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Genetic Predictors of Fatigue in Prostate Cancer Patients Treated with Androgen Deprivation Therapy: Preliminary Findings

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Abstract

Background—Fatigue is a common and distressing side effect of androgen deprivation therapy (ADT) for prostate cancer. The goal of the current study was to examine the relationship between changes in fatigue following initiation of ADT and single nucleotide polymorphisms (SNPs) in three pro-inflammatory cytokine genes: interleukin-1 beta (*IL1B*), interleukin-6 (*IL6*), and tumor necrosis factor alpha (*TNFA*).

Methods—As part of a larger study, men with prostate cancer (n=53) were recruited prior to initiation of ADT. Fatigue was assessed at recruitment and six months after initiation of ADT. DNA was extracted from blood drawn at baseline.

Results—Patients with the *IL6-174* (rs1800795) G/C or C/C genotype displayed greater increases in fatigue intrusiveness, frequency, and duration than the G/G genotype (*p* values 0.05), although inclusion of age, race, and baseline depressive symptomatology in the model attenuated these relationships (*p* values 0.09). Patients with the *TNFA-308* (rs1800629) G/A genotype showed greater increases in fatigue severity than the G/G genotype (*p*=0.02). *IL1B-511* (rs16944) genotype did not significantly predict changes in fatigue (*p* values>0.46). Patients with higher numbers of variants displayed greater increases in fatigue duration and interference (*p* values 0.02) than patients with lower numbers of variants.

Conclusions—Prostate cancer patients treated with ADT who carry variant alleles of the *IL6* and *TNFA* genes are susceptible to heightened fatigue. These preliminary data lend support for the role of genetic variation in the development of cancer-related fatigue secondary to ADT. Findings are relevant to attempts to develop personalized approaches to cancer treatment.

Keywords

prostatic neoplasms; fatigue; polymorphism; single nucleotide

Androgen deprivation therapy (ADT) is indicated for prostate cancer patients with intermediate or high risk of recurrence or local metastasis (National Comprehensive Cancer

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Network, 2011). ADT typically consists of intramuscular injection of luteinizing hormone-releasing hormone agonists (i.e., leuprolide, goserelin), often with bicalutamide, which results in reduction of testosterone to castration levels. Elimination of testosterone slows the growth of prostate cancer. Although multiple randomized trials have demonstrated the effectiveness of ADT in slowing the progression of prostate cancer (Akaza, 2011), it is associated with a number of side effects including fatigue. Studies show that approximately 40% of men treated with ADT experience clinically-significant fatigue (Kyrdalet al., 2010; Storey et al., 2011a). Fatigue is one of the most distressing side effects of cancer treatment and is associated with reduced quality of life (Baker et al., 2005; Storey et al., 2011b). As such, it is important to examine which patients are at greatest risk.

To date, relatively few studies have examined the role of genes in cancer-related fatigue and none to our knowledge have examined genetic variation in fatigue related to receipt of ADT. Available data suggest that cancer-related fatigue may be associated with single nucleotide polymorphisms (SNPs) as well as differential expression in genes involved in the production of pro-inflammatory cytokines. Regarding polymorphisms, results of four studies suggested that SNPs in cytokine genes are predictive of fatigue in human cancers: SNPs of interleukin-1beta (*IL1B*) and interleukin-6 (*IL6*) in breast cancer (Collado-Hidalgo et al., 2008), *IL1B* in lung cancer (Rausch et al., 2010), *IL6* and tumor necrosis factor alpha (*TNFA*) in various cancers during radiation treatment (Aouizerat et al., 2009; Miaskowski et al., 2010). However, evidence is conflicting (Reinertsen et al., 2011). These data are intriguing and suggest the need for additional studies examining the role of genes in the development of cancer-related fatigue.

