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The role of inflammation gene polymorphisms on pain severity in lung cancer patients

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Abstract

Many of the same inflammatory factors that promote tumor growth are also hypothesized to function as pain modulators. There is substantial interindividual variation in pain severity in cancer patients. Therefore, we evaluated 59 SNPs in 37 inflammation genes in newly diagnosed non-Hispanic Caucasian lung cancer patients (n=667) and assessed their association with pain severity. Patients rated their pain "during the past week" on an 11-point numeric scale, (0= 'no pain' and 10= 'pain as bad as you can imagine') at presentation, prior to initiating cancer therapy. Reported analgesic use was abstracted from charts and converted to an equivalent dose of morphine (MEDD). Results showed that 16% of the patients reported severe pain (score ≥ 7). Advanced stage of disease (OR=2.34; 95% CI=1.50-3.65, p-value=0.001), age≤ 50 (OR=2.10; 95%CI=1.32-3.30, p-value=0.002), reports of depressed mood (OR=3.68; 95%CI=1.96-6.93, p-value=0.001); fatigue (OR=3.72; 95% CI=2.36-5.87, p-value=0.001) and MEDD (OR=1.02; 95% C.I=1.01, 1.03) were significantly correlated with severe pain. Controlling for these non-genetic covariates, we found that patients with CC genotypes for *PTGS2* exon10+837T>C (rs5275) were at lower risk for severe pain (OR=0.33; 95% Confidence Interval=0.11-0.97) and an additive model for *TNF α* -308GA (rs1800629) (OR=1.67, 95% CI=1.08,2.58) and *NFKBIA* Ex6+50C>T (rs8904) was predictive of severe pain (OR=0.64, 95% CI=0.43,0.93). In a multi-gene analysis, we found a gene-dose effect, with each protective genotype reducing the risk for severe pain by as much as 38%. This study suggests the importance of inflammation gene polymorphisms in modulating pain severity. Additional studies are needed to validate our findings.

Keywords

Pain; Genes; Inflammation; Epidemiology; Cancer

Introduction

Pain is one of the most devastating, persistent and incapacitating symptoms in patients with lung cancer. Patients with advanced lung cancer suffer from significantly higher levels of physical and mental symptoms compared to patients with most other solid tumors. As many as 80% of patients with newly diagnosed lung cancer present with pain prior to any cancer

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treatment and of whom, 17% report pain of severe intensity(1). Severe pain is reported by 41% of patients with advanced lung cancer(2). Cancer pain often occurs at multiple sites and duration can extend from months to years (3). Because of its high prevalence and the frequency with which patients with lung cancer present in an incurable stage, symptom management is a large component of the care of these patients.

Among cancer patients, chronic inflammation acts as a tumor promoter, resulting in aggressive tumor growth and spread. Many of the same inflammatory factors that promote tumor growth are also hypothesized to function as pain modulators not just in inflamed tissues, but also in damaged peripheral nerves. The activation of inflammatory cells, for example, is classically associated with pain, heat, redness, swelling and loss of function. It is now suggested that following tissue damage or inflammation, inflammatory molecules including cytokines and chemokines also directly sensitize the peripheral terminals of sensory nerves (peripheral sensitization), thus lowering their pain activation threshold (4-6). Elevated cytokine levels, such as interleukin (IL) 6 and IL8 are observed in patients with chronic pain conditions including back pain(7), post-herpetic neuralgia(8), and unstable angina. IL1 and IL2 levels have likewise been implicated in pain response (9,10) and suggested to contribute to variation in postoperative morphine requirements (11) and in complex regional pain syndrome(12). IL4 is correlated with the presence of chronic widespread pain(13) and the association of IL10 level with pain and its potential role in pain therapy has also been suggested(14-17) TNF α has an important role in cancer-related symptoms including pain facilitation and enhancement (18-20). The prolonged presence of increased levels of IFN-gamma in the central nervous system contribute to the generation of central sensitization and persistent pain by reducing inhibitory tone in the dorsal horn(21-23). Taken together, these studies provide evidence of the critical role of the immune system in chronic pain states.

