (d) Patient should have normal cognition as assessed by Memorial Delirium Assessment Scale score of less than 7/30.

Design and Procedures

In this prospective survey, the patient's demographic history, study assessments were performed at the time of opioid rotation for the control of pain (baseline), at the first and second follow up visit. The assessments included Edmonton Symptom Assessment Scale (ESAS), Brief Pain Inventory (BPI), Memorial Delirium Assessment scale (MDAS), and the Edmonton Classification System for Cancer Pain (ECS-CP). Second follow up visit after opioid rotation for pain control was used as a primary endpoint, as in clinical practice it takes at least two follow ups for optimization of pain.

Assessments

A) **Demographic Data:** age, sex, ethnicity, cancer diagnosis, primary symptom, metastatic site, treatment history, medication history, comorbidities including major depression were assessed.

B) **ESAS:** ESAS is a 0-10 validated tool to assess average severity of common cancer related symptoms in the past 24 hours: pain, fatigue, nausea, depression, anxiety, drowsiness, shortness of breath, appetite, feelings of well-being and "Other Problems." (13) Other problems assessed included constipation, dry mouth, hallucination and myoclonus ("jerks"). These are some of the additional dimensions of the ESAS that were identified as target symptoms in our preliminary study. (14)

C) **BPI:** Rates severity of pain for the last 24 hours. The average pain intensity and interference were also assessed. (15)

D) **CAGE questionnaire** (Cut down, Annoyed, Guilty, Eye opener): The CAGE-AID consists of a 4-item questionnaire. (16,17) Patient scores from ≥ 2 to 4 were considered positive for alcoholism, and also raise concern for potential non-medical opioid use and chemical coping. (18-21).

E) **Performance Status:** The Eastern Cooperative Oncology Group performance status (ECOG) was used to assess the patient's level of functioning, how the patient's disease was progressing, and assess how the disease effected the patient's activities of daily living**.** (22)

F) **Delirium:** Patients' delirium was assessed using the Memorial Delirium Assessment Scale (MDAS). (23) MDAS is a clinician rated 10-item severity rating scale. Each item is scored from 0 to 3 depending on its intensity and frequency (possible range, 0-30). A MDAS cut-off score of 7 out of 30 was associated with the diagnosis of delirium with a sensitivity 98% and specificity of 96%. (24) It has been validated in advanced cancer and other settings. (24)

G) **Pain intensity, opioid dose consumption and pain response:** In this study we analyzed the genetic factors associated with specific pain outcomes which are frequently used to evaluate optimal pain management.(1,3,5-7,10,11) Pain intensity: Pain severity was assessed using ESAS pain item and BPI as described above. As different types of opioids were prescribed such as morphine, hydromorphone, oxycodone, fentanyl, hydrocodone and methadone, we translated the daily opioid dose to morphine equivalent daily dose (MEDD). In this study, for the calculation of MEDD we reviewed the electronic medical records and assessed the opioid dose in past 24 hours at time of opioid rotation for pain control (baseline), first follow up clinical visit after the opioid rotation, and second follow up, and the types of opioids. We used a conversion table shown in Appendix A and calculated daily dose of opioids taken over the past 24 hours. Pain response was calculated

change in pain intensity at the first and second follow up compared to the baseline. Pain response at the second follow up was used as a primary outcome, as in clinical practice it takes at least two follow ups for optimization of pain.

H) **ECS-CP:** This assessment tool has been previously used by our group and has been validated. (25,26) It allows staging of the cancer pain syndrome according to the presence of known poor prognostic factors, such as mechanism of pain, incidental pain, psychological distress and addictive behavior and cognitive function.

I) Molecular Analysis: Targeted massively parallel sequencing

20 ml blood sample was collected into a heparinized vacutainer tube from the consenting patient. DNA was extracted from peripheral blood by QiaAmp DNA mini kit (Qiagen) and quantified by fluorescence with the QuantIT Picogreen DNA assay (Life Technology).

We focused on candidate genes since the association of high dimensional data from whole-exome data to a quantitative variable as pain control can be resolved only with very large datasets of thousands of cases. Therefore, we examined not only on “known polymorphisms” since we completely sequenced the whole coding region + introns + upstream and downstream regions, thus being able to identify also new polymorphisms or private variants. The candidate genes (with chromosome involved, and functional role) include PTGS2[prostaglandin G/H synthase and cyclooxygenase, chromosome 1, pain in lung cancer] (27,28); PLA2G4A [phospholipase A2, group 4A, chromosome 1, acute pain, inflammation] (29); IL1F10 [Interleukin 1 family member 10, chromosome 2, cell signaling] (30); IL-1RN [Interleukin 1 receptor type 1, chromosome 2, cancer pain intensity] (31); CXCL8, IL-8 [C-X-C Motif Chemokine Ligand 8; Interleukin -8, chromosome 4, cancer pain](32,33); TNF [Tumor necrosis factor, chromosome 6, cancer pain] (27); IL-6 [Interleukin 6,chromosome 6, cancer pain] (34); OPRM1 [opioid

receptor mu 1, chromosome 6, cancer pain, response to opioids] (35, 36); ABCB1[ATP-binding cassette; chromosome 7; opioid response, neuropathic pain](37); STAT6 [Signal Transducer And Activator Of Transcription 6, chromosome 12, interaction with NFKBIA](39); LRP1[Low density lipoprotein receptor-related protein 1, chromosome 12, migraine] (40); NFKB1A [nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; chromosome 14, cancer pain] (27); SAMD4A [Sterile Alpha Motif Domain 4A, chromosome 14, chronic postsurgical pain] (41]; GCH1[GTP cyclohydrolase 1, chromosome 14, cancer pain] (42); WDHD1 [HMG-box DNA binding protein 1, chromosome 14, pain sensitivity] (41), TCF25 [Transcription factor 25, chromosome 16, chronic postsurgical pain] (43); MCIR [melanocortin 1 receptor, chromosome 16, pain and analgesia] (44); ARRB2[β-arrestin 2, chromosome 17, neuropathic pain, opioid tolerance] (45); TXNRD2 [thioredoxin reductase 2, chromosome 22, temporomandibular disorder pain] (46); COMT[Catechol-O-methyltransferase, chromosome 22, cancer pain, opioid response] (36,47), WDD1[WD repeat and HMG-box DNA binding protein 1, chromosome 14, , chronic postsurgical pain] (41], and CYP2D6 [cytochrome p450, family 2, subfamily D, polypeptide 6, chromosome 22, opioid response] (44). [details of chromosome regions involved are reported in Appendix B]

The custom assay for targeted enrichment was designed with SureSelect XT Custom library prep kit with a total of 2695 probes and a total probe size of 93.5 kbp covering coding sequences, exon-flanking, 5'and 3' UTR, and specific polymorphic intronic regions of 15 genes (Appendix B) associated to pain severity perception, daily opioid dose, and improvement in pain in cancer settings (Agilent Technologies).