Genes regulating pro-inflammatory cytokines are a logical focus of research because of previous data suggesting that inflammation is associated with cancer-related fatigue. Breast cancer survivors with fatigue display higher levels of circulating pro-inflammatory cytokines and their biomarkers, including IL-1 receptor antagonist (IL-1ra) and soluble tumor necrosis factor receptor type II (sTNF-rII), than survivors without fatigue (Bower et al., 2002). Fatigued breast cancer survivors also show greater lipopolysaccharide-induced increases in IL-1B, TNF- α , and IL-6 than non-fatigued survivors (Bower et al., 2007; Collado-Hidalgo et al., 2006). In cancer patients receiving radiation, fatigue has been found to be associated with increases in circulating pro-inflammatory cytokines and their biomarkers, including C-reactive protein (CRP), IL-1ra, IL-1B, IL-6, and soluble tumor necrosis factor receptor type I (sTNF-rI) (Bower et al., 2009; Greenberg et al., 1993; Wang et al., 2010; Wratten et al., 2004), although evidence is mixed (Ahlberg et al., 2004; Geinitz et al., 2001). A quantitative summary of eighteen studies found circulating IL-1ra, IL-6, and neopterin to be significantly correlated with cancer-related fatigue (Schubert et al., 2007). Moreover, fatigue can be induced in humans and animals through therapeutic and experimental administration of cytokines (Bluthe et al., 1994; Kelley et al., 2003; Malik et al., 2001).

Pro-inflammatory cytokines may play a particularly important role in fatigue secondary to ADT because evidence suggests that cytokine production is modulated in part by testosterone. Animal studies indicate that testosterone acts directly on androgen or testosterone receptors on several different types of immune cells, including bone-marrow derived stromal cells, macrophages, and immature T cells (Bellido et al., 1995; Benten et al., 1999a; Benten et al., 1999b; Viselli et al., 1995; Wunderlich et al., 2002). Testosterone attenuates production of pro-inflammatory cytokines such as IL-6 by these immune cells (Bellido, et al., 1995; Kanda et al., 1996; Rettew et al., 2008). Testosterone also inhibits *IL6* gene expression (Bellido, et al., 1995). Castration of male mice typically results in increases in TNF following stimulation with bacterial endotoxin, which is suppressed when testosterone is replaced (Spinedi et al., 1992). Human studies indicate that in vitro

testosterone can suppress production of inflammatory cytokines by macrophages, monocytes, and gingival fibroblasts (D'Agostino et al., 1999; Gornstein et al., 1999; Li et al., 1993). Higher serum testosterone in men is associated with lower levels of serum cytokines and their biomarkers, including CRP, IL-6, and soluble IL-6 receptor (sIL-6r), as well as decreased in vitro production of IL-1B and TNF- α (Garcia et al., 2006; Maggio et al., 2006; Musabak et al., 2003; Van Vliet et al., 2005; Yang et al., 2005). Relative to eugonadal men, hypogonadal men evidence elevated serum levels of inflammatory cytokines, including IL-1B, IL-6, and TNF- α (Khosla et al., 2002; Nettleship et al., 2007). When peripheral blood mononuclear cells from hypogonadal men are treated in vitro with gonadotropin, secretion of IL-1B and TNF- α significantly decreases (Musabak, et al., 2003).

With this as background, the goal of the current study was to examine whether SNPs in genes regulating pro-inflammatory cytokines predicted changes in fatigue in men receiving ADT for prostate cancer using a candidate gene approach. SNPs in the *IL1B*, *IL6*, and *TNFA* genes were selected based on growing evidence for the associations among these SNPs, serum cytokines, and cancer-related fatigue (Aouizerat, et al., 2009; Collado-Hidalgo, et al., 2008; Miaskowski, et al., 2010). In addition, because the selected SNPs are located in predicted transcription factor binding sites, they may differentially affect transcription (National Institute of Environmental Health Sciences, 2011). On the basis of previous evidence, it was hypothesized that patients displaying variants at these sites would display greater increases in fatigue following initiation of ADT than patients who were homozygous for the wild type allele. A cumulative effect of multiple SNPs was also hypothesized, such that patients with more variants would report greater fatigue than patients with fewer variants.