Single nucleotide polymorphisms (SNP) in the inflammation genes have been shown to alter their expressions or functions and thus may be associated with an altered risk for pain severity. Indeed our group and others have shown polymorphisms in *IL6*, *Tumor Necrosis* α, and *IL8* to influence pain severity(1,2). However, these studies only assessed one or a few select candidate genes at a time. Given that pain is a complex trait, multiple genes are likely to influence vulnerability to pain. Therefore, a pathway-based genotyping approach, which assesses polymorphisms in several genes that interact in the same pathway, may provide more robust results. In this study, we therefore evaluated a comprehensive panel of 59 single nucleotide polymorphisms (SNP) in 37 inflammation genes in newly diagnosed non-Hispanic Caucasian lung cancer patients (n=667) and assessed their association with pain severity. We also assessed the extent to which clinical and demographic factors explain pain severity in this population. Because genetic polymorphisms are stable markers, understanding the extent to which genetic variability plays a role in cancer-related pain may prove useful in identifying patients at high-risk for pain and importantly, could help in understanding patients who might benefit most from symptom intervention, and ultimately in developing personalized and more effective pain therapies.

Materials and Methods

Study Subjects

The study sample was drawn from an ongoing previously described case-control study of lung cancer(24). Case patients with newly diagnosed histologically-confirmed non-small cell lung cancer were recruited at the time of initial registration at the Cancer Center prior to initiation of any cancer treatment. There were no restrictions with regard to age, sex, ethnicity, or disease stage. All cases were residents of the United States. The overall response rate for the study was 80%. For this analysis, we used data from patient enrolled from 1999-2005 and for whom pain and genetic data were available. Because of issues associated with population stratification,

we focused our analyses on 667 white Caucasian patients. This study was approved by the Institutional Review Board at M. D. Anderson Cancer Center and all participants provided written informed consent.

Epidemiology, symptoms, and clinical data collection

Trained M.D. Anderson staff interviewers collected demographic, clinical and symptom data prior to initiation of radiotherapy or chemotherapy. Patients rated their pain on an 11-point numeric scale, (0= 'no pain' and 10= 'pain as bad as you can imagine') (25), a standardized method for assessing pain. Because studies show a high correlation between depression, fatigue, and pain, we also assessed depressed mood and fatigue using the following items "during the past 4 weeks, have you felt downhearted and blue?" and "during the past 4 weeks, did you have a lot of energy?" These items were taken from the SF-12. The SF-12 is a validated measure of quality of life and is extensively used in studies of cancer patients(26-29). Data including stage of disease and history of co-morbid conditions (heart disease, stroke, diabetes, etc.) were abstracted from patients' charts.

Pain Medications

Charts were reviewed for information on opioid dose by a Supportive Care Specialist (S.Y.). Due to the different types of opioids reported, we translated the daily opioid dose to a standardized measure, morphine equivalent daily dose (MEDD). We used the conversion factors shown in Table 1 to calculate the total dose of opioids.

Blood collection and molecular analysis

After the interview was completed, a 40 mL blood sample was drawn into coded heparinized tubes. Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion, followed by isopropanol extraction and ethanol precipitation. DNA samples were stored at −80°C. We selected for genotyping SNPs in immune-response genes that met at least two of three criteria: (*a*) minor allele frequency of at least 5%; (*b*) location in the promoter, untranslated region (UTR), or coding region of the gene; and (*c*) previous report of an association with pain severity. All SNPs were genotyped using SNPlex, a technology developed by Applied Biosystems that enables simultaneous genotyping of up to 48 SNPs in a single tube using an oligonucleotide ligation assay and previously described(30).

Statistical analyses

Descriptive statistics were used to summarize patient characteristics. The Kolmogorov-Smirnov Z test was used to assess normality distribution for pain severity. Since normality was not met, we used the National Comprehensive Cancer Network (NCCN) cut-off score for severe pain(31). (A score ≥ 7 is considered as a pain emergency and treatment is initiated with short-acting opioids).

Non-genetic Correlates

We used logistic regression to assess associations between severe pain status and demographic (age and sex), clinical (stage of disease) and symptom variables (depressed mood, fatigue). Variables found to be associated with severe pain at $P<0.05$ were included in subsequent analyses.

