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Associations Between Neurotransmitter Genes and Fatigue and Energy Levels in Women Following Breast Cancer Surgery

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Associations Between Neurotransmitter Genes and Fatigue and Energy Levels in Women Following Breast Cancer Surgery

by

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Abstract

Purpose: This study explores associations between variations in neurotransmitter genes and fatigue and energy levels in a sample of patients following breast cancer surgery. Variations in neurotransmitter genes between the Lower (n=153) and Higher (n=244) Fatigue latent classes as well as between the Higher (n=127) and Lower (n=270) Energy latent classes were evaluated.

Method: This analysis is part of a larger, longitudinal study that evaluated neuropathic pain and lymphedema in women who underwent breast cancer surgery. Patients completed baseline assessments at enrollment and monthly for 6 months following surgery. Growth mixture modeling (GMM) was used to identify distinct latent classes for fatigue severity and energy levels based on Lee Fatigue Scale (LFS) scores. A total of 30 candidate genes involved in various aspects of neurotransmission were evaluated.

Results: Ten genetic associations (i.e., ADRB2 rs1042718, BDNF rs6265, COMT rs9332377, CYP3A4 rs4646437, GALR1 rs949060, GCH1 rs3783642, NOS1 rs9658498, NOS1 rs2293052, NPYR1 Haplotype A04, and SLC6A2 rs17841327) were associated with latent class membership for fatigue. Seven genetic associations (i.e., NOS1 rs471871, SLC6A1 rs2675163, SLC6A1 Haplotype D01, SLC6A2 rs36027, SLC6A3 rs37022, SLC6A4 rs2020942, and TAC1 rs2072100) were associated with latent class membership for energy. Only two (i.e., NOS1, SLC6A2) of thirteen genes were associated with latent class membership for both fatigue and energy.

Conclusions: The molecular findings from this study help support the hypothesis that fatigue and energy are different, yet related symptoms. This study suggests a large number of neurotransmitters (i.e., proteins and receptors) play a role in the development and maintenance of fatigue and energy levels in breast cancer patients.

Key words: fatigue, energy, neurotransmitter genes, growth mixture modeling, breast cancer, candidate genes

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Introduction

Fatigue is the most common symptom associated with cancer and its treatments.[1] Prevalence rates for fatigue range from 59% to 100%.[2] While several studies have examined fatigue in breast cancer patients receiving chemotherapy (CTX)[3] and radiation (RT),[4] studies on the occurrence of and predictors for fatigue following surgery are scarce. In a recent study that examined fatigue in women following breast cancer surgery,[5] women reported relatively high levels of fatigue in the first two months after surgery followed by mild to moderate levels of fatigue that persisted for 12 months after surgery.

The measurement of a patient's level of energy has received little or no attention in the cancer literature. While energy level is commonly thought of as the opposite of fatigue, evidence suggests that fatigue and energy are related, yet distinct concepts.[6,7] In the only study that evaluated energy levels in patients with breast cancer prior to surgery,[8] while 32% of the women reported clinically meaningful levels of fatigue prior to breast cancer surgery, nearly 50% of these women reported clinically meaningful decrements in energy levels. Findings from this study of patients with breast cancer, as well as a study of patients with HIV disease,[9] support the hypothesis that energy is a distinct concept from fatigue.

Factors that contribute to fatigue severity are multidimensional and include numerous biopsychosocial characteristics.[10] Some of the predictors of fatigue following breast cancer surgery include higher levels of anxiety; the personality characteristic of extraversion;[11] increased fatigue prior to surgery;[12] higher levels of emotional distress, mental fatigue, and pain;[13] as well as depressive symptoms and receipt of CTX.[5]

Recent evidence suggests that genetic mechanisms are involved in the modulation of fatigue experienced by breast cancer patients. For example, in one study that examined genetic variations among breast cancer patients,[14] a number of proinflammatory cytokine genes were associated with fatigue. In addition, work by our research team found that variations in interleukin 4 (IL4)[15] and IL6[16] were associated with distinct fatigue trajectories. Polymorphisms

in these cytokine genes may contribute to the severity of fatigue through the modulation of pro- and anti- inflammatory pathways.[15,17]

Although the majority of the literature on genetic associations with fatigue has focused on cytokine dysregulation, a number of additional pathways may influence fatigue and energy levels. Neurotransmitter dysregulation may play an important role in the severity of fatigue and/or changes in energy levels. The most commonly cited neurotransmitter associated with fatigue is serotonin. For example, increased serum levels of serotonin were linked to fatigue following prolonged exercise.[18] However, it is unlikely that a single neurotransmitter is responsible for the development of/or changes in fatigue and/or energy levels. Rather, it is more likely that several neurotransmitters would contribute to inter-individual variability in fatigue and energy.[19] Some neurotransmitter genes that were associated with fatigue and energy in a variety of populations include alterations in the dopaminergic system, specifically polymorphyisms in catechol-o-methyl-transferase (COMT), dopamine-2 receptor (DRD2), and dopamine-1 transporter (DAT1).[20] However, no studies were identified that evaluated for associations between neurotransmitter genes and fatigue and energy levels in patients with breast cancer.

This study of variations in neurotransmitter genes is based on previous work from our research team that used growth mixture modeling (GMM) to identify distinct latent classes for fatigue severity (unpublished data) and energy levels (unpublished data) in women (n=398) prior to and for six months following breast cancer surgery. In the GMM analysis for fatigue, two distinct latent classes were identified (i.e., Lower Fatigue (38.5%) and Higher Fatigue (61.5%)). At enrollment, mean fatigue scores were 1.60 and 3.90 for the Lower and Higher Fatigue classes, respectively. In both fatigue classes, fatigue scores remained relatively constant from the preoperative assessment to 6 months after breast cancer surgery. In the GMM analysis for energy, two distinct latent classes were identified (i.e., Higher Energy (32.0%) and Lower Energy (68.0%)). At enrollment, mean energy scores were 5.82 and 4.35 for the Higher and

Lower Energy classes, respectively. In both energy groups, energy levels remained relatively constant from the preoperative assessment to 6 months after breast cancer surgery.

Given the paucity of research on the role of neurotransmitters in fatigue and energy levels in patients with breast cancer, the purpose of this study was to evaluate for variations in neurotransmitter genes between the Lower and Higher Fatigue latent classes as well as between the Higher and Lower Energy latent classes.

Materials and Methods

Patients and Settings

This analysis is part of a larger, longitudinal study that evaluated neuropathic pain and lymphedema in women who underwent breast cancer surgery. The study methods are described in detail elsewhere.[21-23] In brief, patients were recruited from breast care centers located in a Comprehensive Cancer Center, two public hospitals, and four community practices.

Patients were eligible to participate if they: were adult women (≥18 years) who were scheduled to undergo breast cancer surgery on one breast; were able to read, write, and understand English; agreed to participate; and gave written informed consent. Patients were excluded if they were having breast cancer surgery on both breasts and/or had distant metastasis at the time of diagnosis. A total of 516 patients were approached, 410 were enrolled (response rate 79.5%), and 398 completed the baseline assessment. The most common reasons for refusal were: too busy, overwhelmed with the cancer diagnosis, or insufficient time available to do the baseline assessment prior to surgery.

Instruments

The demographic questionnaire obtained information on age, marital status, education, ethnicity, employment status, and living situation. The Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability.[24] Patients rated their functional status using the KPS scale that ranged from 30 (I feel severely disabled and need to be hospitalized) to 100 (I feel normal; I have no complaints or symptoms).

The Self-Administered Comorbidity Questionnaire (SCQ) is a short and easily understood instrument that was developed to measure comorbidity in clinical and health service research settings.[25] The questionnaire consists of 13 common medical conditions that were simplified into language that could be understood without any prior medical knowledge. Patients were asked to indicate if they had the condition; if they received treatment for it; and did it limit

their activities. The SCQ has well-established validity and reliability and has been used in studies of patients with a variety of chronic conditions.[26,27]

The Lee Fatigue Scale (LFS) consists of 18 items designed to assess physical fatigue and energy.[28] Each item was rated on a 0 to 10 numeric rating scale (NRS). Total fatigue and energy scores were calculated as the mean of the 13 fatigue items and the 5 energy items, with higher scores indicating greater fatigue severity and higher levels of energy. Patients were asked to rate each item based on how they felt "right now". The LFS has been used with healthy individuals[28,29] and in patients with cancer and HIV.[30-33] A cutoff score of $\geq$4.4 indicates high levels of fatigue.[4] A cutoff score of $\leq$4.8 indicates low levels of energy.[4] The LFS has well established validity and reliability. Cronbach's alphas for fatigue and energy scales were .96 and .93, respectively.

Study Procedures

The study was approved by the Committee on Human Research at the University of California, San Francisco and by the Institutional Review Boards at each of the study sites. During the patient's preoperative visit, a clinician explained the study and determined patients' willingness to participate. The research nurse met with interested women, determined eligibility, and obtained written informed consent prior to surgery. After obtaining consent, patients completed the enrollment questionnaires an average of 4 days prior to surgery. Patients completed the LFS at enrollment and monthly for 6 months (i.e., 7 assessments). Medical records were reviewed for disease and treatment information.