Briefly, genomic DNA was enzymatically fragmented, and adaptors were added to ends of the fragments. Then, purified adaptor-tagged DNA libraries were amplified, and target regions were captured by hybridization to specific biotin-labelled oligonucleotide probes. Finally, captured libraries were amplified, indexed and purified, then quantified by picogreen assay and sized with High Sensitivity

DNA kit (Agilent Technologies), pooled and sequenced at 151 bp in paired end using custom QXT sequencing primers on a MiSeq instrument (Illumina). We multiplexed around 70 samples per run on a MiSeq v2 flowcell, including 1% of PhiX library spike-in, thus reaching an average target depth of coverage of 259X (56X - 731X).

Base calling and demultiplexing was performed with the Illumina bcl2fastq conversion software. Burrows-Wheeler Aligner (BWA) was used in order to align the DNA sequenced reads against the human reference genome hg19 and point variants calling was performed with the tool GATK (Haplotype Caller function). Each called variant was annotated with a gene name, gene location, and a "rs" tag from dbSNP data bank adopting the bioinformatic tool Annovar. Allele frequency in human population was achieved from the ExAC project. This work flow allowed to call a total of 851 variants (ABCB1 n=48; ARRB2 n=50; COMT n=86; CXCL8 n=15; CYP2D6 n=98; GCH1 n=43; IL1RN n=58; IL6 n=27; LRP1 n=8; MC1R n=63; NFKBIA n=28; OPRM1 n=230; PTGS2 n=40; STAT6 n=48; TNF n=9).

Statistical Analyses

Summary statistics were performed for all factors. Factors included demographics, ESAS, BPI, ECS-CP, CAGE, ECOG performance status, MEDD and Opioid induced side-effects. Analgesic response to opioids was assessed by opioid daily consumption (MEDD), and pain response. Pain response was defined as a 2-point decrease or 30% in the ESAS pain score.

Logistic regression analysis was used to examine the association of one SNP/variant at a time with pain outcomes. SKATO analysis reported results on the set of SNPs/variants in the targeted region (or the selected gene block), which reflects the significance (or insignificance) of the combined SNPs/variants in the block. The logistic regression model included patients age, sex, MEDD (at the time of opioid rotation), CAGE, ESAS depression, Charleson comorbidity index, tumor type, and SNP, so as to examine

the association of SNP/variant and pain response. We used logistic regression to model change in pain severity and morphine daily dose (as a continuous variable) in a manner similar to the above primary analysis. Optimized Sequence Kernel Association Test [SKATO(Gene-block) analyses] was used to test rare variants, e.g. the allele frequency of the variant is less than 0.01 or lower, in association with the phenotype of interest (48,49). In comparison with standard individual variant test such as GWAS which tests association between of phenotypes with common causal genetic variants, this method usually tests the association of variants in a defined region such as a gene block, and it could capture different variants (in low frequency) in the defined region across a group of individuals (patients) while separate tests of these variants may not be possible. Applying this method to this data set may provide supporting information of the selected gene in association with the phenotypes (pain).

Statistical significance was based on additive genetic model. Due to preliminary nature of the study, correction for multiple testing was not considered. A significance level of 5% (two-sided) was used for all analyses. Statistical analyses will be performed using SPSS 15.0 for Windows (SPSS, Chicago, IL), SAS 9.2 (Cary NC), and R 2.14 (Vienna, Austria).

Sample size and Power: For 94% power to detect an OR of 2.5 (an improvement from 30% response for wildtype (wt) to 52% response for variant) using logistic regression at alpha=0.05 assuming equal numbers of variant and wt for the marker in question, we estimated a sample size of 250 study participants. In addition, since the genes of interest had approximately 20% to 50% variants, we assumed that with 30% variants our power was reduced to 92%, and if we assumed 20% variant our power was reduced to 82%. Due to limited funding, only 174 samples were analyzed. With a sample size of N=174, we still have 80% power to detect an OR of 2.5 at alpha=0.05. Sample size calculation was assessed using NCSS PASS 2005, and PS Version 3.0.

Results

A total of 174/178 (98%) of the patient samples available for genetic analysis were evaluable. Figure 1 shows details of the patients eligible for the study, enrolled, and total number of patients included in the genetic analysis.

Table 1 lists the demographic and clinical characteristics. The median age (IQR) was 60 (49, 66), male gender 49% (n=88), non-Hispanic white 73% (n=131), most common cancer type was lung cancer 35% (N=63). Median (IQR) ESAS pain scores at baseline was 7 (5, 7). The median (IQR) for opioid dose (MEDD) at baseline, first follow-up and 2nd follow-up was 87 (45,135), 90 (54.4, 173.8), and 90 (52.5, 180) respectively. The most common opioid sequences used were (1) Hydrocodone to Morphine (10%), (2) Hydrocodone to Fentanyl (4.5%), (3) Hydromorphone to Oxycodone (4.5%), (4) Oxycodone to Morphine (4.5%), (5) Hydromorphone to Morphine (3.9%), (5) Hydrocodone to Hydromorphone (3.4%), and Hydromorphone to Methadone(3.4%).

Table 2 shows the change in pain scores compared to opioid rotation (baseline) at the 1st and 2nd follow up visits. The median improvement (IQR) in ESAS pain at the 1st supportive care clinic follow-up visit was -1 (-3, 1), and at 2nd follow-up visit (primary endpoint) was -1 (-4, 0).