Genetic Correlates

We used multivariable logistic regression to assess associations between severe pain status and each SNP, adjusting for demographic (age and sex), clinical (stage of disease), and symptom (depressed mood, fatigue) variables found to be associated with severe pain. We focused on

SNPs for which there was a statistically significant ($P < 0.05$) effect in an additive model (i.e., trend in pain risk with increasing copies of the less common, "mutant" allele), or for which there was also a significant association with severe pain for the mutant allele under a dominant model. We also examined recessive models.

Haplotype Analysis

Because there is a high degree of linkage disequilibrium between the three PTGS2 SNPs (D′ was 0.99 between exon10-90C>T and exon10+837T>C, 0.95 between exon10+837T>C and -765G>C, and 0.64 between exon10-90C>T and -765G>C), we inferred the haplotypes consisting of these three SNPs for each patient using the available software PHASE v2.1.1 (32). We assessed for significant associations using the two-sided binomial exact test.

Results

There were a total of 677 white Caucasian patients with previously untreated and histologically-confirmed non-small cell lung cancer. Mean age was 61 years (SD=12). There was about an equal distribution of the sample between early stage (Stage I-IIIA; n=325) and late stage of disease (Stage IIIB-IV; n=321). There were more men (n=351) than women (n=326) and hypertension was the most prevalent co-morbid condition.

Sixteen percent of the patients reported severe pain. MEDD (Table 2) computed as the total dose of opioids from the analgesic use reported at the time of presentation was between 0-1000 mg/24h, mean of 6.05 (SD=47.25). As expected, Table 2 shows that severe pain was more prevalent among those with advanced stage of disease (OR=2.34; 95% CI=1.50-3.65, p-value=0.001), younger age (OR=2.10; 95%CI=1.32-3.30, p-value=0.002), reports of depressed mood (OR=3.68; 95%CI=1.96-6.93, p-value=0.001) and fatigue (OR=3.72; 95% CI=2.36-5.87, p-value=0.001). There was a borderline association for sex (females OR= 1.43, 95% CI=0.99-2.16 (p-value=0.06).

We evaluated 59 SNPs in the 37 immune-response genes, adjusting for the non-genetic correlates (stage of disease, age, sex, MEDD, fatigue, depressed mood). We observed that patients with CC genotypes for *PTGS2* exon10+837T>C (rs5275) were at lower risk for severe pain (OR=0.33; 95% Confidence Interval=0.11-0.97) and an additive model for *TNF α* -308GA (rs1800629) (OR=1.67, 95% CI=1.08,2.58) and *NFKBIA* Ex6+50C>T (rs8904) was predictive of severe pain (OR=0.64, 95% CI=0.43,0.93). In the multigene model for severe pain (Table 3, Panel B), we found that only *TNF α* -308GA significantly predicted severe pain. *PTGS2* exon10+837T>C and *NFKBIA* Ex6+50C>T were borderline significant (p<0.06).

Gene Dose Effect

We also assessed the extent to which the number of protective alleles influences pain severity by combining the allelic information for *TNF α* -308GA, *PTGS2* exon10+837T>C and *NFKBIA* Ex6+50C>T (Table 4, Panel A). Table 4 shows that each protective allele decreased the risk for severe pain by as much as 38% (Table 4, Panel B) which even after adjustment for the non-genetic variables (i.e, MEDD, stage of disease, age, sex, depressed mood and fatigue)., suggesting a gene-dose effect.

Haplotype Analyses

When we assessed for association between a particular haplotype/diplotype status and pain severity, we did not find significant association between the PTGS2 haplotypes and severe pain (Table 5)

Multiple Comparisons

To address the multiple comparison problem, we calculated the false positive report probability (FPRP) for the SNPs (*PTGS2* exon10+837T>C (rs5275), *TNF α* -308GA (rs1800629) and *NFKBIA* Ex6+50C>T (rs8904) that were found to be significant. The FPRP is the probability that the significant finding is false(33). FPRP calculations depend on the observed p-value for the association, prior probability that the association between the genetic variant and the disease is real and the statistical power of the test. For our analyses, we assumed a range of prior probabilities from 0.01 to 0.10. For the statistical power calculations, we used observed odds ratios of each specific SNPs. The noteworthiness of an association is defined as having FPRP value below 0.5 for initial exploratory studies(33). Table 6 gives noteworthiness of our significant association under different prior probabilities. From this table, we see that our observed association is noteworthy for prior probabilities that are greater than 0.05 (for TNF A -308 GA and NFKappa B Ex6+50 C>T) and 0.10 (for PTGS2 exon 10+837 T>C) (considered to be moderate prior probability).