Genomic analyses

Gene selection – A total of 30 candidate genes involved in various aspects of neurotransmission, drug metabolism, or transport of molecules across cell membranes were evaluated. Genes involved in catecholaminergic neurotransmission included alpha-1D-adrenergic receptor (ADRA1D); alpha-2A-adrenergic receptor (ADRA2A); beta-2-adrenergic receptor (ADRB2); beta-3-adrenergic receptor (ADRB3); beta adrenergic receptor kinase 2

(ADRBK2); catecho-o-methyl transferase (COMT); solute-like carrier (SLC) family 6 member 2 – noradrenaline transporter (SLC6A2); and SLC family 5 member 3 – dopamine transporter (SLC6A3). The gene involved in the gabaergic system was SLC family 6 member 1 – GABA transporter (SLC6A1). Genes involved in serotonergic neurotransmission included: GTP cyclohydrolase 1 (GCH1); 5-hydroxytryptamine receptor (HTR) 1A (HTR1A); HTR 1B (HTR1B); HTR 2A (HTR2A); HTR 3A (HTR3A); SLC family 6 member 4 – serotonin transporter (SLC6A4); tyrosine hydroxylase (TH); and tryptophan hydroxylase 2 (TPH2). The two genes involved in molecular transport and drug metabolism that were evaluated were ATP-binding cassette, subfamily B member 1 (ABCB1) and cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4). A number of additional genes that are involved in various aspects of neurotransmission that were evaluated included: brain-derived neurotrophic factor (BDNF); galanin (GAL); galanin receptor 1 (GALR1); galanin receptor 2 (GALR2); nitric oxide synthase 1 (NOS1); nitric oxide synthase 2 (NOS2); neuropeptide Y (NPY); neuropeptide Y receptor 1 (NPYR1); prodynorphin (PDYN); tachykinin precursor 1 (TAC1); and tachykinin receptor 1 (TACR1).

Blood collection and genotyping - Of the 398 patients who completed the baseline assessment, 310 provided a blood sample from which DNA could be isolated from peripheral blood mononuclear cells (PBMCs). Genomic DNA was extracted from PBMCs using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). DNA was quantitated with a Nanodrop Spectrophotometer (ND-1000) and normalized to a concentration of 50 ng/μL (diluted in 10 mM Tris/1 mM EDTA). Genotyping was performed using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina, San Diego, CA). Two blinded reviewers visually inspected signal intensity profiles and resulting genotype calls for each SNP.

SNP selection - A combination of tagging SNPs and literature driven SNPs were selected for analysis. Tagging SNPs were required to be common (defined as having a minor allele

frequency of ≥0.05) in public databases. In order to ensure robust genetic association analyses, quality control filtering of SNPs was performed. SNPs with call rates of <95% or Hardy-Weinberg p-values of <.001 were excluded. As shown in Table 1, a total of 249 SNPs among the 30 candidate genes passed all of the quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with fatigue and energy were examined using PUPASuite 2.0.[34]

Statistical Analyses for the Phenotypic Data

Data were analyzed using SPSS version 20[35] and STATA Version 13.[36] Descriptive statistics and frequency distributions were generated for sample characteristics. Independent sample t-tests (for continuous variables), Mann-Whitney U tests (for continuous variables not normally distributed), and Chi square analyses (for categorical variables) were used to evaluate for differences in demographic and clinical characteristics between the two latent classes for fatigue and energy. All calculations used actual values. Adjustments were not made for missing data. Therefore, the cohort for each analysis was dependent on the largest set of available data between groups.

Unconditional GMM with robust maximum likelihood estimation was carried out to identify latent classes with distinct fatigue and energy trajectories using Mplus Version 5.21. These methods are described in detail elsewhere.[37] In brief, a single growth curve that represented the "average" change trajectory was estimated for the whole sample. Then, the number of latent growth classes for fatigue (unpublished data) and energy (unpublished data) that best fit the data was identified using guidelines recommended in the literature.[38-40]

Statistical Analyses for the Genetic Data

Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher Exact tests. Measures of linkage disequilibrium ((LD), i.e., D' and r^2) were computed from the patients' genotypes with Haploview 4.2. The LD-based haplotype block definition was based on D' confidence interval.[41]

For SNPs that were members of the same haploblock, haplotype analyses were conducted in order to localize the association signals within each gene and to determine if haplotypes improved the strength of the association with the phenotype. Haplotypes were constructed using the program PHASE version 2.1.[42] In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated 5 times using different seed numbers with each cycle. Only haplotypes that were inferred with probability estimates of $\geq$.85, across the five iterations, were retained for downstream analyses. Only inferred haplotypes that occurred with a frequency estimate of $\geq$15% were included in the association analyses, assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[43-45] Homogeneity in ancestry among patients was verified by principal component analysis,[46] using HelixTree (GoldenHelix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought that distinguished the major racial/ethnic groups in the sample by visual inspection of scatter plots of orthogonal PCs (i.e., PC 1 versus PC2, PC2 versus PC3). This procedure was repeated until no discernable clustering of patients by their self-reported race/ethnicity was possible (data not shown). The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including them in all of the logistic regression models. One hundred and six AIMs were included in the analysis.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., delta <10%), the genetic model that best fit the data, by maximizing the significance of the p-value was selected for each SNP. Logistic regression analysis, that controlled for significant covariates, as well as genomic estimates of and self-reported race/ethnicity, was used to evaluate the associations between genotype and Higher Fatigue and Lower Energy class memberships. Only those genetic associations identified as significant from the bivariate analyses were evaluated in the

multivariate analyses. A backwards stepwise approach was used to create a parsimonious model. Except for race/ethnicity, only predictors with a p-value of <.05 were retained in the final model. Genetic model fit and both unadjusted and covariate-adjusted odds ratios were estimated using STATA version 13.[36]

As was done in our previous studies,[15,47,48] based on the recommendations in the literature[49] as well as the implementation of rigorous quality controls for genomic data, the non-independence of SNPs/haplotypes in LD, and the exploratory nature of the analyses, adjustments were not made for multiple testing. In addition, significant SNPs identified in the bivariate analyses were evaluated further using logistic regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variations in other SNPs/haplotypes within the same gene. Only those SNPs that remained significant were included in the final presentation of the results. Therefore, the significant independent associations reported are unlikely to be due solely to chance. Unadjusted associations are reported for all of the SNPs that passed quality control criteria in Table 1, to allow for subsequent comparisons and meta-analyses.

Results

Differences in Demographic and Clinical Characteristics between the Fatigue Latent Classes

As summarized in Table 2, no differences were found between the Lower Fatigue and Higher Fatigue classes for the majority of the demographic and clinical characteristics. However, patients in the Higher Fatigue class were significantly younger, had a lower KPS score, and a higher fatigue severity score at enrollment (all $p<.0001$). In addition, patients in the Higher Fatigue class had a higher SCQ score ($p=.009$), more years of education ($p=.04$), and had a higher number of lymph nodes removed ($p=.016$). A larger percentage of patients in the Higher Fatigue class had received neoadjuvant CTX ($p=.014$) and had received CTX during the first 6 months after breast cancer surgery ($p=.001$).

Candidate Gene Analyses

As shown in Table 1, genotype distributions differed between the Lower and Higher Fatigue classes for: 2 SNPs and one haplotype in ADRB2; 3 SNPs in BDNF; 1 SNP in COMT; 1 SNP in CYP3A4; 1 SNP in GALR1; 1 SNP in GCH1; 5 SNPs and 2 haplotypes in NOS1; 1 SNP and 1 haplotype in NPYR1; 1 SNP and 1 haplotype in SLC6A1; 2 SNPs and 1 haplotype in SLC6A2; 1 SNP in SLC6A3; and 2 SNPs and 1 haplotype in TAC1.

Regression Analyses for ADRB2, BDNF, COMT, CYP3A4, GALR1, GCH1, NOS1, NPYR1, and SLC6A2 Genotypes and Lower Fatigue versus Higher Fatigue Classes

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the odds of belonging to the Higher Fatigue as compared to the Lower Fatigue class, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the phenotypic characteristics that remained significant in the multivariate model were: age (in 5 year increments), KPS score (in 10 point increments), SCQ score, and receipt of CTX within six months after breast cancer surgery.

Ten genetic associations remained significant in the multivariate logistic regression analyses: ADRB2 rs1042718, BDNF rs6265, COMT rs9332377, CYP3A4 rs4646437, GALR1 rs949060, GCH1 rs3783642, NOS1 rs9658498, NOS1 rs2293052, NPYR1 Haplotype A04, and SLC6A2 rs17841327 (Table 4).

In the regression analysis for ADRB2 rs1042718, carrying two doses of the rare A allele (i.e., CC + CA versus AA) was associated with a 87% decrease in the odds of belonging to the Higher Fatigue class (p=.008). In the regression analysis for BDNF rs6265, carrying one or two doses of the rare A allele (i.e., GG versus GA + AA) was associated with a 50% decrease in the odds of belonging to the Higher Fatigue class (p=.020). In the regression analysis for COMT rs9332377, carrying one or two doses of the rare C allele (i.e., TT versus TC + CC) was associated with a 52% decrease in the odds of belonging to the Higher Fatigue class (p=.026). In the regression analysis for CYP3A4 rs4646437, carrying one or two doses of the rare T allele (i.e., CC versus CT + TT) was associated with a 52% decrease in the odds of belonging to the Higher Fatigue class (p=.025). In the regression analysis for GALR1 rs949060, carrying two doses of the rare C allele (i.e., GG + GC versus CC) was associated with a 2.46-fold increase in the odds of belonging to the Higher Fatigue class (p=.020). In the regression analysis for GCH1 rs3783642, carrying one or two doses of the rare C allele (i.e., TT versus TC + CC) was associated with a 53% decrease in the odds of belonging to the Higher Fatigue class (p=.003).

