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Symptom Clusters of Pain, Depressed Mood, and Fatigue in Lung Cancer: Assessing the Role of Cytokine Genes

Cielito C. Reyes-Gibby¹, Michael D. Swartz², Xiaoying Yu², Xifeng Wu¹, Sriram Yennurajalingam¹, Karen O. Anderson¹, Margaret R. Spitz³, and Sanjay Shete¹

¹The University of Texas MD Anderson Cancer Center, Houston

²University of Texas Health Science Center at Houston School of Public Health

³The Dan L. Duncan Cancer Center, Baylor College of Medicine

Abstract

PURPOSE—Symptom clusters, the multiple, co-occurring symptoms experienced by cancer patients, are debilitating and affects quality of life. We assessed if a panel of immune-response genes may underlie the co-occurrence of severe pain, depressed mood and fatigue and help identify patients with severe versus non-severe symptom clusters.

METHODS—Symptoms were assessed at presentation, prior to cancer treatment in 599 newly diagnosed lung cancer patients. We applied cluster analyses to determine the patients with severe versus non-severe symptom clusters of pain, depressed mood, and fatigue.

RESULTS—Two homogenous clusters were identified. One hundred sixteen patients (19%) comprised the severe symptom cluster, reporting high intensity of pain, depressed mood and fatigue and 183 (30%) patients reported low intensity of these symptoms. Using Bayesian model averaging methodology, we found that of the 55 SNPs assessed, an additive effect of mutant alleles in ENOS (-1474 T/A) (Posterior Probability of Inclusion (PPI) = 0.78, OR = 0.54, 95% CI = (0.31, 0.93); IL1B T-31C (PPI = 0.72, OR = 0.55, 95% CI = (0.31, 0.97)); TNFR2 Met¹⁹⁶Arg (PPI = 0.70; OR=1.85;95%CI=(1.03,3.36)); PTGS2 exon 10+837T>C (PPI = 0.69, OR = 0.54, 95%CI = (0.28, 0.99)); and IL10RB Lys⁴⁷Glu (PPI = 0.68; OR=1.74; 95%CI=(1.04,2.92)) were predictive for symptom clusters.

CONCLUSIONS—Genetic polymorphisms may facilitate identification of high risk patients and development of individualized symptom therapies.

Keywords

pain; depression; fatigue; cytokines; symptoms; genes; epidemiology; lung cancer; SNPs

INTRODUCTION

Although steady improvements in the survival rates of patients with lung cancer have been observed in recent years, the majority of patients still experience debilitating physical and psychological symptoms. Symptom burden is greatest in patients in the end stages of life, however many patients living with lung cancer continue to suffer from distressing symptoms. Symptoms in cancer patients can be the subjective expression of the disease

Corresponding author: Cielito C. Reyes-Gibby, DrPH, Department of Emergency Medicine, Unit 1468, The University of Texas MD Anderson Cancer Center, 1155 Pressler Street, Houston, Texas 77030-4009; creyes@mdanderson.org; Phone: (713) 792-1816; Fax: (713)792-8743.

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itself and/or the side-effects or toxicities of disease treatment. These symptoms can include physical (e.g., pain, shortness of breath), affective (e.g., depression, anxiety) and cognitive (e.g., delirium, memory problems, impaired concentration) experiences, which significantly affect the quality of life of these patients. Pain, depression, and fatigue are prevalent symptoms experienced by lung cancer patients[1]. These symptoms are commonly observed to co-occur in a related cluster, however, they are typically treated as separate and mutually exclusive entities. Indeed, a report by the National Cancer Institute called for studies that will help understand the risks for pain, depression, and fatigue in people with cancer[2]. One important gap is the investigation of multiple symptoms of cancer that might identify common biological mechanisms among cancer-related symptoms.