After adjustment for demographic and clinical variables using logistic regression analysis, pain severity was negatively associated with intron variant alleles in OPRM1 rs9322446, P = 0.02; rs2270459, P=0.038; rs62052210, P= 0.038. Opioid daily dose was positively associated with intron variant alleles in NFKBIA rs2233419 P=0.008, rs2233417 P=0.007, rs3138054 P=0.008, rs1050851, P= 0.015 ;ORPM1 rs9479759, P= 0.046, rs2003185, P= 0.047, rs636433, P= 0.044; COMT (rs9306234, P= 0.014, rs165728, P= 0.014, rs2020917, P= 0.036 , rs165728, P= 0.034); ARRB2 (rs1045280, P= 0.045); and pain response with opioids in patients receiving supportive care was negatively associated with OPRM1 rs1319339 p=0.024, rs34427887 P=0.048, and COMT rs4646316 P=0.03, rs35478083 P=0.028 [Table 3]. SKATO analysis

showed association between pain severity and CXCL8 (P=0.0056), and STAT6 (P=0.0297) genes respectively, and pain response with IL-6 (P=0.00499) [Table 4].

Discussion

Advanced cancer patients have significant variability in cancer pain severity, daily opioid requirement, and pain response with opioids receiving supportive care. In this study, unlike previous studies investigating genetic factors associated with pain severity, daily opioid requirement, and pain response were limited to few single nucleotide polymorphisms (SNP), we were the first to examine genetic factors associated with pain severity perception, daily opioid dose, and improvement in pain in advanced cancer patients in a single tertiary cancer center using Burrows-Wheeler Aligner to align the DNA sequenced reads against the human reference genome hg19. This molecular analysis enabled us to not only examine the "known polymorphisms," but also able to identify new polymorphisms or private variants as we were able to completely sequence the whole coding region + UTRs + upstream and downstream regions. This strategy enabled us to capture previously unknown variants that could influence pain severity, MEDD, and improvement in pain. In this study we found that cancer pain severity was negatively associated with intron variant alleles in OPRM1 rs9322446, rs2270459, rs62052210, CXCL2, STAT6 genes, and opioid daily dose (MEDD) was positively associated intron variant alleles in NFKBIA rs2233419, rs2233417, rs3138054, rs1050851, ORPM1 rs9479759, rs2003185, rs636433, COMT (rs9306234,rs165728, rs2020917, rs165728), ARRB2 (rs1045280), and improvement in pain with opioids in patients receiving supportive care was negatively associated OPRM1 rs1319339, rs34427887, COMT rs4646316, rs35478083, and IL-6 gene.

This study was unique in that all patients underwent not only a very thorough genetic assessment but also a very sophisticated clinical evaluation and state of art cancer pain management by the specialists. In contrast to heterogeneity seen in prior studies, the pain phenotypes were uniquely characterized to

capture the most evidence-based understanding of the complex nature of cancer pain. This included rigorous selection of a more homogenous advanced cancer population with cancer pain, and collection of the data prospectively using validated tools at a single setting. These measures were undertaken to effectively reduce phenotypic variability. Moreover, cancer pain treatment for these advanced cancer patients was provided by a homogenous practice: specialist-driven cancer pain management in a single institution. In this context, we were able to leverage more rigorous assessment compared to SNP assessment in prior studies.

Prior studies found significant association of specific SNP's and cancer pain severity. Reyes-Gibby et al., (50) found an association of SNPs in the cytokines gene interleukin (IL)-8 (-251T/A) SNP was significantly associated with pain severity in mixed lung cancer. The same group (34) found that in advanced cancer patients receiving supportive care, there was a significant association between cancer pain severity and SNPs of cytokine genes tumor necrosis factor (TNF) 308GA AA, and IL-6 174 CC. In another study, researchers found that the CC genotypes for PTGS2 gene (10+837T>C (rs5275) SNP was associated with lower pain severity, NFKBIA Ex6+50C>T (rs8904), and TNF-α -308GA (rs 1800629) were associated with pain severity(27). Reyes-Gibby et al., (2009) also found that SNPs in the IL-8 gene (-251T/A) SNP was associated with pain severity in pancreatic cancer patients.(33) Rausch et al. (2012) found that SNPs in PTGS2 (rs5277, rs5275), and LTA (rs1799964) have been associated with increased pain severity in lung cancer patients. (28) McCann et al., (2012) found that SNPs in IL 1-receptor 1 (IL1R1) (rs2110726) were less likely to report pain due to breast cancer, and SNPs IL-13 (rs1295686) were associated with increased pain due to breast cancer. (51) Oliveira et al., found SNPs in IL-*1B* rs1143634 was associated with lower pain severity in metastatic cancer patients. (52) Cajanus et al., (2016) found that SNPs in fatty acid amid hydrolase (FAAH) gene rs 324420, rs 1571138, rs 3766248, and rs 4660928 were significantly associated with cold sensitivity. (53) In contrast, our study we found that SNPs in the OPRM1 gene (rs9322446, rs2270459, rs62052210), CXCL8 (encoding IL-8), and STAT6 gene that were significantly

associated with severity of cancer pain expression in phenotypically homogenous advanced cancer patients. This discovery might represent a target gene for identifying patient populations likely to have a higher nociceptive burden or to express nociception more severely. Importantly, our data provide fodder for additional research to investigate how genetic factors impact phenotypic pain expression.

Our second analysis involved targeted factors associated with the opioid dose, and identified SNPs that were associated with daily opioid dose (MEDD) to achieve improvement in pain. This contrasted with previous studies that found associations between alterations in some of these same genetic factors and opioid dose, but at different sites within the genes. (12, 31,35-37,47,54,55) For example, Klepstad and colleagues found that patients with a homozygous OPRM1 118 A>G polymorphism required more morphine to achieve pain control, compared to heterozygous and homozygous wild-type. (35) Likewise, Rakvag et al., (2005) found that carriers of Val/Val genotype higher MEDD when compared to the Val/Met and the Met/Met genotypes. (56) Reyes-Gibby et al., (2007) found COMT(rs4680), Val/Val and Val/Met required higher MEDD compared to Met/Met genotypes, and *OPRM1(rs1799971)* GG and AG required higher opioid doses compared to AA genotypes(50). The same team (34) found that IL-6 -174C/C genotypes required 4.7 times MEDD for pain relief relative to GG and GC genotypes. Rakvag et al., (2008) found that patients with carriers of A alleles for COMT (rs 4818, rs4680) were associated with lower MEDD requirement. (47) Klepstad et al., (2011) in international multicenter study found no significant association between MEDD and 112 known SNP's associated with cancer pain including OPRM1, and COMT genotypes. (39) Matsuaka et al.,(2012) found MEDD requirement was significantly lower for the A/A genotype of COMT compared to A/G+G/G genotypes.(57) Gutteridge et al., (2018) found that TAOK3 (rs 277441, rs 795484) SNPs were associated with high MEDD (≥800mg) in advanced cancer patients admitted in the palliative care unit. (58) Oliviera et al. (2012) found that COMT (rs4680) Val58Met SNP was associated with higher MEDD requirement(52). Cajanus et al., found that an OPRM1 polymorphism 118A>G (rs1799971) was associated with postoperative oxycodone consumption. (59)