For NOS1, two SNPs (rs9658498, rs2293052) were associated with membership in the Higher Fatigue class. In the regression analysis, including both SNPs, for NOS1 rs9658498, carrying two doses of the rare C allele (i.e., TT + TC versus CC) was associated with a 55% decrease in the odds of belonging to the Higher Fatigue class (p=.029). In the same regression analysis, for NOS1 rs2293052, carrying two doses of the rare T allele (i.e., CC + CT versus TT) was associated with a 4.58-fold increase in the odds of belonging to the Higher Fatigue class (p=.004). In the regression analysis for NPYR1 HapA04, that is composed of alleles at two SNPs (i.e., rs9764 [common T allele], and rs7687423 [common G allele]), each additional dose

of NPYR1 HapA04 was associated with a 1.77-fold increase in the odds of belonging to the Higher Fatigue class (p=.003). In the regression analysis for SLC6A2 rs17841327, carrying two doses of the rare A allele (i.e., CC + CA versus AA) was associated with a 10.31-fold increase in the odds of belonging to the Higher Fatigue class (p=.003).

Differences in Demographic and Clinical Characteristics between the Energy Latent Classes

As summarized in Table 3, no differences were found between the Higher Energy and Lower Energy classes for the majority of the demographic and clinical characteristics. However, patients in the Lower Energy class had a lower KPS score (p=.002), a higher SCQ score (p=.001), and a lower mean energy score at enrollment (p<.0001). In addition, a significant difference was found between the Higher Energy and Lower Energy classes based on stage of disease (p=.040).

Candidate Gene Analysis

As shown in Table 1, genotype distributions differed between the Higher Energy and Lower Energy classes for: 1 SNP in COMT; 2 SNPs in HTR2A; 1 SNP in NOS1; 1 SNP in NOS2A; 4 SNPs and 3 haplotypes in SLC6A1; 4 SNPs in SLC6A2; 1 SNP in SLC6A3; 3 SNPs and 1 haplotype in SLC6A4; 1 SNP in TAC1; and 1 SNP in TACR1.

Regression Analyses for NOS1, SLC6A1, SLC6A2, SLC6A3, SLC6A4, and TAC1 Genotypes and Higher Energy versus Lower Energy Classes

In order to better estimate the magnitude (i.e., odds ratio, OR) and precision (95% confidence interval, CI) of genotype on the odds of belonging to the Lower Energy as compared to the Higher Energy class, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the phenotypic characteristics that remained significant in the multivariate model were: KPS score (in 10 point increments) and receipt of CTX within six months after breast cancer surgery.

Seven genetic associations remained significant in the multivariate logistic regression analyses: NOS1 rs471871, SLC6A1 rs2675163, SLC6A1 Haplotype D01, SLC6A2 rs36027, SLC6A3 rs37022, SLC6A4 rs2020942, and TAC1 rs2072100 (Table 5).

In the regression analysis for NOS1 rs471871, carrying two doses of the rare T allele (i.e., AA + AT versus TT) was associated with a 72% decrease in the odds of belonging to the Lower Energy class (p=.010). For SLC6A1, one SNP (rs2675163) and one haplotype (HapD01) were associated with membership in the Lower Energy class. In the regression analysis, for SLC6A1 rs2675163, carrying one or two doses of the rare C allele (i.e., TT versus TC + CC) was associated with a 1.85-fold increase in the odds of belonging to the Lower Energy class (p=.025). In the same regression analysis, for SLC6A1 HapD01, that is composed of alleles at three SNPs (i.e., rs10514669 [common C allele], rs2697138 [common C allele], and rs1062246 [common A allele]), each additional dose of SLC6A1 HapD01 was associated with a 40% decrease in the odds of belonging to the Lower Energy class (p=.009). In the regression analysis for SLC6A2 rs36027, each additional dose of the rare G allele (i.e., AA versus AG versus GG) was associated with a 41% decrease in the odds of belonging to the Lower Energy class (p=.004). In the regression analysis for SLC6A3 rs37022, carrying two doses of the rare A allele (i.e., TT + TA versus AA) was associated with a 9.75-fold increase in the odds of belonging to the Lower Energy class (p=.036). In the regression analysis for SLC6A4 rs2020942, carrying two doses of the rare A allele (i.e., GG + GA versus AA) was associated with a 64% decrease in the odds of belonging to the Lower Energy class (p=.011). In the regression analysis for TAC1 rs2072100, carrying two doses of the rare G allele (i.e., AA + AG versus GG) was associated with a 2.11-fold increase in the odds of belonging to the Lower Energy class (p=.028).

Discussion

Differences in phenotypic characteristics between the fatigue latent classes as well as between the energy latent classes are described in detail elsewhere (data in preparation). Therefore, this discussion will focus on the genotypic differences.

Polymorphisms Associated With Fatigue

Polymorphisms in the β2-adrenergic receptor (ADRB2) gene may protect individuals from higher levels of fatigue through a number of mechanisms. The ADRB2 receptor, located in musculoskeletal, cardiovascular, respiratory, and metabolic systems, is part of the G-protein-coupled receptor family that influences sympathetic nervous system responses. In addition, this receptor plays a role in the regulation of lipid metabolism. Polymorphisms in the ADRB2 are associated with bronchodilation; insulin secretion; gluconeogenesis and glycogenolysis in skeletal muscle; as well as increased cardiac output; arterial dilation; and lipolysis.[50] Sarpeshkar and Bentley hypothesized that alterations in this gene may be responsible for enhanced aerobic capacity and delayed exercise-induced fatigue.[50] In addition, ADRB2 receptor stimulation inhibits production of type 1 pro-inflammatory cytokines[51] and under-expression of ADRB2 receptors is associated with chronic fatigue syndrome.[52]

In our study, patients who carried two doses of the rare A allele for ADRB2 rs1042718 had a 87% decrease in the odds of belonging to the Higher Fatigue class. Polymorphisms in ADRB2 rs1042718, located on chromosome 5, result in the creation of a synonymous codon (i.e., arginine). No studies were identified that evaluated for associations between rs1042718 and fatigue. However, two studies identified significant associations between rs1042718 and other clinical phenotypes (i.e., enhanced longevity[53] and negative emotions[54]). In the study by Zeng and colleagues,[54] individuals who were heterozygous or homozygous for the rare allele in rs1042718 were less likely to report feelings of uselessness, loneliness, and anxiety. Of note, these results are consistent with our finding that patients who were homozygous for the rare

allele in rs1042718 were less likely to be classified in the Higher Fatigue class because previous studies demonstrated significant associations between psychological distress (e.g. anxiety, depression) and increased fatigue in patients with a variety of cancer diagnoses.[55-57]

BDNF is a neural growth factor found throughout the central nervous system. BDNF is associated with overall brain health because it plays a role in the promotion of neurogenesis, neuroprotection, mental performance, and cognitive function.[58] Altered BDNF levels are associated with Fibromyalgia syndrome, a chronic pain condition that includes fatigue as an associated symptom,[59] as well as chronic fatigue syndrome[60] and depression.[61]

BDNF rs6265 is a missense mutation that results in a functional change in the amino acid sequence from valine (Val) to methionine (Met). In two studies,[61,62] decreases in serum BDNF levels were associated with the Met allele. In our study, carrying one or two doses of the rare allele was associated with a reduction in the odds of belonging to the Higher Fatigue class. One might hypothesize that lower levels of BDNF would be associated with membership in the Higher Fatigue class given that lower levels of BDNF were associated with depression[61] and chronic fatigue syndrome.[60] However, findings regarding changes in serum levels of BDNF associated with the Met allele are inconsistent.[63] In addition, the effect of the Met allele on BDNF levels in the brain, where it may play a greater role in the perception of fatigue, remains unknown. No studies were identified that evaluated for associations between BDNF rs6265 and fatigue.