Studies suggest the important role of cytokine deregulation in the evolution of the malignant process, including symptom production[3]. The chronic inflammation that stimulates tumor growth and spread is hypothesized to contribute to symptom development[4;5]. For example, cytokines associated with inflammation or tissue damage contribute to pain hypersensitivity by modifying the activity of nociceptors[6]. Hyperexcitability in pain transmission neurons may be caused by proinflammatory cytokines produced by glial cells that respond to inflammation or other cancer-produced cytokines. When substance P and excitatory amino acids are released from presynaptic terminals, an exaggerated pain response is produced[2]. Increases in production of proinflammatory cytokines have also been associated with depression in samples of psychiatric patients[7] and patients with cancer[8]. Furthermore, cancer-related fatigue has been related to alterations in cytokine levels caused by cancer or its treatment[3;5]. Additional investigation of the role of cytokines in the pathogenesis of cancer-related symptoms is warranted. In particular, polymorphisms in cytokine genes may play a role in symptom production, perception, and expression[1].

Single nucleotide polymorphisms (SNPs) have been shown to alter the expressions or functions of a gene and thus have been explored as potential biomarkers for disease severity and disease conditions. Indeed, the influence of SNPs in immune-response genes (i.e., PTGS2, TNFalpha, NFKBIA, IL6, IL8) have been shown to influence pain severity in cancer patients[9–15]. However, investigations on the influence of multiple immune-response genes on the co-occurrence of multiple symptoms experienced by cancer patients have been limited. In this study, we assessed the extent to which 55 SNPs in immune-response genes may serve as biomarkers for symptom clusters of pain, depressed mood, and fatigue in 599 patients with non-small cell lung cancer (NSCLC). Because of a hypothesized strong and significant association between pain, depressed mood, and fatigue, we first applied cluster analyses to determine the patients with severe versus non-severe symptom clusters of pain, depressed mood, and fatigue. We also used a Bayesian model averaging approach shown to control false positive findings[16;17] to test our a priori hypothesis that SNPs in immune-response genes may help in identifying patients with severe versus non-severe symptom clusters.

METHODS

Study Subjects

Study subjects were selected from an epidemiological study of lung cancer, as previously described[18]. Patients who were newly diagnosed with NSCLC were recruited to the study at the time of initial registration at the M. D. Anderson Cancer Center. The study response rate was 80%. Because of issues associated with population stratification, we focused our analyses on white Caucasian patients. This study was approved by the Institutional Review Board at M. D. Anderson Cancer Center.

Epidemiology, symptoms, and clinical data collection

Data were collected prior to initiation of cancer treatment. Patients rated their pain on an 11-point numeric scale, (0= 'no pain' and 10= 'pain as bad as you can imagine')[19]. Fatigue was assessed using items from the SF-12: "during the past 4 weeks, did you have a lot of energy?"; likewise, depressed mood was assessed with a different item from the SF-12: "during the past 4 weeks, have you felt downhearted and blue?" [20;21]. Other information, such as stage of disease, medications for symptoms, and history of co-morbid conditions (heart disease, stroke, diabetes, etc.) were abstracted from patients' charts. Because of the different types of opioids prescribed, we translated the daily opioid dose to morphine-equivalent daily dose (MEDD) as in previous studies[13;14].

Blood collection and molecular analysis

Upon completion of the interview, 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.

The selection of Single Nucleotide Polymorphisms for genotyping were based on two of the three following criteria: (a) the minor allele frequency was greater than or equal to 5%; (b) location in coding region, promoter, or untranslated region (UTR) of the gene; and (c) previously reported to be associated with cancer[22] and with symptom production. 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[22].

Statistical analyses

We first summarized the subject characteristics using descriptive statistics. We used the Kolmogorov-Smirnov Z test to assess the normality distribution for pain, fatigue, and depressed mood. Since normality was not met, we used the National Comprehensive Cancer Network (NCCN) cut-off score of 7 for severe pain[23]. We also combined responses to the SF-12 questionnaire[12;13;15], for the question, "During the past 4 weeks, have you been feeling downhearted and blue?," responses of "most of the time" and "all of the time" were combined to indicate severe levels of depressed mood, and "none of the time," "little of the time," "some of the time," and "good bit of the time" were combined to indicate non-severe levels of depressed mood. For the question on fatigue ("During the past 4 weeks, have you had a lot of energy?"), we combined the responses "most of the time," "all of the time," "some of the time," and "good bit of the time" to indicate non-severe levels of fatigue and "none of the time" and "little of the time" to indicate severe levels of fatigue.