Hajj et al. found that AG genotype c.118A>G *OPRM1* needed a higher MEDD requirement than AA patients. (60) These variation in the MEDD requirement may be due variability in opioid receptors such as having fewer responsive receptors, or due to variability in the rate of development of tolerance or hyperalgesia, or other opioid induced side-effects such as drowsiness, confusion, hallucinations and myoclonus with adequate number of mu receptors resulting higher opioid dose required for same improvement in pain. Future studies are needed to better characterize the genetic factors associated with MEDD required for improvement in pain based on the preliminary results found in our study.

Finally, we identified unique SNPs associated with improvement of pain with opioids in cancer patients receiving supportive care to achieve personalized pain therapy including OPRM1 rs1319339, rs34427887, and COMT rs4646316, rs35478083, and STAT6 gene. In contrast, prior studies found that improvement in pain was associated with SNPs in *IL-6* −174GC and *IL-8 −251T/A* (34), 3435C>T SNPs of the *ABCB1/MDR1* gene (a major determinant of morphine bioavailability), A118G polymorphism of *OPRM1* (50), SNPs of *RHBDF2* gene(rs12948783). (9) However, our study was unable to compare the differential improvement in pain to various mu agonist receptor agonists. Future well-powered studies should characterize whether rotation to specific opioids, and the genetic factors identified in this study will help in refining those clinical trials that lead to the development of personalized opioid therapies in advanced cancer patients with complex pain. In addition, further well powered studies are required to determine the strength of these association and percentage of genetic factors contribution overall cancer improvement in pain.

Are we ready to translate the findings to clinic?

From a clinical perspective, it is important to consider that there are many clinical (stage of disease, previous opioid treatment, particularly undertreatment, drug-specific response), psychological, therapeutic decisions (changing dose or drug, slowly or rapidly, depends on a clinical individual

decision), and other confounding factors that may influence the pain severity, daily opioid dose, and pattern of the clinical pain response overlapping the possible direction provided by individual genetic profile. Therefore, this study which offers a tantalizing glimpse into the mechanisms and targets of pain, and genetic variants represent only one important facet that may contribute to decision making in cancer pain management. The current challenge is that we are only on the cusp of comprehensively analyzing the genetic factors influencing cancer pain in the advanced cancer setting. Our study used a more comprehensive method, and although there is a need to replicate the findings in larger, well-powered studies, it represents a major step toward bringing personalized care to advanced cancer patients with pain and designing intelligent, effective analgesic strategies. Additionally, one-point decrease in pain severity (0-10 ESAS scale) found in our study may be a limitation in patients with higher pain severity (e.g., 7/10 or more). Also, in situations of more severe pain patients more intensive approach in an inpatient setting may be required. Further studies are needed.

Conclusions

This study identified SNPs of OPRM1, COMT, NFKBIA, CXCL8, IL-6, STAT6, and ARRB2 genes were associated with pain severity, daily opioid dose, and pain response in advanced cancer patients receiving outpatient supportive care consultation by a supportive/palliative care specialist. Additional studies are needed to validate our findings for personalized pain therapy.

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Table 1. Demographic and Clinical Characteristics (N=178)	
Age (Median-IQR)	60 (49, 66)
Gender % (N)	**N (%)**
Male	88 (49)
Race/Ethnicity % (N)	
Black Non-Hispanic	20(11.2)
Hispanic	22(12.4)
White Non-Hispanic	131 (73.16)
Cancer Diagnosis	**N(%)**
Breast	11 (6.2)
Gastrointestinal	62 (34.8)
Genitourinary	11(6.2)
Gynecological	10(5.6)
Head & Neck	11(6.2)
Lung	63(35.4)
Leukemia	2(1.1)
Myeloma	2(1.1)
Sarcoma	1(.6)
Skin	5(2.8)
ECOG	
0-2	124(71)
3-4	51(29)
Current Treatments	
Chemotherapy	80(44.9)
Radiation	41(23.0)
Targeted Therapy	54(30.3)
Immunotherapy	18(10.1)
Clinical Characteristics	**Median (IQR)**
MDAS score	1 (0, 2)
Morphine Equivalent Daily Dose (MEDD)	87 (45-135)
CAGE (Positive/Negative)	
Positive (≥2/4)	23.6%
Negative (<2/4)	76.4%
ECS-CP	
Mechanism of Pain	
No- no pain syndrome	1.1%
Nc- any nociceptive combination of visceral and/or bone or soft tissue pain	98.9%

Table 1. Demographic and Clinical Characteristics (N=178)	
Ne- Neuropathic pain syndrome with or without any combination of nociceptive pain	9.0%
Incident Pain	
Io- no incident pain	63.5%
Ii- incident pain present	32.6%
Psychological Distress	
Po-no psychological distress present	71.9%
Pp- psychological distress present	23.6%
Addictive Behavior	
Ao- No addictive behavior	89.9%
Aa- addictive behavior present	5.1%
Cognitive Function	
Co- no impairment	92.1%
Ci-partial impairment	2.2%
Baseline symptoms	**Median (IQR)**
BPI (Severity)	4.5 (3,5.75)
BPI (Interference)	5.1 (2.6,6.8)
Charleston Comorbidity Index	10 (8,12)
ESAS Symptoms	
Pain	7 (5,7)
Fatigue	6 (4,8)
Nausea	1 (0,5)
Depression	2 (0,4)
Anxiety	2 (0,5)
Drowsy	4 (1.25,6)
Appetite	4 (2,7)
Feeling of Well Being	5 (3,6)
Shortness of Breath	2 (0,5)
Sleep	5 (2,7)
Financial Distress	2 (0,5)
Spiritual Pain	0 (0,2)
OIN ESAS Symptoms	
Dry Mouth	3.0 (0,6)
Jerking	0.0 (0,1)
Hallucination	0.0(0,1)
Constipation	0.0(0,5)

Table 1. Demographic and Clinical Characteristics (N=178)

Abbreviations:

ECOG: assessment of performance status using Eastern Cooperative Oncology Group scale; MDAS: Memorial Delirium Assessment Scale; CAGE: measure for alcoholism, Cut down, Annoyed, Guilty, Eye opener; ECS-CP: The Edmonton Classification System for Cancer Pain; BPI: Brief Pain Inventory; ESAS: Edmonton Symptom Assessment Scale. OIN: Opioid induced neurotoxicity symptoms.