Catechol-o-methyltransferase (COMT) is a key enzyme responsible for the metabolism and inactivation of dopamine, norepinephrine, and epinephrine.[64] Alterations in the COMT gene, located on chromosome 22, were associated with fatigue and pain in breast cancer patients through interactions with two stress pathways (i.e., hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic nervous system (SNS)).[64-66] A specific polymorphism in the COMT gene (i.e., rs4650) that results in a Val to Met substitution has been studied extensively. This SNP has functional consequences for the COMT enzyme that results in altered levels of dopamine[67] and

catecholamines. However, it is unlikely that only one polymorphism in the COMT gene would contribute to fatigue. Indeed a number of studies found associations between other SNPs in the COMT gene and fatigue.[68]

In our study, patients who carried one or two doses of the rare C allele for COMT rs9332377 had a 52% decrease in the odds of belonging to the Higher Fatigue class. This intronic SNP is located near the 3' UTR of the COMT gene. Its location near the 3' UTR suggests that this polymorphism has a regulatory function and might affect COMT expression.[69] Only three studies have reported significant associations between COMT rs9332377 and clinical phenotypes (i.e., hearing loss,[70] suicidal ideation,[67] nicotine dependence[69]). No studies have evaluated for associations between COMT rs9332377 and fatigue. Of note, in a study of patients with mood disorders,[67] individuals who were homozygous for the rare C allele of COMT rs9332377 reported lower irritability scores on the Questionnaire for Measuring Factors of Aggression. This finding supports our association between rs9332377 and increased fatigue when one considers COMT's role in the manifestation of emotions, a possible marker for chronic fatigue syndrome.[68]

The CYP3A4 gene, located on chromosome 7, encodes for a hepatic enzyme that is a part of the cytochrome P450 superfamily. Cytochrome P450 enzymes are responsible for catalyzing multiple reactions involved in lipid synthesis and drug metabolism. In addition, CYP3A enzymes are responsible for the metabolism of approximately one-third of anticancer drugs.[71]

The rs4646437 SNP is located in intron 7 of the CYP3A4 gene. No studies have evaluated for associations between CYP3A4 rs4646437 and fatigue. However, a recent study reported an association between CYP3A4 rs4646437 and in vitro CYP3A expression and activity.[72] In this study, women who carried the rare T allele of rs4646437 had higher expression and activity of the CYP3A4 enzyme. Considering CYP3A4's role in metabolizing anti-cancer drugs, one can hypothesize that women who are able to more effectively metabolize CTX

agents would be less likely to experience higher levels of fatigue. This hypothesis is supported by our findings that carrying one or two doses of the rare T allele for rs4646437 was associated with a 52% decrease in the odds of belonging to the Higher Fatigue class.

Galanin, a neuropeptide found throughout the CNS, has an inhibitory effect on multiple neurotransmitters including serotonin and norepinephrine.[73] Polymorphisms in the galanin gene are associated with a number of clinical conditions including eating disorders,[74] cancer,[75] Alzheimer's disease,[76,77] depression, and anxiety.[73]

Within the CNS, the functional effects of galanin are mediated by three G-protein-coupled galanin receptor subtypes, including GALR1. The GAL1 receptor has an inhibitory effect on adenylate cyclase through coupling with the G proteins Gi/Go. This inhibition affects ATP metabolism and plays an important role in cellular energy pathways.[77] Of note, Staines[78] hypothesized that dysfunctions in G protein-coupled receptors, such as GALR1, contribute to the development of fatigue-related conditions. In our study, patients who carried two doses of the rare C allele for GALR1 rs949060 had a 2.46-fold increase in the odds of belonging to the Higher Fatigue class. GALR1 rs949060 is located on chromosome 18 approximately 3000 bps upstream of the GALR1 gene in the promoter region. No studies were identified that report on polymorphisms in GALR1 rs949060 and fatigue.

Guanosine triphosphate cyclohydrolase (GCH1) is the rate-limiting enzyme involved in the synthesis of tetrahydrobiopterin (BH4). BH4 plays a role in nitric oxide (NO) production and hydroxylation of aromatic amino acids. The GCH1 gene is located on chromosome 14. Polymorphisms in GCH1 are associated with pain,[79] altered cognitive performance,[80] and dopa-responsive dystonia.[81] In our study, carrying one or two doses of the rare C allele for GCH1 rs3783642 was associated with a 53% decrease in the odds of belonging to the Higher Fatigue class. No studies have reported on GCH1 rs3783642. However, one study did find a protective association between other polymorphisms in GCH1 and fibromyalgia syndrome, which is characterized by pain, fatigue, and mood disturbances.[82]

Neuropeptide Y receptor Y1 (NPYR1) is part of a family of G protein-coupled receptors that binds neuropeptide Y (NPY). NPY acts in both the central and peripheral nervous systems. Peripherally, NPY is a neurotransmitter that is released from sympathetic nerve endings. In the CNS, NPY acts on receptors present in those areas of the brain that are involved with emotion.[83] NPY is involved in sleep regulation, anxiety, memory, pain, and energy homeostasis.[84,85] Alterations in NPY are implicated in chronic fatigue syndrome[83] and depression.[86] Alterations in NPY signaling through variations in NPYR1 may have an effect on any of the aforementioned processes, including fatigue.

In our study, each additional dose of NPYR1 HapA04, that is composed of alleles at two SNPs (i.e., rs9764 [common T allele], and rs7687423 [common G allele]), was associated with a 1.77-fold increase in the odds of belonging to the Higher Fatigue class. HapA04 is located on chromosome 4 and is comprised of a 3-prime UTR SNP (rs9764) and one intronic SNP (rs7687423). Although no studies were identified that reported on NPYR1 HapA04, the polymorphisms rs9764 and rs7687423 were associated with nicotine[87] and methamphetamine[88] dependence, respectively. No studies were identified that reported on associations with either SNP and fatigue.

Polymorphisms Associated With Energy

The solute carrier family 6, member 1 (SLC6A1) gene, located on chromosome 3, encodes for one of the four GABA transporters found in the brain. The role of this transporter is to remove GABA from the synaptic cleft which decreases extracellular levels of GABA. The inhibitory neurotransmitter GABA is responsible for normal brain function. Based on studies of knockout mice,[89] deficiencies in SLC6A1 are associated with depression, reduced aggression, and reduced anxiety. Furthermore, research by Thoeringer and colleagues[90] demonstrated an association between polymorphisms in the SLC6A1 gene and anxiety disorders. In a recent genome-wide association study,[91] an association was found between a SNP in SLC6A1 and symptoms of inattention and hyperactivity in attention-deficit/hyperactivity disorder (ADHD).

In our study, one SNP (rs2675163) and one haplotype (HapD01) in the SLC6A1 gene were associated with membership in the Lower Energy class. Carrying one or two doses of the rare C allele of SLC6A1 rs2675163 was associated with a 1.85-fold increase in the odds of belonging to the Lower Energy class, while each additional dose of SLC6A1 HapD01, that is composed of alleles at three SNPs (i.e., rs10514669, rs2697138, and rs1062246), was associated with a 40% decrease in the odds of belonging to the Lower Energy class. No studies were identified that reported on polymorphisms in SLC6A1 rs2675163, rs10514669, rs2697138, or rs1062246.

The solute carrier family 6, member 3 (SLC6A3) gene, located on chromosome 5, encodes for a dopamine transporter. The dopamine transporter protein is responsible for re-uptake of dopamine from the synaptic cleft which results in decreased extracellular levels of dopamine.[92] Decreased levels of dopamine are hypothesized to play a role in the development of central fatigue because of dopamine's known effects on initiation of movement.[93] Therefore, alterations in dopaminergic circuits, including its transport receptors, may affect an individual's energy level and fatigue.

The majority of the literature on polymorphisms in the SLC6A3 gene has focused on ADHD.[94,95] In addition, associations were found between dopaminergic polymorphisms and fatigue,[20] as well as decreases in mental energy and sustained attention.[96] In our study, carrying two doses of the rare A allele of SLC6A3 rs37022 was associated with a 9.75-fold increase in the odds of belonging to the Lower Energy class. No studies were identified that reported on polymorphisms in this SNP.

The solute carrier family 6, member 4 (SLC6A4) gene, located on chromosome 17, encodes for a membrane protein that is responsible for re-uptake of serotonin from the synaptic cleft. The serotonergic neurotransmitter system is hypothesized to play a role in cancer-related fatigue.[97,98] Serotonin is involved in various human behaviors including sleep, mood, appetite, memory, and learning. Increased levels of serotonin in the brain are hypothesized to contribute

to fatigue through its interaction with the HPA axis leading to a sensation of reduce potential to perform physical activity.[97] Yamamoto et al.[99] demonstrated a reduced density of serotonin transporters in the rostral subdivision of the anterior cingulate of patients with chronic fatigue syndrome versus healthy controls. In addition, in one study, an association was found between polymorphisms in the promoter of the SLC6A4 gene and chronic fatigue syndrome.[100]

In our study, polymorphisms in the SLC6A4 gene were not associated with Higher Fatigue class membership. In our study, carrying two doses of the rare A allele of SLC6A4 rs2020942 was associated with a 64% decrease in the odds of belonging to the Lower Energy class. The rs2020942 polymorphism has been linked with obsessive-compulsive symptoms[101] and risk for nonsyndromic cleft lip with or without cleft palate.[102] No studies were identified that reported on associations between SLC6A4 rs2020942 and energy level.

The tachykinin, precursor 1 (TAC1) gene, located on chromosome 7, encodes for a group of tachykinin peptide hormones (i.e., substance P, neurokinin A, neuropeptide K, neuropeptide γ) that function as neurotransmitters. Substance P plays a role in inflammation in both the central and peripheral nervous systems.[103] Substance P is implicated in fibromyalgia syndrome, which is characterized by symptoms including pain, fatigue, anxiety, and depression.[104] In addition, Substance P is associated with fatigue and other negative mood states.[105] Therefore, polymorphisms in the tachykinin pathway genes may have an effect on fatigue and energy levels.

In our study, carrying two doses of the rare G allele of TAC1 rs2072100 was associated with a 2.11-fold increase in the odds of belonging to the Lower Energy class. The rs2072100 polymorphism has been linked with increased risk for colorectal cancer[106] and susceptibility to multiple sclerosis.[107] No studies were identified that reported on associations with rs2072100 and energy.