Methods

Participant Eligibility and Recruitment

Men with prostate cancer were recruited for a larger IRB-approved study examining side effects of ADT. Eligibility criteria were that participants: 1) be at least 18 years of age or older, 2) be able to speak and read English, 3) have at least an eighth grade education, 4) be diagnosed with non-metastatic or asymptomatic metastatic prostate cancer, 5) be free of a secondary diagnosis of brain cancer, 6) be disease-free or in remission for any secondary cancers, 7) be free of treatment for other cancers in the 12 months prior to recruitment, 8) be free of previous treatment with cranial irradiation, 9) be scheduled to be treated with goserelin or leuprolide for at least 12 months, 10) be free of previous treatment with goserelin or leuprolide in the past 12 months and bicalutamide in the past 6 months prior to recruitment, 11) be free of history of cerebrovascular accident, 12) be free of cognitive impairment as assessed by the Short Portable Mental Status exam (score < 3), and 13) be able to provide informed consent. Patients included in the current analyses were recruited between September 2008 and October 2010.

Eligibility was determined by chart review and consultation with the attending physician. Eligible patients were recruited and informed consent was obtained during an outpatient clinic visit on or before the day they started ADT. All participants completed a baseline assessment at this time (i.e., Time 1). Participants completed a follow-up assessment six months after their first ADT injection (i.e., Time 2).

One hundred and twenty-three men were approached for study participation and 97 (79%) signed consent. Thirteen patients were found to be ineligible before consent and 13 refused. Reasons for refusal included: not interested (n=6), too busy (n=3), upset about cancer progression (n=1), or no reason given (n=3). Of the 97 patients who signed consent, 27 were later found to be ineligible (e.g., ADT discontinued) or withdrew prior to the Time 2

assessment. Three patients did not provide a blood sample and 14 patients were not yet due for their Time 2 assessment at the time of data analysis, leaving a final sample of 53 participants with complete fatigue and genetic data for the current analyses.

Measures

Demographic and clinical data—Age, education, race, marital status, and annual household income were assessed at Time 1 via self-report. Cancer treatment information (i.e., concurrent bicalutamide, concurrent radiation, previous brachytherapy, previous bicalutamide previous androgen deprivation therapy, and previous prostatectomy) was collected via medical chart review.

Fatigue—The 14-item Fatigue Symptom Inventory (FSI) was used to assess fatigue at Time 1 and Time 2 (D. M. Hann et al., 1998). Analyses focused on participants' ratings of fatigue severity (0 = not at all fatigued, 10 = as fatigued as I could be), which was the average of ratings of fatigue: 1) on the day they felt most fatigued in the past week, 2) on the day they felt least fatigued in the past week, 3) on average in the past week, and 4) right now. Analyses were also conducted using ratings of the number of days fatigued in the past week (0 to 7) (i.e., fatigue frequency), how much of the day patients felt fatigued (0 = none, 10 = entire) (i.e., fatigue duration), and the average rating of the degree to which fatigue interfered (0 = no interference, 10 = extreme interference) with general activity, ability to bathe and dress, normal work activity, ability to concentrate, relations with others, enjoyment of life, and mood (i.e., fatigue interference). Previous research has demonstrated the reliability and validity of the FSI in cancer patients (Broeckel et al., 1998; D. M. Hann et al., 2000; D. M. Hann, et al., 1998).

Depressive symptomatology—The Center for Epidemiological Studies – Depression Scale (CES-D) (Radloff, 1977) was used to assess depressive symptomatology at Time 1. The CES-D is a 20-item measure in which higher scores indicate greater depressive symptomatology. The validity of the CES-D has been demonstrated with a wide range of populations, including cancer patients (Beeber et al., 1998; D. Hann et al., 1999). Depressive symptoms were examined as a potential confound of the relationship between genotype and fatigue.

DNA Collection and Genotyping

Genomic DNA samples were extracted from whole blood specimens using the FlexGene DNA Kit (Qiagen, Chatsworth, CA). DNA samples from participants were screened for the presence of SNPs in *IL1B* (rs16944), *IL6* (rs1800795), and *TNFA* (rs1800629) genes by 5'-exonuclease assay (TaqMan) using the ABI Prism 7900HT sequence detection system (Applied Biosystems) according to the manufacturer's instructions. In this assay, different probes are used in a polymerase chain reaction (PCR) based assay to distinguish between variant DNA sequences at a single locus. Primers and probes were supplied directly by Applied Biosystems as Assays-By-Design.