Discussion

Although previous studies have shown the influence of inflammation-related genes on pain severity in several disease conditions, these studies only assessed a few candidate genes and with small sample sizes. In this study, we conducted a systematic assessment of the influence of a larger number of polymorphisms in inflammation related genes on pain severity in a large sample of newly-diagnosed, previously untreated patients with non-small cell lung cancer. We found that functional variants of the *PTGS2* exon10+837T>C (rs5275), *TNF α* 308GA (rs1800629) and *NFKBIA* Ex6+50C>T (rs8904) contribute to pain severity. The most significant finding was that in analysis of the joint effects, the number of observed protective genotypes was associated with a reduced risk in a dose-response manner, with each protective genotype reducing the risk for severe pain by as much as 38%.

We also observed a significant association with polymorphisms in TNF-308 G/A and pain severity. The -308 polymorphism is a G → A substitution and reportedly affects gene expression, the rare A allele resulting in higher TNF production(34). TNF-*α* has been suggested to be critical for the development of inflammatory pain behavior in animal models. The novel therapeutic potential of TNF inhibitors has also been suggested for conditions such as brain cancer, epilepsy, and chronic pain(35-38). Anti-TNF therapy has also been shown to be profoundly analgesic, with an efficacy similar to that of cyclooxygenase 2 inhibition, and reduced astrocyte activity in collagen induced arthritis(37).

Importantly, carriers of the homozygous variant genotype (CC) of PTGS2 exon10+837T>C exhibited significantly protective effect (OR=0.32) for severe pain. Specifically, carriers of CC genotypes had 64% reduced risk for severe pain relative to carriers of the TT and TC genotypes, even when demographic, clinical and other symptom variables were taken into account.

The PTGS2 gene encodes the proinflammatory cyclooxygenase (COX) 2 enzyme. Exon10 +837T>C of the PTSG2 gene is a functional SNP, that modulates expression of COX2. Subjects with the variant genotypes of exon10+837T>C were observed to have lower steady-state PTGS2 mRNA level than those with the homozygous wild-type [mean ± SE: 15.96 ± 2.82 versus 33.02 ± 14.66](39). COX-2 is inducible and upregulated during an inflammatory response. Cox-2 is rapidly induced by growth factors, cytokines, and proinflammatory molecules, and is involved in prostanoid production under acute and chronic inflammatory conditions as well as in neurodegenerative processes, ischemia, normal neuronal functioning, neurotoxicity, and synaptic plasticity(40). Peripheral elevation of COX-2 after tissue injury contributes to increased prostaglandin E(2) at the site of injury and leads to pain onset. Indeed,

COX-2 is a therapeutic target for pain. Inhibition of COX-2 enzymatic activities is responsible for the anti-inflammatory properties of aspirin, indomethacin, ibuprofen and related NSAIDs, such as Vioxx (Rofecoxib) and Celebrex (Celecoxib).

We found that polymorphisms in NF-kappa-B inhibitor alpha (NFKappaBIA) gene were also predictive of severe pain. NF-κB is activated upon noxious stimulation and contributes to pain hypersensitivity by increasing the transcription of 'pain-related' genes such as Cox 2 and proinflammatory cytokines. Animal studies show that NF-κB inhibition attenuates the nociceptive response in models of neuropathic pain.(41,42) Intrathecal pre-treatment of rats with NF-κB inhibitors reduced spinal NF-κB activation and subsequent expression of COX-2 mRNA thereby suppressing hyperalgesia following unilateral hind paw inflammation(43)

Consistent with our previous studies(44), we found depressed mood and fatigue were also significant correlates of pain. Several studies have addressed the relationship between depression, fatigue and pain and found these symptoms to co-occur. Although the causal relationship between these symptoms remains debatable, studies have shown that symptoms such as pain, are in fact, associated with depressive disorders or psychological distress and anxiety (45-47). It has also been hypothesized that a shared biological mechanism may underlie the co-occurrence of these symptoms(48). Among the implications of these findings is the need to address symptoms such as depressed mood and fatigue in order to improve upon pain severity as well as study of potential common underlying genetic mechanisms for both pain and depressed mood.