Polymorphisms Associated With Both Fatigue and Energy

Two genes (i.e., NOS1, SLC6A2) were associated with latent class membership for both fatigue and energy. Nitric oxide synthase 1 (NOS1) is part of a group of nitric acid synthases (NOS) responsible for the synthesis of NO. NO mediates several biological processes including vasodilation, neural regulation of skeletal muscle, and neurotransmission.[108] Elevated NO levels are implicated in several fatigue-related disorders including chronic fatigue syndrome,[109] fatigue in patients with muscular dystrophies,[110,111] and post-radiation syndrome.[112] NOS gene polymorphisms that alter regulation of NO synthesis may influence a patient's susceptibility for the development of fatigue. While no studies were identified on associations between NOS polymorphisms and fatigue, other studies found associations between polymorphisms in the NOS1 gene and depression[113] and anxiety.[114]

In our study, two SNPs (i.e., rs9658498, rs2293052) in the NOS1 gene were associated with membership in the Higher Fatigue class. Carrying two doses of the rare C allele of rs9658498 was associated with a 55% decrease in the odds of belonging to the Higher Fatigue class, while carrying two doses of the rare T allele of rs2293052 was associated with a 4.58-fold increase in the odds of belonging to the Higher Fatigue class. No studies were identified that reported on NOS1 rs9658498. However, in one study an association was found between rs2293052 and Parkinson's disease (PD).[115] These results support our findings of an association between this SNP and increased fatigue because similar to the aforementioned fatigue-syndromes, PD is associated with increased NO levels.[116] In addition, fatigue is a common symptom associated with PD[117] and may share similar susceptibility gene polymorphisms.

In addition, a different SNP (rs471871) in the NOS1 gene was associated with energy level. Carrying two doses of the rare T allele of rs471871 was associated with a 72% decrease in the odds of belonging to the Lower Energy class. No studies were identified that reported on NOS1 rs471871.

The solute carrier family 6, member 2 (SLCA2) gene encodes for the norepinephrine transporter (NET) protein. The NET found on noradrenergic synapses, is responsible for the removal of NE from the synaptic cleft and plays a major role in NE homeostasis.[118] Impairments in the NET protein may contribute to the development of fatigue.[119] Mutations in the SLCA2 gene are associated with orthostatic intolerance, a syndrome that includes fatigue as a significant symptom.[118,120] In addition, polymorphisms in the SLCA2 gene are associated major depression, a condition that includes fatigue as a major symptom.[121] In our study, carrying two doses of the rare A allele of SLC6A2 rs17841327 was associated with a 10.31-fold increase in the odds of belonging to the Higher Fatigue group. No studies were identified that reported on SLC6A2 rs17841327. In addition, a different SNP (rs36027) in the SLC6A2 gene was associated with energy level. Each additional dose of the rare G allele of SLC6A2 rs36027 was associated with a 41% decrease in the odds of belonging to the Lower Energy class. No studies were identified that reported on SLC6A2 rs36027.

Limitations and Directions for Future Research

The molecular findings from this study support the hypothesis that fatigue and energy are different, yet related symptoms. Only 2 of the 13 genes identified in this study were associated with membership in both the fatigue and energy latent classes. Additional support for this hypothesis comes from a recent study that explored the concepts of fatigue and energy in a sample of women with HIV.[122] Lerdal et al.[122] concluded that fatigue and energy are distinct constructs and should not be used interchangeably, neither clinically nor in research. Additional studies are needed that determine which phenotypic and genotypic characteristics differentiate differences in fatigue and energy. Findings from these types of studies will provide insights into the mechanism that underlie one or both of these symptoms and facilitate the development and testing of interventions to decrease fatigue and/or increase energy levels of patients undergoing cancer treatment.

A number of limitations must be acknowledged. While our sample size was sufficient, additional studies with independent samples are needed to confirm the latent classes as well as the genetic associations. In order to increase the generalizability of these results, women were recruited from 7 different centers and approximately 30% of the patients were ethnic minorities. However, the single diagnosis of breast cancer limits the generalizability of the findings to other cancer diagnoses. Lastly, longer prospective studies may reveal a potential effect of hormonal therapy on the trajectories of fatigue and energy following breast cancer surgery.

Despite these limitations, the findings from this study suggest that higher levels of fatigue and decrements in energy are significant symptoms for women following breast cancer surgery. The molecular findings suggest a large number of neurotransmitters (i.e., proteins and receptors) play a role in the development and maintenance of fatigue and energy levels in breast cancer patients. If these genetic associations are confirmed in independent samples, these findings may help identify individuals at higher risk for experiencing higher fatigue and lower energy levels.

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Table 1 – Summary of Single Nucleotide Polymorphisms Analyzed for Neurotransmitter Genes and the Growth Mixture Model Analyses for Fatigue (i.e., Lower versus Higher) and Energy (i.e., Higher versus Lower)

Gene	SNP	Position	Chr	MAF	Alleles	Fatigue			Energy		
						Chi Square	p-value	Model	Chi Square	p-value	Model
ATP-BINDING CASSETTE, SUBFAMILY B (MDR/TAP) MEMBER 1											
ABCB1	rs2235048	86976447	7	.471	T>C	0.100	.951	A	0.297	.862	A
ABCB1	rs6961419	87010072	7	.400	T>C	0.994	.608	A	0.379	.828	A
ABCB1	rs1128503	87017537	7	.433	C>T	1.306	.520	A	0.129	.938	A
ABCB1	rs1922241	87023830	7	.299	G>A	2.837	.242	A	3.502	.174	A
ABCB1	rs10264990	87040551	7	.293	T>C	0.868	.648	A	1.805	.405	A
ABCB1	rs1989830	87043599	7	.309	C>T	2.162	.339	A	1.293	.524	A
ABCB1	rs1858923	87059152	7	.445	T>C	0.027	.987	A	0.960	.619	A
ABCB1	rs9282564	87067376	7	.089	A>G	2.773	.250	A	0.744	.689	A
ABCB1	rs13233308	87082896	7	.438	C>T	0.438	.803	A	1.249	.535	A
ABCB1	rs10267099	87116696	7	.213	A>G	4.187	.123	A	0.050	.975	A
ABCB1	HapA01					1.328	.515		0.104	.949	
ABCB1	HapA05					2.796	.247		3.493	.174	
ABCB1	HapB01					0.574	.751		1.448	.485	
ABCB1	HapB02					0.312	.855		1.712	.425	
ALPHA-1D ADRENERGIC RECEPTOR											
ADRA1D	rs3787441	4153060	20	.268	T>C	2.162	.339	A	0.886	.642	A
ADRA1D	rs6084664	4155930	20	.159	T>C	0.421	.810	A	1.962	.375	A
ADRA1D	rs2326478	4156247	20	.326	C>T	2.629	.269	A	1.064	.587	A
ADRA1D	rs835880	4156895	20	.225	A>G	2.733	.255	A	0.373	.830	A
ADRA1D	rs8183794	4158448	20	.182	C>T	1.239	.538	A	0.565	.754	A
ADRA1D	rs6116268	4159440	20	.480	C>T	0.231	.891	A	0.382	.826	A
ADRA1D	rs946188	4163316	20	.236	A>G	1.912	.385	A	1.632	.442	A
ADRA1D	rs1556832	4163557	20	.461	C>T	0.036	.982	A	1.230	.541	A
ADRA1D	rs8118409	4164663	20	.229	G>A	1.469	.480	A	1.247	.536	A
ADRA1D	rs4815670	4164864	20	.467	G>A	1.559	.459	A	0.012	.994	A
ADRA1D	rs6076639	4167258	20	.206	C>T	0.024	.988	A	1.069	.586	A

ADRA1D	rs4815675	4171454	20	.423	T>C	0.441	.802	A	2.968	.227	A
ADRA1D	HapA01					1.244	.537		0.303	.859	
ADRA1D	HapA03					2.644	.267		0.360	.835	
ADRA1D	HapB02					1.672	.433		1.494	.474	
ADRA1D	HapB03					0.272	.873		0.321	.852	
ADRA1D	HapC01					1.608	.448		0.020	.990	
ADRA1D	HapC02					0.668	.716		0.408	.816	
ADRA1D	HapC03					1.469	.480		1.247	.536	
ADRA1D	HapD01					0.217	.897		2.698	.260	
ADRA1D	HapD02					0.477	.788		0.252	.881	
ALPHA 2A ADRENERGIC RECEPTOR											
ADRA2A	rs521674	112825580	10	.364	A>T	n/a	n/a	n/a	n/a	n/a	n/a
ADRA2A	rs3750625	112829591	10	.079	C>A	2.186	.335	A	2.644	.267	A
BETA 2 ADRENERGIC RECEPTOR											
ADRB2	rs2400707	148185245	5	.401	G>A	3.901	.142	A	1.770	.413	A
ADRB2	rs11168070	148186120	5	.357	C>G	FE	.046	D	2.522	.283	A
ADRB2	rs1042718	148187110	5	.203	C>A	FE	.023	R	2.519	.284	A
ADRB2	rs1042719	148187640	5	.315	G>C	3.870	.144	A	1.158	.560	A
ADRB2	HapA01					0.754	.686		0.538	.764	
ADRB2	HapA02					8.497	.014		1.642	.440	
ADRB2	HapA05					4.652	.098		2.265	.322	
BETA 3 ADRENERGIC RECEPTOR											
ADRB3	rs4994	37942955	8	.092	T>C	2.520	.284	A	0.959	.619	A
BETA ADRENERGIC RECEPTOR KINASE 2											
ADRBK2	rs1008673	24324013	22	.148	A>G	0.651	.722	A	1.077	.584	A
ADRBK2	rs3817819	24405188	22	.421	C>T	0.264	.876	A	2.243	.326	A
ADRBK2	rs5761159	24432308	22	.438	G>T	0.210	.901	A	0.325	.850	A
ADRBK2	rs9608416	24441018	22	.468	A>G	1.498	.473	A	1.133	.567	A
ADRBK2	HapA01					1.253	.534		1.533	.465	
ADRBK2	HapA04					0.378	.828		0.364	.834	
BRAIN DERIVED NEUROTROPHIC FACTOR											
BDNF	rs7124442	27633617	11	0.290	T>C	4.385	.112	A	2.754	.252	A
BDNF	rs6265	27636492	11	.222	G>A	FE	.042	D	2.636	.268	A
BDNF	rs11030101	27637320	11	.409	A>T	2.614	.271	A	0.792	.673	A