Table 2. Change in Edmonton Symptom Assessment Scale (ESAS) Scores at First and Second Supportive Care Clinic Follow-up Visit.

ESAS items	**(1st Follow-up visit – Baseline)**			**(2nd Follow-up visit – Baseline)**		
	Median	IQR		Median	IQR	
		25	75		25	75
Pain	-1.00	-3.00	1.00	-1.00	-4.00	0.00
Fatigue	-0.50	-2.00	1.00	0.00	-2.00	1.00
Anxiety	0.00	-2.00	1.00	0.00	-2.00	1.00
Depression	0.00	-1.00	0.00	-3.00	-5.75	-1.00
Anorexia	0.00	-2.00	1.00	0.00	-2.00	2.00
Drowsiness	0.00	-2.00	2.00	0.00	-2.00	2.00
Feeling of Well Being	0.00	-2.00	1.00	0.00	-2.00	1.00
Sleep Disturbance	0.00	-2.00	1.00	0.00	-2.50	1.50
Financial Distress	0.00	-1.00	0.50	0.00	-2.00	1.00
Spiritual Pain	0.00	0.00	0.00	0.00	-1.00	0.00

Table 3. Association between Genetic factors and Pain severity, Daily opioid dose, Improvement in pain

SNP	Stat	CI (LCL, UCL)	p-value
Pain Severity			
OPRM1 (rs9322446)	-2.37	(-1.33, -0.12)	.019
OPRM1 (rs2270459)	2.09	(.04, 1.352)	.038
OPRM1 (rs62052210)	2.09	(.04, 1.352)	.038
Opioid Daily Dose (MEDD)			
NFKBIA (rs2233419)	2.69	(10.38, 65.73)	.008
NFKBIA (rs2233417)	2.73	(11, 66.73)	.007
ORPM1 (rs9479759)	2.00	(.94, 83.56)	.046
ORPM1 (rs2003185)	2.00	(.43, 43.46)	.047
ORPM1 (rs636433)	2.01	(.98, 78.76)	046
NFKBIA (rs3138054)	2.67	(10.33, 66.93)	.008
NFKBIA (rs1050851)	2.46	6.62, 58.85)	.015
ARRB2 (rs1045280)	2.01	(.61, 46.88)	.045
s2020917	-2.12	(-51.33, -2.00)	.036
COMT (rs4646317)	-2.20	(-45.93, -2.64)	.292
COMT (rs9306234)	-2.49	(-49.15, -5.83)	.014
COMT (rs165728)	-2.13	(75.03, 2.13)	.034
Improvement in pain			
OPRM1 (rs1319339)	-2.27	(-2.15, -0.16)	.024
OPRM1 (rs34427887)	-1.99	(-3.21, -0.02)	.048
COMT (rs4646316)	-2.09	(-1.54, -0.05)	.038
COMT (rs35478083)	-2.21	(-3.30, -0.19)	.029

Abbreviations:

SNP: Single nucleotide polymorphism; Cl-confidence interval; MEDD: Morphine equivalent daily dose. OPRM1: opioid μ 1 receptor protein coding gene; NFKBIA: NF-kappa-B inhibitor alpha coding gene; COMT: Catechol-O-methyltransferase coding gene

Table 4. SKATO-Gene Block Analysis

Chr	Gene	Pain Severity	MEDD	Pain response	No. of SNPs in the Gene block	No. of SNPs Tested
		p-values	p-values	p-values		
1	PTGS2	0.87	0.65	0.28	17	17
2	IL-1R	0.80	0.51	0.68	35	35
4	CXCL8 (IL-8)	**0.0056**	1.00	0.72	4	4
6	TNF	1.00	0.78	0.63	4	4
6	OPRM1	0.83	1.00	0.59	87	86
7	IL-6	0.33	1.00	**0.0499**	11	11
7	ABCB1	0.53	0.73	0.65	18	18
12	STAT6	**0.0297**	1.00	1.00	18	18
12	LRP1	0.55	0.42	0.72	2	2
14	NFKBIA	0.17	0.87	0.117	11	11
14	GCH1	0.355	0.324	0.865	17	17
16	MC1R	0.51	0.55	0.86	25	25
17	ARRB2	0.16	0.90	1.00	18	18
22	COMT	0.28	0.88	0.0814	45	45
22	CYP2D6	0.674	1.00	0.82	43	34

Abbreviations:
SKATO: Optimized Sequence Kernel Association Test; SNP: Single nucleotide polymorphism; Chr: Chromosome; MEDD: Morphine equivalent daily dose; PTGS2: prostaglandin G/H synthase and cyclooxygenase ; IL-1R: Interleukin 1 receptor; CXCL8 encoding IL-8: C-X-C Motif Chemokine Ligand 8; TNF: Tumor necrosis factor; IL-6: Interleukin 6; OPRM1: opioid receptor mu 1; ABCB1: ATP-binding cassette; STAT6: Signal Transducer And Activator Of Transcription 6; LRP1: Low density lipoprotein receptor-related protein 1; NFKB1A: nuclear factor of kappa light polypeptide gene enhancer; GCH1: GTP cyclohydrolase 1; MCIR: melanocortin 1 receptor; ARRB2: β-arrestin 2; COMT: Catechol-O-methyltransferase, and CYP2D6 (cytochrome p450, family 2, subfamily D, polypeptide 6.