While this study has a relatively large number of patients, there remains concern about the issue of false negative findings (failed to detect SNPs with small contribution to pain severity). One could also argue that pain is a heterogeneous outcome, with a variety of causes - e.g., neuropathic pain is different from pain related to pressure from a large tumor, which is different from pain related to stretching of a capsule; thus a 0-10 pain severity/intensity rating is a global measure of pain, that does not delineate if the pain measured is of a neuropathic or nociceptive type of pain. However, evidence suggests that cancer pain is typically of a mixed pain mechanism, with only a small proportion of cancer patients suffering from pure neuropathic pain at diagnosis. A review of pain studies(49) in lung cancer patients, for example, found that neuropathic pain accounted for 30% (range 25-32%) of cases, with nociceptive pain as the major pathophysiological subtype in lung cancer pain. Furthermore, while neuropathic pain may occur due to a malignant invasion of neurological structures (including pancoast tumours), NP in cancer patients occurs as a late effect of treatment with vinca alkaloids, taxanes, platinum-derived compounds, radiotherapy, or surgery. Given that our study focused on newly diagnosed lung cancer patients, who have not had any cancer treatment, misclassification of the type of cancer pain (of whether nociceptive or neuropathic) was greatly attenuated. We also acknowledge that there is more genetic variation for each gene than is captured in this study. The selective choice of SNPs for each gene limited our ability to perform more extensive haplotype analyses. In conducting the FPRP analyses, we found that our observed association is noteworthy for initial studies, and therefore, should be assessed in confirmatory studies.

In conclusion, despite advances in pain treatment and management for cancer, a significant number of patients continue to suffer from severe and persistent pain. While epidemiological, clinical and psychological factors have been shown to influence pain and its treatment, we have also shown in a preliminary fashion that variation in pain severity and pain treatment response may be partially attributed to host genetic variability. Future studies with larger cohorts are needed to validate our findings.

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Appendix A. Single Nucleotide Polymorphisms and Severe Pain

Proinflammatory cytokines, receptors, and related molecules		**p-value***
IL1A C-889T	rs1800587	0.47
IL1A Ala^{114}Ser	rs17561	0.46
IL1B C-511T	rs16944	0.11
IL1B T-31C	rs1143627	0.15
IL1B C3954T	rs1143634	0.37
IL1R1 Ala^{124}Gly	rs2228139	0.36
IL2 T-330G	rs2069762	0.03
IL2RB Asp^{391}Glu	rs228942	0.84
IL6 G-174C	rs1800795	0.36
IL6R Asp^{358}Ala	rs8192284	0.94
IL8 T-251A	rs4073	0.06
IL8RA Ser^{276}Thr	rs2234671	0.21
IL12B A1188C	rs3212227	0.38
IL12RB Met^{365}Thr	rs375947	0.47
IL16 T-295C	rs4778889	0.56
IL16 Asn^{446}Lys	rs17875535	0.55
TNFA T-1031C	rs1799964	0.15
TNFA T-857C	rs1799724	0.38
TNFA G-308A	rs1800629	0.03
TNFA A-238C	rs361525	0.35
TNFB Arg^{13}Cys	rs2857713	0.03
TNFB His^{51}Pro	rs3093543	0.73
TNFR1 G-610T	rs4149570	0.96
TNFR1 Arg^{121}Gln	rs4149584	0.35
TNFR2 Met^{196}Arg	rs1061622	0.46
TNFR2 Glu^{232}Lys	rs5746026	0.06
IFNAR1 Val^{168}Leu	rs2257167	0.80
IFNAR2 Phe^{10}Val	rs7279064	0.98
IFNG T-1615C	rs2069705	0.34
IFNG A874T	rs2430561	0.97
GM-CSF T-1916C	rs2069614	0.91
GM-CSF Ile^{117}Thr	rs25882	0.10
MCP1 A-2518G	rs1024611	0.45
MIF G-173C	rs755622	0.32
Anti-inflammatory cytokines, receptors, and related molecules		