Symptom clusters

We performed hierarchical clustering, a methodology which groups individuals according to similarities in severity scores for pain, depression and fatigue, using the SAS statistical package (Version 9.2, SAS institute, Cary, NC). Average linkage clustering was accomplished using the Euclidean distance metric for the standardized symptom scores. We identify homogenous symptom burden clusters to define a dichotomous phenotype described below in the results section.

Genetic and Non-Genetic Factors: In order to determine which SNPs in immune-response genes are associated with pain, depressed mood and fatigue symptom cluster, we used stochastic search variable selection (SSVS) applied to logistic regression. SSVS is a Bayesian model averaging approach to variable selection that uses Markov chain Monte

Carlo to search through the set of possible models to find the most likely variables associated with an outcome.

We simultaneously searched through all 55 SNPs as well as the non-genetic covariates (sex, age, stage of disease) while adjusting for morphine equivalent daily dose, coded as a dichotomous variable (any dose coded as “1”, versus none coded as “0”). In order to control for both false positives and false negatives, we used Bayesian methods for model selection which uses both prior probabilities of inclusion and decision rules based on the posterior probability of inclusion. For the priors in SSVS, we used an uninformative prior probability of inclusion of 0.50. This prior was shown to be the best balance between reducing false positives and detecting true positives when not all possible factors were measured. For our decision rule, we considered variables as candidate for our final model if the posterior probability of inclusion (PPI) was greater than 50% [17;24] and if the 95% credible interval (CI) excluded 1.0[16]. For those variables whose PPI was greater than 0.5, the odds ratios (OR) were estimated as the posterior mean[17], conditional on inclusion, and the 95% CIs were computed using the 2.5th percentile of the posterior distribution as lower bound and 97.5th percentile of the posterior distribution for the upper bound, also conditional on inclusion. We set SSVS to perform 200,000 iterations to determine the important genetic and non-genetic covariates for predicting cluster membership. (NOTE: Data available upon request and compliance to Institutional Review Board Requirements of MD Anderson)

RESULTS

There were a total of 599 white Caucasian patients with previously untreated and histologically-confirmed NSCLC comprising our study sample (Table 1). Mean age was 61 years (SD=12). There were more men (n=316) than women (n=283). There was about an equal distribution of the sample between early stage (Stage I-IIIa; n=287) and late stage of disease (Stage IIIB-IV; n=285) and hypertension was the most prevalent co-morbid condition. Table 1 also shows the characteristics of the patient sample by severe levels of pain (Panel A), depressed mood (Panel B), and fatigue (Panel C). Severe pain varied by age ($p<0.007$) and stage of disease ($p<0.001$). Whereas, severe fatigue only varied by stage of disease ($p<0.01$).

Figure 1 and table 2, together describe the results of our cluster analysis. Figure 1 shows the dendrogram that identifies the average euclidean distance between our clusters, while table 2 shows the mean and median of the symptoms in each cluster. Cluster one and two are the most homogenous across symptoms. Cluster 1, comprising 19% of our sample, can be best described as a “high” symptom burden group. Specifically, patients in cluster one reported combined high intensity of pain, depressed mood and fatigue; whereas, patients in cluster two (n=183; 30%), the low symptom burden group, reported combined low intensity of pain, depressed mood and fatigue. The remaining clusters three, four, and five, of patients are more heterogeneous for symptom burden, where one symptom is reported higher or lower than the remaining two. For example individuals comprising cluster three reported higher pain and lower fatigue and depressed mood. For this study, we focus on the homogeneous symptom clusters of reported high and low total symptom burden (i.e. cluster 1 versus cluster 2).

Model Building

We evaluated 55 SNPs in the 37 genes from the immune- response pathway for predicting high versus low symptom burden. In this Bayesian approach, we consider candidate variables as significant if both the Posterior Probability of Inclusion (PPI) is greater than 50% and the 95% CI excludes 1.0. Out of the SNPs of interest, only a few had a posterior probability greater than 0.5 (see Table 3). Of those with probability greater than 0.5, even

fewer had ORs with 95% CIs which did not include 1, composing our final model (see table 4). Specifically, ENOS (-1474 T/A) (PPI = 0.78, OR = 0.54, 95% CI = (0.31, 0.93); IL1B T-31C (PPI = 0.72, OR = 0.55, 95% CI = (0.31, 0.97)); TNFR2 Met¹⁹⁶Arg (PPI = 0.70; OR=1.85; 95% CI=(1.03,3.36)); PTGS2 exon 10+837T>C (PPI = 0.69, OR = 0.54, 95% CI = (0.28, 0.99)); and IL10RB Lys⁴⁷Glu (PPI = 0.68; OR=1.74; 95% CI=(1.04,2.92)).