BDNF	rs11030102	27638172	11	.205	C>G	6.132	.047	A	3.223	.200	A
BDNF	rs11030104	27641093	11	.233	A>G	4.247	.120	A	3.035	.219	A
BDNF	rs2049045	27650817	11	.156	G>C	2.743	.254	A	1.505	.471	A
BDNF	rs11030107	27651411	11	.205	A>G	6.253	.044	A	3.303	.192	A
BDNF	rs7103411	27656701	11	.243	T>C	5.365	.068	A	3.811	.149	A
BDNF	rs16917237	27658959	11	.231	G>T	4.987	.083	A	2.871	.238	A
BDNF	rs6484320	27659764	11	.243	A>T	5.365	.068	A	3.811	.149	A
BDNF	rs7127507	27671460	11	.295	T>C	3.149	.207	A	2.151	.341	A
BDNF	rs2049046	27680351	11	.464	A>T	2.727	.256	A	1.592	.451	A
BDNF	HapA01					2.384	.304		0.673	.714	
CATECHOL-O-METHYLTRANSFERASE											
COMT	rs5748489	18307146	22	.388	C>A	1.476	.478	A	0.394	.821	A
COMT	rs2020917	18308884	22	.263	C>T	1.755	.416	A	0.401	.818	A
COMT	rs737866	18310109	22	.265	A>G	1.569	.456	A	0.293	.864	A
COMT	rs1544325	18311668	22	.397	G>A	1.210	.546	A	0.246	.884	A
COMT	rs5993882	18317533	22	.234	T>G	1.818	.403	A	FE	.034	R
COMT	rs5993883	18317638	22	.495	T>G	0.294	.863	A	0.739	.691	A
COMT	rs740603	18325177	22	.495	G>A	0.581	.748	A	0.730	.694	A
COMT	rs4646312	18328337	22	.371	T>C	2.192	.334	A	0.899	.638	A
COMT	rs165656	18328863	22	.489	C>G	0.689	.709	A	2.150	.341	A
COMT	rs6269	18329952	22	.391	A>G	2.017	.365	A	0.770	.680	A
COMT	rs4633	18330235	22	.472	C>T	0.669	.716	A	2.295	.317	A
COMT	rs6267	18330263	22	.002	G>T	n/a	n/a	n/a	n/a	n/a	n/a
COMT	rs740601	18330763	22	.399	A>C	2.671	.263	A	1.847	.397	A
COMT	rs5031015	18331103	22	.001	G>A	n/a	n/a	n/a	n/a	n/a	n/a
COMT	rs4818	18331207	22	.387	C>G	1.935	.380	A	0.704	.703	A
COMT	rs4680	18331271	22	.475	G>A	0.919	.632	A	2.802	.246	A
COMT	rs165774	18332561	22	.288	G>A	0.705	.703	A	1.270	.530	A
COMT	rs174699	18334458	22	.098	T>C	3.521	.172	A	0.875	.646	A
COMT	rs9332377	18335692	22	.129	T>C	FE	.029	D	1.310	.519	A
COMT	rs165599	18336781	22	.338	A>G	0.173	.917	A	0.123	.940	A
COMT	HapA01					0.624	.732		0.106	.949	
COMT	HapA06					1.618	.445		5.354	.069	

COMT	HapA10					1.939	.379		0.260	.878	
COMT	HapB02					0.789	.674		1.102	.576	
COMT	HapB20					1.404	.496		0.244	.885	
COMT	HapC01					0.173	.917		0.123	.940	
COMT	HapC02					4.286	.117		1.028	.598	
COMT	PAIN LPS					2.839	.242		1.586	.452	
COMT	PAIN APS					0.707	.702		1.737	.420	
COMT	PAIN HPS					3.552	.169		2.015	.365	
COMT	PAIN DIPLO					6.161	.291		4.338	.502	
COMT	PAIN RECODE A					FE	.634		FE	.901	
CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 4											
CYP3A4	rs4646437	99203019	7	.163	C>T	FE	.031	D	1.013	.602	A
GALANIN											
GAL	rs694066	68209561	11	.104	G>A	0.433	.805	A	0.571	.751	A
GAL	rs3136540	68212986	11	.249	C>T	1.936	.380	A	0.753	.686	A
GAL	rs1042577	68215046	11	.334	G>A	2.473	.290	A	3.691	.158	A
GAL	HapA01					2.443	.295		3.508	.173	
GAL	HapA04					1.838	.399		0.762	.683	
GALANIN RECEPTOR 1											
GALR1	rs949060	73087926	18	.381	G>C	FE	.017	R	4.518	.104	A
GALANIN RECEPTOR 2											
GALR2	rs2443168	71578042	17	.443	T>A	1.118	.572	A	0.066	.968	A
GALR2	rs2598414	71578694	17	.391	C>T	0.043	.979	A	1.314	.518	A
GALR2	HapA01					0.043	.979		1.314	.518	
GALR2	HapA03					1.333	.513		0.085	.958	
GTP CYCLOHYDROLASE 1											
GCH1	rs7142517	54376554	14	.297	C>A	1.053	.591	A	0.077	.962	A
GCH1	rs841	54380242	14	0.236	C>T	1.116	.572	A	2.634	.268	A
GCH1	rs752688	54381319	14	.236	C>T	1.116	.572	A	2.634	.268	A
GCH1	rs7155309	54392601	14	.234	T>C	1.085	.581	A	2.556	.279	A
GCH1	rs12587434	54395333	14	.236	T>G	0.675	.713	A	2.942	.230	A
GCH1	rs9671371	54398385	14	.337	C>T	3.455	.178	A	0.920	.631	A
GCH1	rs2183081	54406501	14	.409	T>C	0.566	.754	A	3.866	.145	A
GCH1	rs17128050	54413629	14	.148	T>C	2.307	.316	A	3.276	.194	A

GCH1	rs3783637	54417868	14	.155	C>T	3.040	.219	A	3.939	.140	A
GCH1	rs3783638	54418123	14	.187	G>A	4.716	.095	A	2.287	.319	A
GCH1	rs998259	54424781	14	.168	C>T	4.400	.111	A	2.700	.259	A
GCH1	rs3783642	54429953	14	.461	T>C	FE	.003	D	3.142	.208	A
GCH1	HapA01					1.855	.395		2.752	.253	
GCH1	HapA05					1.122	.571		2.616	.270	
GCH1	HapA06					1.100	.577		0.091	.955	
GCH1	HapB01					1.922	.383		2.672	.263	
GCH1	HapB03					5.712	.058		2.942	.230	
5-HYDROXYTRYPTAMINE RECEPTOR 1A											
HTR1A	rs6449693	63291774	5	.437	A>G	1.721	.423	A	0.092	.955	A
5-HYDROXYTRYPTAMINE RECEPTOR 1B											
HTR1B	rs6296	78228979	6	.313	G>C	1.927	.382	A	3.466	.177	A
5-HYDROXYTRYPTAMINE RECEPTOR 2A											
HTR2A	rs6314	46307035	13	.078	C>T	4.127	.127	A	0.589	.745	A
HTR2A	rs7322347	46308104	13	.420	T>A	0.976	.614	A	1.289	.525	A
HTR2A	rs1923882	46309662	13	.223	C>T	1.334	.513	A	3.683	.159	A
HTR2A	rs7997012	46309986	13	.380	G>A	1.799	.407	A	0.053	.974	A
HTR2A	rs3742278	46317578	13	.189	A>G	0.134	.935	A	0.032	.984	A
HTR2A	rs1923884	46319837	13	.167	C>T	0.410	.815	A	0.083	.959	A
HTR2A	rs1923886	46321292	13	.427	T>C	3.229	.199	A	0.284	.868	A
HTR2A	rs7330636	46321593	13	.364	C>T	2.559	.278	A	2.276	.320	A
HTR2A	rs9567739	46322945	13	.374	G>C	0.777	.678	A	0.180	.914	A
HTR2A	rs2296972	46326472	13	.330	G>T	2.576	.276	A	1.113	.573	A
HTR2A	rs9534495	46327229	13	.114	A>G	FE	.889	A	FE	.384	A
HTR2A	rs9534496	46329109	13	.182	G>C	4.086	.130	A	6.131	.047	A
HTR2A	rs4942578	46330611	13	.264	G>T	0.672	.715	A	0.393	.822	A
HTR2A	rs2770292	46333107	13	.162	C>G	1.095	.578	A	0.787	.675	A
HTR2A	rs1928042	46335217	13	.218	A>C	1.288	.525	A	2.168	.338	A
HTR2A	rs2770293	46336975	13	.376	C>T	1.724	.422	A	2.884	.236	A
HTR2A	rs1328674	46339708	13	.044	G>A	n/a	n/a	n/a	n/a	n/a	n/a
HTR2A	rs2770298	46344848	13	.260	T>C	1.500	.472	A	0.512	.774	A
HTR2A	rs1928040	46345237	13	.480	T>C	3.163	.206	A	FE	.044	R