Proinflammatory cytokines, receptors, and related molecules		**p-value***
IL4 C-590T	rs2243250	0.20
IL4 5′-UTR(C/T)	rs2070874	0.70
IL4R Ile^{75}Val	rs1805010	0.30
IL4R Glu^{400}Ala	rs1805011	0.99
IL4R Ser^{503}Pro	rs1805015	0.79
IL4R Gln^{576}Arg	rs1801275	0.90
IL4R Ser^{752}Ala	rs1805016	0.96
IL5 C-745T	rs2069812	0.10
IL10 A-1082G	rs1800896	0.07
IL10 C-819T	rs1900871	0.67
IL10 C-592A	rs1800872	0.33
IL10RA Ser^{159}Gly	rs3135932	0.18
IL10RB Lys^{47}Glu	rs2834167	0.92
IL13 C-1112T	rs1800925	0.63
IL13 Arg^{130}Gln	rs20541	0.63
Prostaglandins and nitric oxide		
PTGS2 G-765C	rs20417	0.45
PTGS2 exon10+837T>C	rs5275	0.022
PTGS2 exon10-90C>T	rs689470	0.80
INOS Leu^{608}Ser	rs2297518	0.47
ENOS Glu^{298}Asp	rs1799983	0.14
Intracellular signaling molecules		
IKB C-420T	rs2233409	0.26
IKB 3′-UTR(C/T)	rs8904	0.01
PPARA Leu1^{162}Val	rs1800206	0.97
PPARD 5′-UTR(T/C)	rs2016520	0.96
PPARG Pro^{12}Ala	rs1801282	0.09

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Table 1
Morphine equivalent daily dose (MEDD) conversion factors

Opioid with route and dose	Conversion Factor	MEDD
Morphine PO 1 mg	1	1 mg
Morphine IV 1 mg	3	3 mg
Hydromorphone PO 1 mg	5	5 mg
Hydromorphone IV 1 mg	10	10 mg
Oxycodone PO 1 mg	1.5	1.5 mg
Methadone PO 1 mg	10	10 mg
Methadone IV 1 mg	10	10 mg
Fentanyl transdermal 1 μg/h	2	2 mg
Fentanyl IV 1 μg	0.3	0.3 mg

Note: The total dose of opioids reported at the time of presentation was converted to an equivalent oral morphine dose in milligrams using the conversion factors shown above. The conversion factor for methadone is variable, and there is no single consensus conversion factor for this drug. For the purpose of this study, we used a conversion factor of 10.

Abbreviations: IV, intravenous; PO, oral.

Table 2

Characteristics of the non-Hispanic Caucasians lung cancer cases (*N* = 677)

Variable	Pain Severity		
	Severe/Non-Severe	**Odds Ratio (95% Confidence Interval)**	**p-value**
Stage of Disease			
Early Stage	34/291	1.0	
Advanced Stage	69/252	2.34 (1.50-3.65)	0.001
Age			
>50	71/462	1.0	
≤ 50	35/109	2.10 (1.32-3.30)	0.002
Sex			
Male	47/304	1.0	
Female	59/267	1.43 (0.99-2.16)	0.06
COMORBIDITIES			
Heart disease			
No	65/371	1.0	
Yes	27/115	1.34 (0.82-2.19)	0.15
Diabetes			
No	87/447	1.0	
Yes	57/39	0.66 (0.25-1.72)	0.39
Hypertension			
No	61/301	1.0	
Yes	31/185	0.83 (0.51-1.32)	0.42
Stroke			
No	88/461	1.0	
Yes	4/25	0.84 (0.28-2.46)	0.54
Lung Disease			
No	68/340	1.0	
Yes	24/146	0.82 (0.49-1.36)	0.44
SYMPTOMS			
Depressed Mood**			
None-to-Mild	84/499	1.0	

Variable	Pain Severity		
	Severe/Non-Severe	**Odds Ratio (95% Confidence Interval)**	**p-value**
Moderate to Severe	18/29	3.68 (1.96-6.93)	0.001
Fatigue***			
None to Mild	32/327	1.0	
Moderate to Severe	70/192	3.72 (2.36-5.87)	0.001
Opiod dose	Range= 0, 1000		
MEDD	Mean = 6.05	1.02 (1.01, 1.03)	0.001
	SD=47.25		

* Pain was measured using the item from the Brief Pain Inventory "During the past week, please rate your pain on a scale of 0 to 10. (0 is no pain and 10 is pain as bad as you can imagine)?" None-to-moderate pain=score of 0-6; severe pain= score of 7 to 10.