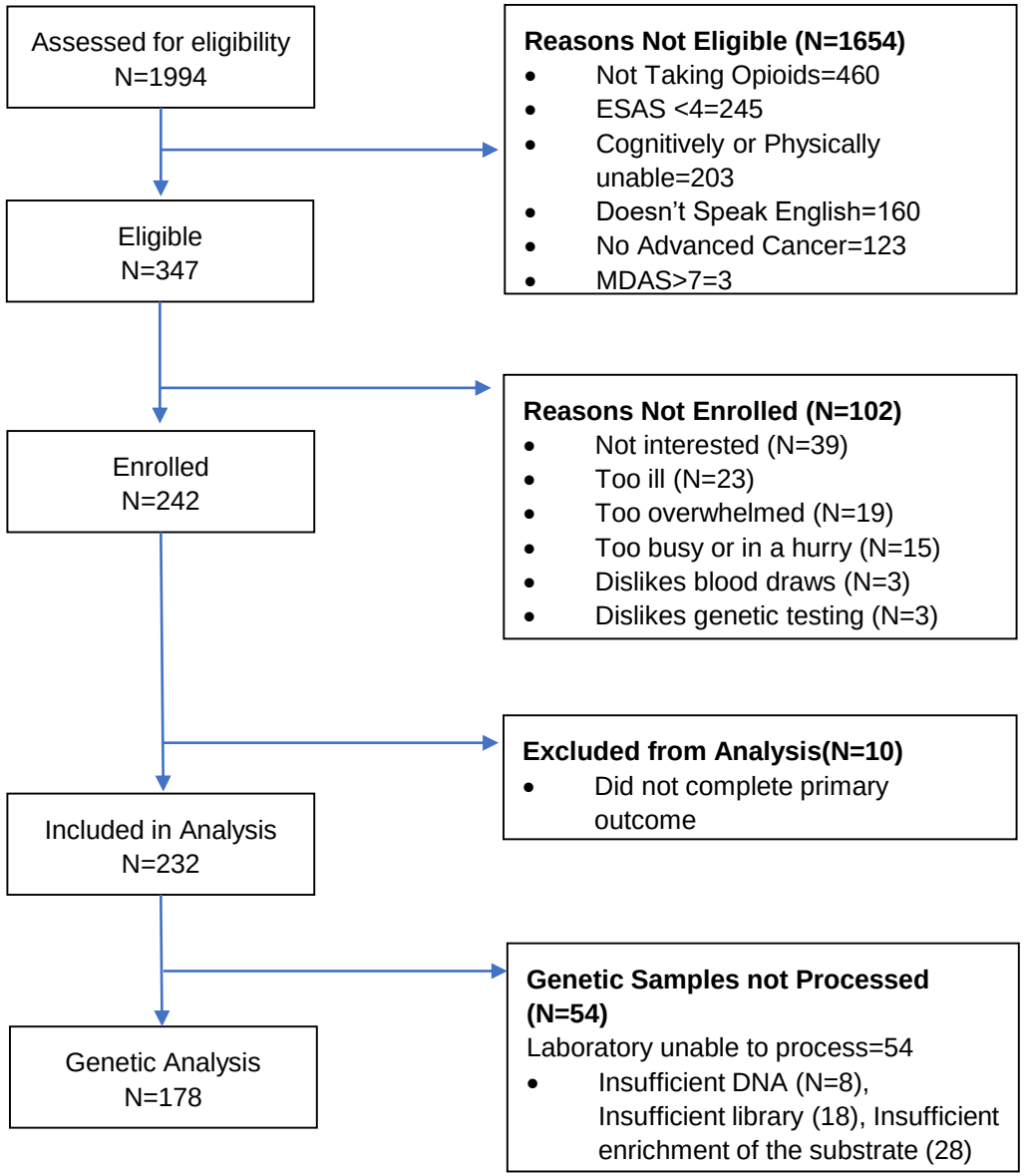

Figure 1.
Study Enrollment and Analysis

Appendix A

Morphine Equivalent Daily Dose in Milligrams (MEDD) Calculation

Opioid Dose Conversion Table

Opioid	PO	IV
Morphine	1	2.5
Hydrocodone	1	NA
Hydromorphone	5	10
Oxycodone	1.5	NA
Oxymorphone	3	NA
Fentanyl	NA	If Patch, multiply patch dose in mcg by 2 to get MEDD in mg
		If IV, divide 24 h dose in mcg, multiply by 10, and then multiple by 2.5 to get MEDD in mg
Methadone	5	10
Tramadol	0.1	NA
Codeine	0.15	NA

Appendix B. Cancer Pain Related Genes included in the Target Panel for Genetic Analysis				
Chromosome	**Start position**	**End position**	**Gene**	**Location**
chr1	186640911	186641271	PTGS2	UTR3
chr1	186641375	186643415	PTGS2	UTR3
chr1	186643433	186643913	PTGS2	exonic
chr1	186644360	186645852	PTGS2	exonic
chr1	186645916	186646096	PTGS2	exonic
chr1	186646751	186646991	PTGS2	exonic
chr1	186647344	186647584	PTGS2	exonic
chr1	186648141	186648381	PTGS2	exonic
chr1	186648391	186648631	PTGS2	exonic
chr1	186649344	186649584	PTGS2	exonic
chr1	186650231	186650411	PTGS2	upstream
chr1	186651786	186651966	PTGS2(dist=2227),PLA2G4A(dist=146066)	intergenic
chr2	113864756	113864996	IL1F10(dist=31329),IL1RN(dist=10474)	intergenic
chr2	113868644	113868824	IL1F10(dist=35217),IL1RN(dist=6646)	intergenic
chr2	113870486	113870726	IL1F10(dist=37059),IL1RN(dist=4744)	intergenic
chr2	113873054	113873294	IL1F10(dist=39627),IL1RN(dist=2176)	intergenic
chr2	113874987	113875167	IL1RN	upstream
chr2	113875184	113875364	IL1RN	upstream
chr2	113875417	113875657	IL1RN	exonic
chr2	113877626	113877819	IL1RN	exonic
chr2	113878989	113879229	IL1RN	UTR5
chr2	113885107	113885347	IL1RN	exonic

chr2	113886824	113889093	IL1RN	exonic
chr2	113890215	113890875	IL1RN	exonic
chr2	113891133	113891683	IL1RN	UTR3
chr4	74605893	74606073	IL8	upstream
chr4	74606210	74606450	IL8	exonic
chr4	74607238	74607509	IL8	exonic
chr4	74607528	74608103	IL8	exonic
chr4	74608146	74608326	IL8	exonic
chr4	74608422	74608637	IL8	UTR3
chr4	74608697	74608949	IL8	UTR3
chr4	74609053	74609473	IL8	UTR3
chr6	31542941	31543121	TNF	upstream
chr6	31543313	31543733	TNF	exonic
chr6	31544243	31544423	TNF	exonic
chr6	31544477	31544657	TNF	exonic
chr6	31544879	31545779	TNF	exonic
chr6	31545790	31546150	TNF	UTR3
chr6	154331062	154331242	OPRM1	upstream
chr6	154331591	154332191	OPRM1	exonic
chr6	154348515	154348695	OPRM1	intronic
chr6	154360212	154360992	OPRM1	exonic
chr6	154367746	154367948	OPRM1	intronic
chr6	154367993	154368113	OPRM1	intronic
chr6	154393048	154393228	OPRM1	intronic
chr6	154394628	154394868	OPRM1	intronic
chr6	154395114	154395338	OPRM1	intronic
chr6	154404607	154404795	OPRM1	intronic
chr6	154407598	154408968	OPRM1	UTR5
chr6	154410864	154411434	OPRM1	exonic