HTR2A	rs972979	46347165	13	.373	G>A	0.861	.650	A	0.310	.856	A
HTR2A	rs731779	46350039	13	.171	T>G	2.539	.281	A	0.810	.667	A
HTR2A	rs2770304	46353366	13	.333	A>G	0.124	.940	A	0.749	.688	A
HTR2A	rs927544	46354052	13	.255	T>C	0.687	.709	A	1.617	.445	A
HTR2A	rs594242	46356053	13	.169	C>G	2.157	.340	A	0.357	.837	A
HTR2A	rs4941573	46362858	13	.447	A>G	2.597	.273	A	0.611	.737	A
HTR2A	rs1328684	46364231	13	.314	T>C	2.964	.227	A	1.412	.494	A
HTR2A	rs6304	46364550	13	.010	A>G	n/a	n/a	n/a	n/a	n/a	n/a
HTR2A	rs2296973	46364782	13	.281	G>T	0.486	.784	A	0.144	.930	A
HTR2A	rs2070037	46365071	13	.216	T>C	0.222	.895	A	0.603	.740	A
HTR2A	rs9534511	46366581	13	.445	C>T	3.026	.220	A	0.195	.907	A
HTR2A	rs6313	46367941	13	.450	C>T	3.482	.175	A	0.891	.640	A
HTR2A	HapA03					1.394	.498		3.597	.166	
HTR2A	HapA07					1.790	.409		0.517	.772	
HTR2A	HapB01					0.296	.862		0.034	.983	
HTR2A	HapB02					2.558	.278		2.390	.303	
HTR2A	HapB03					3.229	.199		0.284	.868	
HTR2A	HapC01					1.400	.497		2.117	.347	
HTR2A	HapC05					2.004	.367		0.646	.724	
HTR2A	HapD01					0.672	.715		0.393	.822	
HTR2A	HapD02					0.617	.734		0.810	.667	
HTR2A	HapE01					1.288	.525		2.168	.338	
HTR2A	HapF01					3.173	.205		4.584	.101	
HTR2A	HapF02					4.177	.124		1.625	.444	
HTR2A	HapF03					1.500	.472		0.512	.774	
HTR2A	HapG01					0.456	.796		0.687	.709	
HTR2A	HapH01					2.201	.333		0.306	.858	
HTR2A	HapH06					1.304	.521		2.825	.244	
HTR2A	HapI01					3.916	.141		0.835	.659	
5-HYDROXYTRYPTAMINE RECEPTOR 3A											
HTR3A	rs1985242	113353483	11	.370	T>A	1.548	.461	A	0.170	.919	A
HTR3A	rs11214796	113359889	11	.261	T>C	0.845	.655	A	1.080	.583	A
HTR3A	rs10160548	113361891	11	.378	T>G	2.139	.343	A	0.480	.787	A

HTR3A	HapA01					1.214	.545		0.206	.902	
HTR3A	HapA04					1.218	.544		0.763	.683	
NITRIC OXIDE SYNTHASE 1											
NOS1	rs2682826	116137221	12	.311	C>T	0.946	.623	A	0.143	.931	A
NOS1	rs816361	116139514	12	.318	C>G	1.353	.508	A	0.359	.836	A
NOS1	rs816363	116144850	12	.458	C>G	FE	.042	D	1.261	.532	A
NOS1	rs9658498	116152908	12	.409	T>C	FE	.041	R	1.186	.553	A
NOS1	rs1353939	116159736	12	.261	G>A	1.739	.419	A	1.016	.602	A
NOS1	rs1047735	116169653	12	.346	C>T	1.896	.387	A	1.227	.542	A
NOS1	rs12829185	116178403	12	.243	C>T	FE	.025	R	0.295	.863	A
NOS1	rs2293054	116186097	12	.299	G>A	3.410	.182	A	3.123	.210	A
NOS1	rs6490121	116192578	12	.364	A>G	2.852	.240	A	0.548	.760	A
NOS1	rs2293052	116200003	12	.358	C>T	FE	.001	R	1.925	.382	A
NOS1	rs7977109	116214723	12	.418	A>G	0.957	.620	A	1.830	.401	A
NOS1	rs3782206	116229472	12	.116	C>T	4.155	.125	A	1.577	.455	A
NOS1	rs7295972	116231751	12	.445	G>A	1.150	.563	A	0.033	.984	A
NOS1	rs11068447	116232070	12	.124	C>T	2.235	.327	A	1.408	.495	A
NOS1	rs547954	116238889	12	.206	C>T	1.188	.552	A	1.085	.581	A
NOS1	rs3782212	116239785	12	.270	C>T	0.320	.852	A	0.069	.966	A
NOS1	rs12578547	116247730	12	.266	T>C	2.973	.226	A	0.642	.726	A
NOS1	rs471871	116249901	12	.246	A>T	3.255	.196	A	FE	.039	R
NOS1	rs545654	116261432	12	.496	T=C	0.913	.633	A	1.220	.543	A
NOS1	rs1552227	116263418	12	.257	C>T	3.750	.153	A	0.096	.953	A
NOS1	rs10507279	116264657	12	.122	G>A	0.206	.902	A	1.890	.389	A
NOS1	rs693534	116269101	12	.382	G>A	2.797	.247	A	0.826	.662	A
NOS1	rs1123425	116270488	12	.439	A>G	12.001	.002	A	3.429	.180	A
NOS1	rs3782221	116280264	12	.270	G>A	4.488	.106	A	1.189	.552	A
NOS1	HapA02					3.993	.136		0.878	.645	
NOS1	HapA04					1.299	.522		0.429	.807	
NOS1	HapB02					1.739	.419		1.016	.602	
NOS1	HapB03					5.382	.068		1.186	.553	
NOS1	HapC01					1.896	.387		1.227	.542	
NOS1	HapC03					6.036	.049		0.295	.863	

NOS1	HapD01					9.804	.007		2.102	.350	
NOS1	HapD02					2.376	.305		0.208	.901	
NOS1	HapD03					1.204	.548		1.340	.512	
NOS1	HapE01					5.117	.077		1.019	.601	
NOS1	HapE03					1.150	.563		0.033	.984	
NOS1	HapF01					1.914	.384		2.593	.273	
NOS1	HapF02					4.204	.122		1.325	.516	
NOS1	HapF04					1.045	.593		1.299	.522	
NOS1	HapF06					3.648	.161		2.065	.356	
NITRIC OXIDE SYNTHASE 2											
NOS2A	rs9906835	23113501	17	.413	A>G	0.061	.970	A	1.357	.507	A
NOS2A	rs2297512	23116682	17	.385	A>G	1.117	.572	A	1.156	.561	A
NOS2A	rs2297516	23119857	17	.416	A>C	1.368	.505	A	0.896	.639	A
NOS2A	rs2297518	23120724	17	.145	G>A	1.666	.435	A	3.351	.187	A
NOS2A	rs2248814	23124448	17	.393	G>A	0.840	.657	A	2.349	.309	A
NOS2A	rs1137933	23130059	17	.170	C>T	0.889	.641	A	0.817	.665	A
NOS2A	rs4795067	23130802	17	.278	A>G	0.266	.876	A	0.278	.870	A
NOS2A	rs3729508	23133157	17	.422	G>A	1.730	.421	A	2.031	.362	A
NOS2A	rs944725	23133698	17	.382	C>T	0.689	.709	A	FE	.034	D
NOS2A	rs3730013	23150045	17	.342	C>T	2.534	.282	A	0.021	.990	A
NOS2A	rs10459953	23151645	17	.366	G>C	4.128	.127	A	1.975	.372	A
NOS2A	rs2779248	23151959	17	.347	T>C	0.452	.798	A	0.340	.844	A
NOS2A	HapA01					1.518	.468		1.018	.601	
NOS2A	HapA04					1.088	.580		1.051	.591	
NOS2A	HapB01					2.660	.265		3.648	.161	
NOS2A	HapB02					1.177	.555		1.658	.436	
NOS2A	HapC01					0.649	.723		0.520	.771	
NOS2A	HapC02					3.667	.160		1.396	.498	
NOS2A	HapC03					2.487	.288		0.123	.940	
NEUROPEPTIDE Y											
NPY	rs16148	24288863	7	.424	T>C	0.669	.716	A	1.443	.486	A
NPY	rs16147	24289935	7	.496	A>G	0.401	.818	A	0.807	.668	A
NPY	rs16478	24291133	7	.290	C>T	1.867	.393	A	2.658	.265	A