** Depressed mood was measured using the item from the SF-12 "during the past 4 weeks have you been feeling downhearted and blue?" Response options were "none of the time; little of the time; some of the time; good bit of the time; most of the time; all of the time" None to mild: "none of the time; little of the time; some of the time; good bit of the time; Moderate to severe= combined response options "most of the time; all of the time".

*** Fatigue was measured using the item from the SF-12 ""During the past 4 weeks, have you had a lot of energy?" Response options were "none of the time; little of the time; some of the time; good bit of the time; most of the time; all of the time" None to mild: "most of the time; all of the time; some of the time; good bit of the time; Moderate to severe= combined response options "none of the time; little of the time".

Table 3

Single Nucleotide Polymorphisms and Severe Pain

		Panel A: Single SNP		Panel B: Multivariable SNPs	
Single Nucleotide Polymorphisms	**Severe/Non-Severe**	**Odds Ratio 95% Confidence Interval**	**p-value**	**Odds Ratio 95% Confidence Interval**	**p-value**
***NFKBIA* Ex6+50C>T rs8904**					
CC	**48/215**	0.64 (0.43, 0.93)	0.02	0.69 (0.47, 1.02)	0.06
CT	**47/269**				
TT	**8/83**				
PTGS2 exon10+837T>C rs5275					
TT	**45/243**	0.33 (0.11, 0.97)	0.04	0.35 (0.12, 1.05)	0.06
TC	**48/236**				
CC	**7/74**				
***TNFA* G-308A rs1800629**					
GG	**64/398**	1.67 (1.08, 2.58)	0.02	1.64 (1.05, 2.56)	0.02
GA	**34/155**				
AA	**5/13**				

Adjusting for stage of disease, age, sex, MEDD, depressed mood and fatigue

Table 4

Gene Dose Effect of TNF A -308 GA, PTGS2 exon10+837T>C, and NFKappa B Ex6+50C>T on Severe Pain.

PANEL A: Unadjusted			
Variables	**P-value**	**Odds Ratio**	**95% C.I.**
Number of Protective Allele (0-5)	0.001	0.83	0.71-0.96
PANEL B: Adjusted			
Variables	P-value	Odds Ratio	95% C.I.
Number of Protective Allele (0-5)	0.001	0.62	0.47-0.81
Fatigue (Reference= None to mild)	.0001	2.83	1.70-4.75
Depressed mood (Reference= None to mild)	.011	2.73	1.26-5.94
Stage of Disease (Reference= Early stage)	.023	1.84	1.08-3.10
Sex (Reference=Male)	.037	1.71	1.03-2.90
Age (≤50; > 50)	0.32	0.75	0.42-1.33
MEDD	0.004	1.02	1.01,1.03

Table 5

PTGS2 Haplotype Status and Severe Pain

Haplotype*	Severe Pain %	Non-Severe Pain %	p-value**
GTC	0.707	0.645	0.08
GCC	0.122	0.173	0.06
CCC	0.132	0.141	0.70
GCT	0.004	0.002	
CTC	0.0	0.008	
CCT	0.033	0.028	

* PTGS2 G-765c; PTGS2 exon 10+837 T>C; PTGS2 exon 10-90C>T

** Based on two-sided binomial exact test

The haplotypes for which the p-value is missing is not computed because too few are observed (<5%).

Table 6

False Positive Reporting Probabilities f or significant SNPs

Priors	PTGS2 exon 10+837 T>C OR= 0.33	TNF A -308 GA OR=1.67	NFKappa B Ex6+50 C>T OR=0.64
0.10	**0.441**	**0.273**	**0.257**
0.08	0.502	**0.324**	**0.307**
0.05	0.625	**0.442**	**0.422**
0.03	0.739	0.574	0.555
0.01	0.897	0.805	0.792

Bold- indicates noteworthy findings for a given Odds Ratio for a specific SNP suggesting that the observed association is noteworthy for initial studies.