Those genes with a PPI greater than 0.5 that did not make it into the final model due to their odds ratio estimates having too much variation may be worth further study (Table 3). These genes had enough evidence in the data for stochastic search to include them in the model for more than half the model visits, however, the variation in the odds ratio estimates led to exclusion from the final model. Although these SNPs had a PPI above our threshold, the 95% CI marginally included 1, and therefore further investigation is necessary to make more definitive inference. The non-genetic factors including stage of disease (PPI = 0.996, OR = 3.60, 95% CI = (1.80, 7.50)) and sex (PPI = 0.94, OR = 2.63, 95% CI = (1.33, 5.34)) were both strongly predictive of high symptom burden.

DISCUSSION

We found an additive effect of mutant alleles in ENOS (-1474 T/A) (Posterior Probability of Inclusion (PPI) = 0.78, OR = 0.54, 95% CI = (0.31, 0.93); IL1B T-31C (PPI = 0.72, OR = 0.55, 95% CI = (0.31, 0.97)); TNFR2 Met¹⁹⁶Arg (PPI = 0.70; OR=1.85; 95% CI=(1.03,3.36)); PTGS2 exon 10+837T>C (PPI = 0.69, OR = 0.54, 95% CI = (0.28, 0.99)); and IL10RB Lys⁴⁷Glu (PPI = 0.68; OR=1.74; 95% CI=(1.04,2.92)) were predictive for the symptom cluster of pain, depressed mood and fatigue in lung cancer patients. To our knowledge, this study is among the first to simultaneously and systematically examine a comprehensive number of immune-response genes as potential markers for symptom clusters of pain, depressed mood and fatigue.

This paper is also one of the first to apply SSVS to genetic data for symptom clusters. We showed that whereas the usual frequentist maximum likelihood estimates would have yielded unstable estimates with a reduced sample size (n=206), using the Bayesian model averaging with uninformative priors enabled us to find potentially important genes and non-genetic factors associated with symptom burden. Recent research has shown that models built using SSVS exhibited more evidence for detecting the true risk model compared to using standard stepwise-type selection methods on simulated data[17;25]. Specifically, using simulated data, where the true underlying disease model is known, Swartz, Yu, and Shete[17] have shown that using SSVS with both informed and uninformed priors had a higher probability of correct associations, and lower probability of incorrect associations than stepwise, forward or backward selection. By jointly considering the PPI and the 95% CIs, our Bayesian posterior fully includes the uncertainty surrounding the model selection process itself, leading to a more accurate reflection of the precision of the estimates.

In this study, we found that IL1B T-31C to be associated with high symptom burden. The T-31C substitution, located in a TATA-box motif of IL1 β , markedly affects the binding of several transcription factors and influences the transcription activity of IL1 β [26;27]. Consistent with our findings, IL1 β has been implicated in pain response and variation in postoperative morphine consumption[28;29], depression[30], and fatigue[31]. Studies involving intraplantar injections of IL1 β have shown that increased levels of IL1 β produce hyperalgesia at distant peripheral sites[32] and that conversely, subcutaneous injection of IL-1 receptor antagonist blocks nociceptive response[33]. Animal, human, and in vitro studies have shown IL-1 β to be related to the diagnosis of major depressive disorder, specific symptoms of depression, and response to antidepressant treatment[30;34]. In a recent study, two variants of the IL-1 β gene (rs16944, rs114643) were shown to be

associated with nonremission after antidepressant treatment and decreased amygdala and anterior cingulate cortex function[30]. IL1 β levels also predicted cancer-related fatigue severity before adjuvant therapy in women with breast cancer[31]. However, a number of studies show that IL1 is not associated with fatigue[35;36]. Additional studies are needed to further explore the role of IL1 in symptom burden outcomes.