Briefly, 25- μ L PCR reactions were performed in a 96-well plate using (per well) 6.5 ng of genomic DNA, 300 nM of the sense and 900 nM of the antisense primers, 1 x TaqMan Universal PCR Master Mix, 100 nM of the wild-type 6-carboxyfluorescein (FAM)-labeled and minor groove-binding fluorescent quencher (MGBNFQ)-conjugated probe and the polymorphic VIC-labeled and MGBNFQ-conjugated probe (Applied Biosystems). The VIC-labeled probe was homologous to the polymorphic-type; the FAM-labeled probe was homologous to the wild-type DNA. PCR was performed for one cycle at 50°C for 2 minutes, one cycle at 95°C for 10 minutes, and 40 amplification cycles of 95°C for 15 seconds and 60°C for 1 minute using the ABI Prism 7900HT. Study subjects with either the homozygous

wild-type, homozygous variant, or heterozygous genotype were identified by Sequence Detection System (version 2.3), enabling us to distinguish polymorphic from wild type alleles. Assays included three negative controls and 5% duplicates per assay. Quality control guidelines were followed as previously described (Kote-Jarai et al., 2008).

Statistical Analyses

Mean-level changes in fatigue by genotype were examined using mixed-two factor repeated measures ANOVAs. Our primary interest was genotype by time interactions, which would indicate differential changes in fatigue by genotype. Four outcomes were examined: fatigue severity, fatigue interference, fatigue frequency, and fatigue duration. Fatigue outcomes were examined among patients with homozygous wild type, heterozygous, and homozygous variant genotypes (results shown in an online appendix). Based on these data, a dominant genetic model was selected such that minor allele carriers were compared to patients homozygous for the major allele (i.e., *IL1B*: G/A or A/A versus G/G, *IL6*: G/C or C/C versus G/G, *TNFA*: G/A or A/A versus G/G). The cumulative effect of multiple SNPs on residualized change in fatigue was examined by summing the number of variants (i.e., *IL1B*: G/A or A/A, *IL6*: G/C or C/C, *TNFA*: G/A or A/A) for each patient. Linear regression was then used to predict fatigue at Time 2 from the number of variants (i.e., 0 to 3), controlling for fatigue at Time 1. Consistent with previous literature (Aouizerat, et al., 2009; Collado-Hidalgo, et al., 2008; Miaskowski, et al., 2010; Rausch, et al., 2010), we also analyzed relationships among genotype and change in fatigue while controlling for potential sociodemographic and behavioral confounds (i.e., age, race, depressive symptomatology at baseline).

Results

The sample (N=53) had a mean age of 67 years (range: 45–90). The majority of participants was Caucasian (87%), non-Hispanic (98%), married (66%), had completed high school (96%), and had an annual household income of \$40,000 a year or more (51%). Fifty-two percent of participants were receiving ADT adjuvant to radiation, 42% were receiving ADT due to progressive disease, and 6% were receiving ADT as primary treatment. Regarding previous treatment, 49% of participants had received bicalutamide, 21% goserelin or leuprolide, 25% radical retropubic prostatectomy, 26% radiotherapy, and 8% brachytherapy. Genotype frequencies are displayed in Table 1. All SNPs were in Hardy-Weinberg equilibrium (p values > 0.16).

Means and standard deviations for fatigue by genotype are presented in Table 2. For the SNP on the *IL1B* gene, genotype by time interactions were non-significant for all fatigue outcomes (p values > 0.46). For the SNP on the *IL6* gene, significant interactions were observed for fatigue interference ($F=5.71$, $p=0.02$), fatigue frequency ($F=4.17$, $p=0.05$), and fatigue duration ($F=4.18$, $p=0.05$). Patients with the G/C or C/C genotype reported greater increases in fatigue interference, frequency, and duration over time than patients with the G/G genotype (see Figure 1). For the SNP on the *TNFA* gene, a significant interaction was observed for fatigue severity ($F=5.67$, $p=0.02$). Patients with the G/A genotype reported greater increases in fatigue severity over time than patients with the G/G genotype (see Figure 2).