chr6	154411914	154413129	OPRM1	exonic
chr6	154413584	154413764	OPRM1	intronic
chr6	154414379	154414679	OPRM1	exonic
chr6	154415278	154415518	OPRM1	intronic
chr6	154420967	154421147	OPRM1	intronic
chr6	154428569	154428749	OPRM1	exonic
chr6	154428895	154429015	OPRM1	exonic
chr6	154429342	154429462	OPRM1	UTR3
chr6	154429759	154430214	OPRM1	UTR3
chr6	154431405	154431681	OPRM1	exonic
chr6	154439779	154443979	OPRM1	exonic
chr6	154444283	154444403	OPRM1	UTR3
chr6	154444432	154444612	OPRM1	UTR3
chr6	154445187	154446927	OPRM1	UTR3
chr6	154447217	154447984	OPRM1	UTR3
chr6	154448236	154450104	OPRM1	UTR3
chr6	154450602	154450722	OPRM1	UTR3
chr6	154450770	154451006	OPRM1	UTR3
chr6	154451010	154451310	OPRM1	UTR3
chr6	154451583	154453591	OPRM1	UTR3
chr6	154567793	154568033	OPRM1	exonic
chr7	22765381	22765978	IL6	upstream
chr7	22766555	22766735	IL6	upstream
chr7	22766736	22767276	IL6	exonic
chr7	22768083	22768449	IL6	exonic

chr7	22769093	22770231	IL6	exonic
chr7	22771013	22771313	IL6	exonic
chr7	22771331	22771553	IL6	UTR3
chr7	87133139	87133799	ABCB1	exonic
chr7	87135184	87135484	ABCB1	exonic
chr7	87138573	87138813	ABCB1	exonic
chr7	87144523	87144763	ABCB1	exonic
chr7	87145782	87146022	ABCB1	exonic
chr7	87148147	87148327	ABCB1	intronic
chr7	87148615	87148855	ABCB1	exonic
chr7	87150072	87150498	ABCB1	exonic
chr7	87160591	87161071	ABCB1	exonic
chr7	87165725	87165905	ABCB1	exonic
chr7	87166326	87166506	ABCB1	intronic
chr7	87168532	87168712	ABCB1	exonic
chr7	87170606	87170846	ABCB1	exonic
chr7	87173397	87173637	ABCB1	exonic
chr7	87174106	87174346	ABCB1	exonic
chr7	87175139	87175379	ABCB1	exonic
chr7	87178628	87178868	ABCB1	exonic
chr7	87179149	87179629	ABCB1	exonic
chr7	87179718	87179958	ABCB1	exonic
chr7	87179977	87180217	ABCB1	exonic
chr7	87183042	87183282	ABCB1	exonic
chr7	87190520	87190760	ABCB1	exonic
chr7	87195351	87195591	ABCB1	exonic
chr7	87196076	87196316	ABCB1	exonic
chr7	87199423	87199603	ABCB1	exonic
chr7	87214791	87215031	ABCB1	exonic
chr7	87225015	87225195	ABCB1	exonic
chr7	87229379	87229559	ABCB1	exonic
chr7	87230052	87230412	ABCB1	UTR5
chr7	87232245	87232485	ABCB1	intronic
chr7	87342437	87342677	ABCB1	UTR5
chr12	57489175	57490572	STAT6	exonic
chr12	57490576	57491079	STAT6	exonic
chr12	57492243	57492423	STAT6	exonic
chr12	57492515	57493235	STAT6	exonic
chr12	57493497	57493915	STAT6	exonic

chr12	57496055	57496295	STAT6	exonic
chr12	57496505	57496745	STAT6	exonic
chr12	57497628	57498397	STAT6	exonic
chr12	57498462	57498642	STAT6	exonic
chr12	57498907	57499147	STAT6	exonic
chr12	57499227	57500132	STAT6	exonic
chr12	57500246	57500664	STAT6	exonic
chr12	57500965	57501145	STAT6	exonic
chr12	57501336	57501576	STAT6	exonic
chr12	57501893	57502133	STAT6	exonic
chr12	57503713	57504365	STAT6	UTR5
chr12	57504838	57505078	STAT6	UTR5
chr12	57505083	57505323	STAT6	UTR5
chr12	57522699	57522939	LRP1	exonic
chr12	57525742	57525982	LRP1	intronic
chr14	35870690	35871290	NFKBIA	exonic
chr14	35871562	35872102	NFKBIA	exonic
chr14	35872188	35872668	NFKBIA	exonic
chr14	35872711	35873191	NFKBIA	exonic
chr14	35873195	35873435	NFKBIA	intronic
chr14	35873611	35873971	NFKBIA	exonic
chr14	55306367	55306547	SAMD4A(dist=46334),GCH1(dist=2177)	intergenic
chr14	55306714	55306894	SAMD4A(dist=46681),GCH1(dist=1830)	intergenic
chr14	55308690	55310893	GCH1	exonic
chr14	55312437	55312617	GCH1	exonic
chr14	55313761	55313941	GCH1	exonic
chr14	55326336	55326516	GCH1	exonic
chr14	55331979	55332219	GCH1	exonic
chr14	55358575	55358755	GCH1	intronic
chr14	55360049	55360229	GCH1	intronic
chr14	55369004	55369604	GCH1	exonic
chr14	55371489	55371669	GCH1(dist=1947),WDHD1(dist=33987)	intergenic
chr14	55373580	55373760	GCH1(dist=4038),WDHD1(dist=31896)	intergenic
chr14	55378901	55379081	GCH1(dist=9359),WDHD1(dist=26575)	intergenic
chr16	89978489	89979089	TCF25	downstream
chr16	89979611	89980331	TCF25(dist=1819),MC1R(dist=3956)	intergenic
chr16	89981391	89981631	TCF25(dist=3599),MC1R(dist=2656)	intergenic
chr16	89984260	89987408	MC1R	exonic
chr17	4613761	4614061	ARRB2	exonic