We also found that mutant alleles of PTGS2 exon 10+837T>C influence symptom burden. The PTGS2 gene encodes the proinflammatory cyclooxygenase (COX) 2 enzyme. Exon10+837T>C of the PTGS2 gene is a functional SNP[10], that modulates expression of COX2. As 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. The effect of COX 2 on depression has also been observed. Patients who have been treated with rofecoxib, a COX2 inhibitor, due to other indications showed clinical improvement of depressive mood[9]. More recently, a randomized placebo controlled trial showed that as an add-on strategy for treatment of depression, COX 2 inhibitors (Celecoxib) resulted in a significant reduction of Hamilton Depression Scale scores[37].

We also found that a SNP (rs1800783) in the ENOS gene may influence symptom burden. Nitric Oxide Synthase (NOS) generates NO, which contributes to oxidative stress and induces cytotoxicity. Several cytokines, (IFN- γ , TNF- α , IL-1 β) induce NO generation. To date, NOS inhibitors are actively being investigated as therapeutic targets for inflammatory and pain conditions[38], neurodegenerative disorders, and fatigue[39].

We also showed the potential roles of TNFR2 Met¹⁹⁶Arg (rs1061622) and IL10RB Lys⁴⁷Glu (rs2834167) on symptom burden. Polymorphisms in TNFR2 have been found to be previously associated with paranoid schizophrenia[40], but to our knowledge, there are no reports describing prior associations with pain, depressed mood or fatigue. Similarly, there have not yet been reports of an association between polymorphisms in the IL10RB gene and pain, depressed mood, or fatigue. However, we have a small data set, and because the credible intervals for the odds ratios were close to 1, further investigation into their association with symptom burden is required.

Not surprisingly, we found that stage of disease and sex were important factors for symptom burden. We found that women and those with advanced stage of disease were at a higher risk for symptom burden. These findings are consistent with the results of previous studies of lung cancer patients that found sex and disease stage were significant predictors of symptom severity[12;15].

There are limitations to this study. We only used one-item to measure each of the symptoms of pain, depressed mood, and fatigue. However, although multiple items may be preferred, studies have shown that symptoms measured by a single-item correlate highly with multi-item measures of pain, fatigue and depression[41;42]. Furthermore, as with all genetic studies, the SNPs genotyped for this study may be merely in linkage disequilibrium with the true functional SNP, rather than being the causal SNP itself. Additionally, it could be argued that cytokine activity falls into multiple biological networks influencing gene activation and suppression and thus more genes with functional significance should be assessed.

In conclusion, we found an additive effect of mutant alleles in ENOS (-1474 T/A) (Posterior Probability of Inclusion (PPI) = 0.78, OR = 0.54, 95% CI = (0.31, 0.93); IL1B T-31C (PPI = 0.72, OR = 0.55, 95% CI = (0.31, 0.97)); TNFR2 Met¹⁹⁶Arg (PPI = 0.70; OR=1.85;95%CI=(1.03,3.36)); PTGS2 exon 10+837T>C (PPI = 0.69, OR = 0.54, 95% CI = (0.28, 0.99)); and IL10RB Lys⁴⁷Glu (PPI = 0.68; OR=1.74; 95%CI=(1.04,2.92)) were associated with high symptom burden for pain, depressed mood and fatigue symptom cluster in lung cancer patients. The non-genetic factors that were predictive of high symptom

burden included sex and stage of disease. Further studies are required to validate these important preliminary findings as these genetic polymorphisms may facilitate identification of patients at risk of high symptom burden for pain, depressed mood and fatigue symptom cluster and development of individualized therapies.

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Figure 1.

Dendrogram for symptom clusters. This figure shows the dendrogram for the clusters of symptom burden, whose numerical summaries (mean and median) are given in table 2. The horizontal bars at the top of each box containing the cluster name shows the Euclidian distance for the merge into that cluster. The horizontal bars continuing up the tree represent the average Euclidean distance between the 5 clusters just before they merge. We see that clusters 1 and 4 are “closer” together and clusters 3, 5, and 2 are closer together based on average Euclidean distance. This dendrogram shows that the severe (cluster 1) and non-severe (cluster 2) clusters are well separated. At the bottom of the dendrogram, we show the mean pain, fatigue and depressed mood scores for each cluster.