The additive effects of multiple SNPs on change in fatigue were examined using linear regression. The number of variants (i.e., *IL1B*: G/A or A/A, *IL6*: G/C or C/C, *TNFA*: G/A or A/A) for each participant was summed and entered as a predictor of fatigue at Time 2, controlling for fatigue at Time 1. Four patients had 0 variants, 24 patients had 1, 19 patients had 2, and 6 patients had 3. Regression analyses indicated that the number of variants significantly predicted increases in fatigue interference (Std. B=0.25, $p=0.01$) and duration

(Std. B=0.23, $p=0.02$). The effects of multiple SNPs on fatigue severity and frequency were marginally significant (p values=0.08).

Genotype was then examined as a predictor of residualized change in fatigue independent of potential sociodemographic and behavioral confounds. Linear regressions controlling for age, race (i.e., non-Caucasian=0, Caucasian=1), and baseline depressive symptomatology continued to demonstrate a significant effect of *TNFA* genotype on fatigue severity ($F=5.39$, $p=0.02$). Inclusion of covariates in analyses rendered marginally significant the effect of *IL6* genotype on fatigue interference ($F=3.85$, $p=0.06$), frequency ($F=2.91$, $p=0.09$), and duration ($F=3.04$, $p=0.09$). The number of variants continued to significantly predict increases in fatigue duration (Std. B=0.21, $p=0.02$) and interference (Std. B=0.23, $p=0.01$) when covariates were added to the model. The effects of multiple SNPs on fatigue severity and frequency were marginally significant ($p=0.10$) and non-significant ($p=0.12$), respectively, when controlling for age, race, and baseline depressive symptomatology.

Discussion

This preliminary study is the first to our knowledge to examine whether SNPs in genes regulating production of pro-inflammatory cytokines predict fatigue following initiation of ADT for prostate cancer. Selected SNPs are located in predicted transcription factor binding sites, regions with putative regulatory control over gene expression. Results indicate that patients with the G/C or C/C *IL6-174* genotype reported greater increases in fatigue severity, frequency, and duration after initiation of ADT than patients with the G/G genotype, although inclusion of age, race, and depressive symptomatology as control variables attenuated the relationship between *IL6* genotype and these outcomes. Patients with the G/A *TNFA-308* genotype reported greater increases in fatigue severity than patients with the G/G genotype. Genetic variation at *IL1B-511* was not predictive of changes in fatigue. The current study also demonstrated a cumulative effect of genetic variants on increases in fatigue, such that patients with a greater number of variants reported larger increases in fatigue interference and duration. These findings are noteworthy because they lend additional support for the role of genetic variation in the development of fatigue in cancer patients.

It is unclear why *IL6* and *TNFA* genotype affected some aspects of fatigue, but not others. One possibility may be that our sample size was not large enough to detect a true effect for every outcome. Patients with the *IL6* G/C or C/C genotype displayed higher scores on every aspect of fatigue than patients with the G/G genotype although not all comparisons were statistically significant; the same was true for patients with the *TNFA* G/A genotype. Several comparisons demonstrated small p values that were nevertheless above the p 0.05 cutoff, such as differences in fatigue severity by *IL6* genotype ($p=0.11$) and differences in frequency ($p=0.10$) and duration of fatigue ($p=0.08$) by *TNFA* genotype. Alternately, pro-inflammatory cytokines may differentially affect various aspects of the fatigue experience in prostate cancer patients. Further studies with larger sample sizes are needed to examine this question.