chr17	4617821	4618361	ARRB2	exonic
chr17	4618418	4618658	ARRB2	intronic
chr17	4619014	4619374	ARRB2	exonic
chr17	4619412	4619952	ARRB2	exonic
chr17	4619954	4620149	ARRB2	exonic
chr17	4620472	4621068	ARRB2	exonic
chr17	4621153	4621693	ARRB2	exonic
chr17	4621854	4622034	ARRB2	exonic
chr17	4622526	4622766	ARRB2	exonic
chr17	4623486	4623786	ARRB2	exonic
chr17	4623799	4623979	ARRB2	exonic
chr17	4624217	4624817	ARRB2	exonic
chr17	4625069	4625249	ARRB2	downstream
chr22	19928002	19928182	TXNRD2	intronic
chr22	19928794	19928974	TXNRD2	intronic
chr22	19929067	19929482	TXNRD2	exonic
chr22	19930019	19930211	COMT	intronic
chr22	19938378	19938618	COMT	UTR5
chr22	19938925	19939266	COMT	UTR5
chr22	19942907	19943087	COMT	intronic
chr22	19945087	19945267	COMT	intronic
chr22	19948014	19948254	COMT	intronic
chr22	19948676	19948856	COMT	UTR5
chr22	19948923	19949103	COMT	intronic
chr22	19949188	19949428	COMT	intronic
chr22	19949726	19950518	COMT	exonic
chr22	19950790	19950970	COMT	intronic
chr22	19951065	19951305	COMT	exonic
chr22	19951617	19952778	COMT	exonic
chr22	19953143	19953323	COMT	intronic
chr22	19954651	19955251	COMT	intronic
chr22	19956028	19957528	COMT	exonic
chr22	42522484	42526924	CYP2D6	whole gene

Abbreviations: Chr: chromosome; UTR: untranslated region; PTGS2: prostaglandin G/H synthase and cyclooxygenase ; PLA2G4A: phospholipase A2, group 4A; IL1F10: Interleukin 1 family member 10; IL-1RN: Interleukin 1 receptor antagonist 1; IL-8: Interleukin -8; TNF: Tumor necrosis factor; IL-6: Interleukin 6; OPRM1: opioid receptor mu 1; ABCB1: ATP-binding cassette; STAT6: Signal Transducer And Activator Of Transcription 6; LRP1: Low density lipoprotein receptor-related protein 1; NFKB1A: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; SAMD4A: Sterile Alpha Motif Domain 4A; GCH1: GTP cyclohydrolase 1; WDHD1: WD repeat and HMG-box DNA binding protein 1; TCF25: Transcription factor 25; MCIR: melanocortin 1 receptor; ARRB2: β-arrestin 2;

TXNRD2: thioredoxin reductase 2; COMT: Catechol-O-methyltransferase, and CYP2D6 (cytochrome p450, family 2, subfamily D, polypeptide 6.

Appendix C

Allele Frequencies of all the Single Nucleotide Polymorphisms (variants) Included in Genetic Analysis

Chromosome	Single Nucleotide Polymorphism	Base Pair	Minor Allele	Major Allele	MAF (minor allele freq)
6	rs1319339	154,010,049	C	T	0.1222
6	rs589046	154,072,003	T	C	0.2921
6	rs9322446	154,087,567	A	G	0.1433
6	rs9479759	154,094,300	T	C	0.0791
6	rs2003185	154,126,837	C	T	0.4633
6	rs636433	154,131,351	A	G	0.09322
6	rs34427887	154,246,729	T	C	0.05337
12	rs56214329	57,104,187	A	G	0.0565
12	rs73118440	57,110,414	T	G	0.0565
14	rs2233419	35,402,754	A	G	0.1751
14	rs2233417	35,402,888	T	C	0.1695
14	rs3138054	35,403,101	T	C	0.1723
14	rs2233416	35,403,559	A	G	0.05932
14	rs1050851	35,403,720	A	G	0.2062
14	rs12885400	54,891,921	C	T	0.05932
14	rs7147286	54,891,947	A	G	0.3305
14	rs3783642	54,893,485	C	T	0.3904
14	rs3759664	54,904,861	T	C	0.2175
14	rs3759665	54,904,887	T	C	0.3305
14	rs8007267	54,912,273	T	C	0.236
16	rs2270459	89,913,443	A	C	0.1102
16	rs8060848	89,913,551	G	A	0.4548
16	rs62052210	89,913,750	G	A	0.1102

16	rs62052211	89,913,908	T	C	0.4463
16	rs3212354	89,917,962	T	C	0.4463
16	rs3212357	89,918,196	T	C	0.4466
16	rs3212358	89,918,331	A	G	0.4435
16	rs1805005	89,919,436	T	G	0.113
16	rs1805008	89,919,736	T	C	0.06742
17	rs1045280	4,719,343	C	T	0.3551
22	rs2020917	19,941,361	T	C	0.2388
22	rs6269	19,962,429	G	A	0.387
22	rs2239393	19,962,905	G	A	0.3876
22	rs4646316	19,964,609	T	C	0.2599
22	rs4646317	19,964,645	A	G	0.3933
22	rs9306234	19,965,665	C	A	0.4011
22	rs4646318	19,967,324	A	G	0.05056
22	rs35478083	19,969,362	C	T	0.05337
22	rs165728	19,969,500	C	T	0.08146
22	rs1135840	42,126,611	C	G	0.4576
22	rs4987144	42,127,001	A	G	0.3146
22	rs28371730	42,127,207	T	C	0.3174
22	rs2004511	42,127,209	C	T	0.1921
22	rs1985842	42,127,407	T	G	0.4574
22	rs1058172	42,127,526	T	C	0.06369
22	rs2267447	42,128,694	C	T	0.191
22	rs3892097	42,128,945	T	C	0.1348
22	rs28371705	42,129,796	C	G	0.1364
22	rs28371704	42,129,809	C	T	0.1364
22	rs28371703	42,129,819	T	G	0.1343
22	rs28371702	42,129,950	A	C	0.4645

22	rs1081000	42,130,547	C	T	0.1911
22	rs1065852	42,130,692	A	G	0.1921
22	rs769258	42,130,761	T	C	0.0618