Table 1

Patient Characteristics of the Study Sample

Variables	Total	Panel A. Pain Severe/non-severe	Panel B. Depressed Mood Severe/non-severe	Panel C. Fatigue Severe/non-severe
Age	(Mean = 61; SD = 12)			
>50	465	65/400*	31/434	195/270
<= 50	134	32/102	13/121	65/69
Sex				
Male	316	43/273	18/298	132/184
Female	283	54/229	26/257	128/155
Stage of Disease				
Early	287	32/253**	23/262	109/176***
Advanced	285	62/225	19/268	140/147
Heart Disease				
Yes	128	24/104	12/116	62/66
No	380	59/321	26/354	165/215
Hypertension				
Yes	188	29/159	13/175	78/110
No	320	54/266	25/295	149/171
Diabetes				
Yes	57	5/32	1/36	17/20
No	471	78/393	37/434	210/261
Stroke				
Yes	23	4/19	4/19	12/11
No	485	79/406	34/451	215/270
MEDD				
Yes	98	43/55	17/81	52/46
No	477	52/425	66/411	202/275

SD= standard deviation

*
p<0.007**
p<0.001***
p<0.01

Table 2

Clusters based on Pain, Depressed Mood, and Fatigue

VARIABLES	TOTAL SAMPLE	HOMOGENEOUS GROUPS			HETEROGENEOUS GROUPS		
		N=599	Severe N=116	Non-severe N=183	Cluster 3 N=95	Cluster 4 N=120	Cluster 5 N=85
Pain (0–10 scale)							
Mean	3.01		5.72	0.92	5.57	3.42	0.36
Median	2.00		5.50	0.00	5.00	3.00	0
Depressed Mood (1–6 scale)							
Mean	2.41		3.82	1.50	2.51	1.63	3.47
Median	2.00		3.00	2.00	5.00	2.00	3.00
Fatigue (1–6 scale)							
Mean	4.13		5.59	2.85	3.25	5.41	4.06
Median	4.00		6.00	3.00	4.00	5.00	4.00

Cluster 1 and 2 are homogeneous for symptom burden with Cluster 1 best described as a “high” symptom burden group, consisting of higher scores of pain, depressed mood and fatigue; whereas, cluster 2 consists of lower scores of pain, depressed mood and fatigue. Clusters 3, 4 and 5 are heterogeneous for symptoms, where one symptom is scored higher or lower than the other two symptoms measured.

Table 3

Posterior Probability of Inclusion for Specific SNPs

Proinflammatory cytokines, receptors, and related molecules	RS numbers	PPI
<i>IL1A</i> C-889T	rs1800587	0.36
<i>IL1A</i> Ala ¹¹⁴ Ser	rs17561	0.41
<i>IL1B</i> T-31C	rs1143627	0.72 [*]
<i>IL1B</i> C3954T	rs1143634	0.27
<i>IL1R1</i> Ala ¹²⁴ Gly	rs2228139	0.63 [*]
<i>IL2</i> T-330G	rs2069762	0.22
<i>IL2RB</i> Asp ³⁹¹ Glu	rs228942	0.35
<i>IL6</i> G-174C	rs1800795	0.27
<i>IL6R</i> Asp ³⁵⁹ Ala	rs8192284	0.20
<i>IL8</i> T-251A	rs4073	0.22
<i>IL8RA</i> Ser ²⁷⁶ Thr	rs2234671	0.48
<i>IL12B</i> A1188C	rs3212227	0.27
<i>IL12RB</i> Met ³⁶⁵ Thr	rs375947	0.57 [*]
<i>IL16</i> T-295C	rs4778889	0.49
<i>IL16</i> Asn ⁴⁴⁶ Lys	rs17875535	0.50 [*]
<i>TNFA</i> T-1031C	rs1799964	0.31
<i>TNFA</i> T-857C	rs1799724	0.22
<i>TNFA</i> G-308A	rs1800629	0.48
<i>TNFA</i> A-238C	rs361525	0.39
<i>TNFB</i> Arg ¹³ Cys	rs2857713	0.32
<i>TNFB</i> His ⁵¹ Pro	rs3093543	0.47
<i>TNFR2</i> Met ¹⁹⁶ Arg	rs1061622	0.70 [*]
<i>TNFR2</i> Glu ²³² Lys	rs5746026	0.40
<i>IFNAR1</i> Val ¹⁶⁸ Leu	rs2257167	0.29
<i>IFNAR2</i> Phe ¹⁰ Val	rs7279064	0.22
<i>IFNG</i> T-1615C	rs2069705	0.28
<i>IFNG</i> A874T	rs2430561	0.26
<i>GM-CSF</i> T-1916C	rs2069614	0.55 [*]
<i>GM-CSF</i> Ile ¹¹⁷ Thr	rs25882	0.50 [*]
<i>MCP1</i> A-2518G	rs1024611	0.29
<i>MIF</i> G-173C	rs755622	0.30
Anti-inflammatory cytokines, receptors, and related molecules		
<i>IL4</i> C-590T	rs2243250	0.39
<i>IL4</i> 5'-UTR(C/T)	rs2070874	0.42
<i>IL4R</i> Ile ⁷⁵ Val	rs1805010	0.35