Nevertheless, results from the current study augment the small existing literature examining pro-inflammatory cytokine genes as predictors of cancer-related fatigue (Aouizerat, et al., 2009; Collado-Hidalgo, et al., 2008; Miaskowski, et al., 2010; Rausch, et al., 2010). Regarding the *IL1B* gene, our findings of no association between genetic variants and cancer-related fatigue conflict with previous literature demonstrating a significant relationship. To our knowledge, three studies have examined SNPs in *IL1B* as predictors of fatigue in cancer patients (Collado-Hidalgo, et al., 2008; Rausch, et al., 2010; Reinertsen, et al., 2011). Of these, two studies examined the same SNP as the current study (rs16944)

(Collado-Hidalgo, et al., 2008; Reinertsen, et al., 2011); one found a significant effect of genotype (Collado-Hidalgo, et al., 2008) while the other did not (Reinertsen, et al., 2011). The third study examined five other SNPs, of which only one (i.e., rs1143627) was in high linkage disequilibrium ($r^2 > 0.80$) with the SNP we examined (Rausch, et al., 2010). This SNP was also not significantly associated with fatigue (Rausch, et al., 2010).

Regarding the *IL6* gene, three other studies have examined genetic variation as a predictor of cancer-related fatigue (Collado-Hidalgo, et al., 2008; Miaskowski, et al., 2010; Reinertsen, et al., 2011). Two studies examined the same SNP as in the current study (i.e., rs1800795). One of these studies found no association between genetic variation at that site and fatigue in breast cancer survivors (Reinertsen, et al., 2011). The other found that breast cancer survivors with the G/G and C/C genotype reported higher fatigue than survivors with the G/C genotype (Collado-Hidalgo, et al., 2008). In contrast, we found that patients with the G/C or C/C genotype reported greater increases in fatigue than survivors with the G/G genotype. Thus, in our study the G/G genotype appeared to have a protective effect on fatigue. Mixed findings regarding the at-risk genotype are congruent with conflicting literature examining the *IL6-174* genotype as a predictor of circulating IL-6 levels. Several studies have reported that the C allele is associated with higher circulating IL-6 (Boiardi et al., 2006; Brull et al., 2001; Ravaglia et al., 2005), although other studies have found no difference or lower IL-6 among C allele carriers (Bennermo et al., 2011; Fishman et al., 1998; Garg et al., 2006; Hulkkonen et al., 2001; Walston et al., 2005).

Regarding the *TNFA* gene, our findings of a significant association between variation at rs1800629 and fatigue are consistent with a study of radiotherapy patients examining the same SNP (Aouizerat, et al., 2009). Nevertheless, the direction of effects was opposite, with G/A genotype associated with greater increases in fatigue in our study and less fatigue in the other study (Aouizerat, et al., 2009). The directionality of our findings is consistent with the majority of studies indicating that the A allele at rs1800629 is associated with increased gene expression and higher circulating levels of TNF- α (Abraham and Kroeger, 1999; Kroeger et al., 1997; Kroeger et al., 2000; Wilson et al., 1997).

Taken together, our results and those from previous studies suggest that genetic variation may play a role in cancer related fatigue. For some SNPs, however, it is unclear which genetic variants place patients at risk. While SNPs in the current study were located in predicted transcription factor binding sites which regulate gene expression, a single gene can have many regulatory sites scattered across the genome. As such, the influence of variants examined in the current study may be moderated by other regulatory regions. The regulatory sites for *IL1B*, *IL6*, and *TNFA* genes have not yet been fully identified. As basic research on genome fine mapping progresses, new SNPs may be uncovered that will help to clarify the mechanisms of inflammatory gene transcription as they relate to fatigue in cancer patients. Fine mapping research should be pursued concurrently with biobehavioral studies examining the role of downstream gene products in cancer-related fatigue, such as mRNA (Bower et al., 2011; Reinertsen, et al., 2011).

In the meantime, preliminary findings from the current study represent an important first step in identifying genetic variation as a predictor of fatigue secondary to ADT. A strength of the study was the longitudinal design with a pre-ADT baseline and uniform timing of assessments. Limitations of the study, however, include a small sample that was relatively homogenous in terms of race. Additional research is needed to confirm these findings in larger and more diverse samples of men starting treatment with ADT. Race was assessed via self report; ancestry informative markers (AIMs) were not collected. We did not collect data regarding participants' body mass index (BMI), a correlate of inflammation (O'Connor et al., 2009). Future research should control for race using AIMs and also control for BMI. We

also did not assess the functional relevance of these SNPs using circulating cytokines, which will be important in future research. Our study was exploratory in nature; thus we did not control for multiple statistical tests. Consideration of Type I error will be important as the field moves forward. Additional research should be conducted as part of a larger effort to identify additional polymorphisms in candidate genes and gene-by-gene interactions as well as to elucidate the relationship between gene expression and cancer-related fatigue.

