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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.

Editorial Note: David Casarett MD MA

This is an interesting and provocative study of genetics and opioid effects, which is likely to be controversial as it raises more questions than it answers.

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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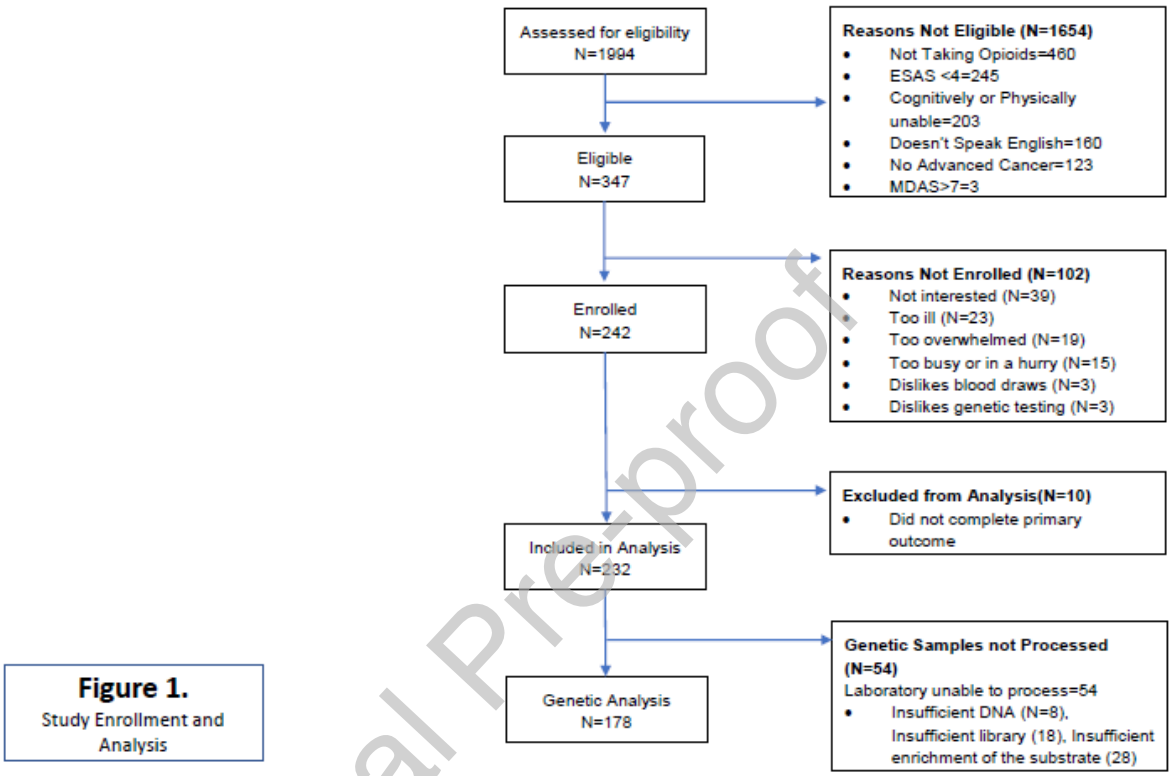

Figure 1.
Study Enrollment and Analysis

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)

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

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

Table 1. Demographic and Clinical Characteristics (N=178)	
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	-2.21	(-3.30, -0.19)	.029

(rs35478083)

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.