Proinflammatory cytokines, receptors, and related molecules	RS numbers	PPI
<i>IL4R</i> Ser ⁵⁰³ Pro	rs1805015	0.31
<i>IL4R</i> Gln ⁵⁷⁶ Arg	rs1801275	0.29
<i>IL4R</i> Ser ⁷⁵² Ala	rs1805016	0.35
<i>IL5</i> C-745T	rs2069812	0.28
<i>IL10</i> A-1082G	rs1800896	0.26
<i>IL10</i> C-592A	rs1800872	0.31
<i>IL10RA</i> Ser ¹⁵⁹ Gly	rs3135932	0.33
<i>IL10RB</i> Lys ⁴⁷ Glu	rs2834167	0.68*
<i>IL13</i> C-1112T	rs1800925	0.56*
<i>IL13</i> Arg ¹³⁰ Gln	rs20541	0.40
Prostaglandins and nitric oxide		
<i>PTGS2</i> G-765C	rs20417	0.41
<i>PTGS2</i> exon10+837T>C	rs5275	0.69*
INOS Intron	rs2072324	0.33
<i>INOS</i> Leu ⁶⁰⁸ Ser	rs2297518	0.33
<i>ENOS</i> Glu ²⁹⁸ Asp	rs1799983	0.24
ENOS (-1474 T/A)	rs1800783	0.78*
Intracellular signaling molecules		
<i>IKB</i> C-420T	rs2233409	0.23
<i>IKB</i> 3'-UTR(C/T)	rs8904	0.28
<i>PPARA</i> Leu ¹⁶² Val	rs1800206	0.33
<i>PPARD</i> 5'-UTR(T/C)	rs2016520	0.23
<i>PPARG</i> Pro ¹² Ala	rs1801282	0.30

* denotes gene or covariate with PPI greater than 0.5, and is a candidate for the final model.

Table 4

Odds ratios and 95% Credible interval (CI) for the final model*

Covariate/Gene	rs number	PPI	Odds Ratio	95% CI
Stage of disease Reference (early stage)	-	0.99	3.60	(1.80, 7.50)
Sex Reference (males)	-	0.94	2.63	(1.33, 5.34)
ENOS -1474 T/A**	rs1800783	0.78	0.54	(0.31, 0.93)
<i>IL1B</i> T-31C**	rs1143627	0.72	0.55	(0.31, 0.97)
TNFR2 Met ¹⁹⁶ Arg**	rs1061622	0.70	1.85	(1.03, 3.36)
<i>PTGS2</i> exon10+837T>C**	rs5275	0.69	0.54	(0.28, 0.99)
<i>IL10RB</i> Lys ⁴⁷ Glu**	rs2834167	0.68	1.74	(1.04, 2.92)

* included in the final model: candidate factors with an odds ratio different from 1 (95% CI excludes 1) after adjusting for Morphine Equivalent Daily Dose

** additive genetic model