A detailed understanding of genetic mechanisms involved in the development and maintenance of cancer-related fatigue has high clinical relevance. Evidence suggests that behavioral interventions can prevent or reduce fatigue secondary to cancer and its treatment (Jacobsen et al., 2007). Additional evidence points to the promise of pharmacologic interventions for fatigue in cancer patients (Breitbart and Alici, 2010). Early identification of patients with genetic risk factors can enable clinicians to provide timely behavioral or pharmacologic intervention to prevent or reduce fatigue. This goal is consistent with personalized cancer treatment, or treatment tailored to individuals' genetic profiles to maximize therapeutic benefit and minimize side effects (National Cancer Institute, 2008). Treatment delivered in this way will help to ensure that cancer patients achieve the best possible outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Unadjusted Mean Fatigue Levels and 95% Confidence Intervals by *IL6* Genotype.

Figure 2.
Unadjusted Mean Fatigue Levels and 95% Confidence Intervals by *TNFA* Genotype.

Table 1

Genotype Frequencies.

	rs16944 (<i>IL1B</i>)	rs1800795 (<i>IL6</i>)	rs1800629 (<i>TNFA</i>)
Homozygous major allele	28 (53%) G/G	18 (34%) G/G	33 (62%) G/G
Heterozygous	17 (32%) G/A	29 (55%) G/C	20 (38%) G/A
Homozygous minor allele	8 (15%) A/A	6 (11%) C/C	0 (0%) A/A
Minor allele frequency	0.31	0.39	0.19

Table 2

Means and Standard Deviations for Fatigue By Genotype.

	IL1β				Genotype* Time F	p
	G/G		G/A or A/A			
	Time 1	Time 2	Time 1	Time 2		
Fatigue severity	2.13 (2.11)	3.21 (1.95)	1.94 (1.96)	2.60 (2.46)	.56	.46
Fatigue interference	1.48 (2.21)	2.11 (1.95)	1.29 (2.42)	2.30 (3.12)	.54	.46
Frequency of fatigue	2.21 (2.25)	3.43 (2.57)	1.96 (2.35)	2.80 (2.68)	.35	.56
Duration of fatigue	2.68 (2.57)	3.39 (2.71)	2.20 (2.96)	2.88 (3.15)	.00	.96
	IL6					
	G/G		G/C or C/C			
	Time 1	Time 2	Time 1	Time 2		
Fatigue severity	2.24 (2.26)	2.49 (2.44)	1.94 (1.92)	3.15 (2.08)	2.72	.11
Fatigue interference	1.71 (2.76)	1.68 (2.56)	1.23 (2.03)	2.47 (2.54)	5.71	.02
Frequency of fatigue	2.28 (2.47)	2.44 (2.64)	2.00 (2.21)	3.49 (2.57)	4.17	.05
Duration of fatigue	2.67 (3.01)	2.50 (2.68)	2.34 (2.63)	3.49 (3.00)	4.18	.05
	TNFα					
	G/G		G/A			
	Time 1	Time 2	Time 1	Time 2		
Fatigue severity	2.10 (2.08)	2.48 (2.00)	1.95 (1.98)	3.65 (2.39)	5.67	.02
Fatigue interference	1.35 (2.25)	1.92 (2.50)	1.46 (2.42)	2.66 (2.63)	1.43	.24
Frequency of fatigue	2.09 (2.34)	2.73 (2.53)	2.10 (2.25)	3.80 (2.69)	2.77	.10
Duration of fatigue	2.30 (2.73)	2.58 (2.77)	2.70 (2.81)	4.10 (2.95)	3.19	.08

Note: Unadjusted means, standard deviations, and repeated measures ANOVAs are shown.