NPY	rs16139	24291404	7	.029	A>G	n/a	n/a	n/a	n/a	n/a	n/a
NPY	rs1468271	24293506	7	.027	A>G	n/a	n/a	n/a	n/a	n/a	n/a
NPY	rs5574	24295658	7	.429	C>T	0.466	.792	A	0.667	.717	A
NPY	HapA01					0.621	.733		0.501	.779	
NPY	HapA04					2.576	.276		1.807	.405	
NPY	HapA05					1.867	.393		2.658	.265	
NEUROPEPTIDE Y RECEPTOR Y1											
NPYR1	rs9764	164464855	4	.282	T>C	2.934	.231	A	4.479	.107	A
NPYR1	rs7687423	164470247	4	.410	G>A	FE	.008	D	2.179	.336	A
NPYR1	HapA01					3.258	.196		4.432	.109	
NPYR1	HapA04					7.788	.020		2.296	.317	
PRODYNORPHIN											
PDYN	rs6045868	1915278	20	.334	G>A	2.867	.239	A	0.441	.802	A
PDYN	rs2235751	1917934	20	.361	G>A	0.229	.892	A	1.737	.420	A
SOLUTE CARRIER FAMILY 6 MEMBER 1 – GABA TRANSPORTER											
SLC6A1	rs2697149	11011480	3	.221	T>G	4.406	.110	A	1.138	.566	A
SLC6A1	rs2601126	11011624	3	.407	C>T	7.247	.027	A	9.249	.010	A
SLC6A1	rs1710885	11013807	3	.192	T>C	0.932	.627	A	1.105	.575	A
SLC6A1	rs1710886	11014655	3	.333	G>C	1.273	.529	A	2.153	.341	A
SLC6A1	rs1710887	11014960	3	.395	G>T	0.483	.786	A	0.833	.659	A
SLC6A1	rs9990174	11015439	3	.326	G>T	3.317	.190	A	0.073	.964	A
SLC6A1	rs1568072	11016606	3	.220	C>T	0.107	.948	A	0.725	.696	A
SLC6A1	rs1728811	11016870	3	.426	C>T	1.060	.589	A	FE	.019	D
SLC6A1	rs11718132	11020020	3	.134	G>T	2.251	.324	A	2.314	.314	A
SLC6A1	rs2697144	11026099	3	.251	A>G	1.457	.483	A	2.461	.292	A
SLC6A1	rs2928079	11030114	3	.425	A>T	0.727	.695	A	5.034	.081	A
SLC6A1	rs1170695	11030338	3	.309	T>C	0.011	.994	A	3.093	.213	A
SLC6A1	rs2933308	11030624	3	.366	G>A	0.001	.999	A	5.113	.078	A
SLC6A1	rs10510403	11041670	3	.141	A>G	1.210	.546	A	3.984	.136	A
SLC6A1	rs2675163	11050014	3	.231	T>C	4.566	.102	A	FE	.007	D
SLC6A1	rs10514669	11050912	3	.194	C>T	3.846	.146	A	0.410	.815	A
SLC6A1	rs2697138	11051907	3	.145	C>A	3.498	.174	A	0.902	.637	A
SLC6A1	rs1062246	11055169	3	.417	A>G	0.752	.686	A	6.731	.035	A

SLC6A1	HapA01					7.675	.022		9.703	.008	
SLC6A1	HapA02					5.233	.073		3.699	.157	
SLC6A1	HapA04					4.102	.129		0.977	.614	
SLC6A1	HapB01					0.350	.840		3.012	.222	
SLC6A1	HapB03					1.060	.589		7.111	.029	
SLC6A1	HapC01					0.001	.999		5.113	.078	
SLC6A1	HapC02					0.039	.981		1.084	.582	
SLC6A1	HapC03					0.011	.994		3.093	.213	
SLC6A1	HapD01					0.142	.932		9.291	.010	
SLC6A1	HapD02					0.083	.960		4.475	.107	
SOLUTE CARRIER FAMILY 6 MEMBER 2 – NORADRENALINE TRANSPORTER											
SLC6A2	rs2242446	54247926	16	.242	T>C	5.004	.082	A	2.579	.275	A
SLC6A2	rs17841327	54251754	16	.321	C>A	FE	.001	R	FE	.038	D
SLC6A2	rs3785143	54252607	16	.087	C>T	3.800	.150	A	FE	.034	D
SLC6A2	rs192303	54257725	16	.291	G>C	1.667	.434	A	2.487	.288	A
SLC6A2	rs6499771	54258172	16	.155	A>G	1.543	.462	A	1.102	.576	A
SLC6A2	rs36027	54260281	16	.439	A>G	4.109	.128	A	6.269	.044	A
SLC6A2	rs36024	54263892	16	.403	C>T	FE	.033	D	FE	.009	D
SLC6A2	rs36021	54269451	16	.416	T>A	1.343	.511	A	2.959	.228	A
SLC6A2	rs40147	54274341	16	.323	C>T	0.664	.717	A	0.969	.616	A
SLC6A2	rs1814270	54274578	16	.404	T>C	3.383	.184	A	0.264	.876	A
SLC6A2	rs36017	54276319	16	.438	C>G	2.140	.343	A	0.073	.964	A
SLC6A2	rs3785155	54279891	16	.138	G>A	5.059	.080	A	2.325	.313	A
SLC6A2	rs47958	54283963	16	.433	C>A	1.663	.435	A	0.937	.626	A
SLC6A2	rs5568	54287625	16	.315	A>C	1.182	.554	A	0.153	.926	A
SLC6A2	rs1566652	54289076	16	.321	G>T	3.038	.219	A	0.768	.681	A
SLC6A2	rs5569	54289336	16	.303	C>T	0.526	.769	A	0.289	.866	A
SLC6A2	rs998424	54289447	16	.303	C>T	1.076	.584	A	0.760	.684	A
SLC6A2	HapA01					11.238	.004		4.147	.126	
SLC6A2	HapC01					2.585	.275		0.913	.634	
SLC6A2	HapC10					3.172	.205		0.141	.932	
SLC6A2	HapD01					0.585	.746		0.235	.889	
SLC6A2	HapD04					0.955	.620		0.912	.634	

SOLUTE CARRIER FAMILY 6 MEMBER 3 – DOPAMINE TRANSPORTER											
SLC6A3	rs3863145	1445711	5	.219	C>T	3.510	.173	A	0.973	.615	A
SLC6A3	rs40184	1448077	5	.419	G>A	0.951	.621	A	1.541	.463	A
SLC6A3	rs11564773	1449813	5	.052	A>G	FE	.706	A	FE	.557	A
SLC6A3	rs6876225	1459036	5	.035	C>A	n/a	n/a	n/a	n/a	n/a	n/a
SLC6A3	rs6347	1464412	5	.265	A>G	4.120	.127	A	0.673	.714	A
SLC6A3	rs37022	1468629	5	.216	T>A	2.452	.294	A	FE	.015	R
SLC6A3	rs2975292	1472932	5	.447	C>G	3.724	.155	A	0.318	.853	A
SLC6A3	rs11564758	1473588	5	.323	G>C	1.653	.438	A	0.783	.676	A
SLC6A3	rs464049	1476905	5	.465	T>C	0.512	.774	A	0.122	.941	A
SLC6A3	rs10053602	1481135	5	.213	T>C	1.840	.399	A	0.207	.902	A
SLC6A3	rs463379	1484164	5	.253	C>G	2.283	.319	A	2.907	.234	A
SLC6A3	rs403636	1491354	5	.207	G>T	0.333	.846	A	0.064	.969	A
SLC6A3	rs6350	1496199	5	.060	C>T	FE	.212	A	FE	.851	A
SLC6A3	rs2937639	1496728	5	.471	G>A	FE	.032	R	1.541	.463	A
SLC6A3	HapA01					0.635	.728		0.220	.896	
SLC6A3	HapA07					2.116	.347		3.043	.218	
SLC6A3	HapA09					1.570	.456		0.437	.804	
SLC6A3	HapA10					1.786	.409		0.684	.710	
SOLUTE CARRIER FAMILY 6 MEMBER 4 – SEROTONIN TRANSPORTER											
SLC6A4	rs3813034	25548930	17	.476	A>C	0.931	.628	A	1.254	.534	A
SLC6A4	rs1042173	25549137	17	.478	T>G	0.808	.667	A	1.473	.479	A
SLC6A4	rs4325622	25550601	17	.473	T>C	0.846	.655	A	0.974	.614	A
SLC6A4	rs3794808	25555919	17	.469	G>A	0.394	.821	A	2.851	.240	A
SLC6A4	rs140701	25562658	17	.464	G>A	0.270	.874	A	3.873	.144	A
SLC6A4	rs140700	25567515	17	.089	G>A	0.995	.608	A	1.979	.372	A
SLC6A4	rs2020942	25571040	17	.346	G>A	0.611	.737	A	FE	.016	R
SLC6A4	rs8076005	25571336	17	.214	A>G	2.102	.350	A	FE	.018	D
SLC6A4	rs6354	25574024	17	.180	A>C	0.617	.735	A	FE	.041	D
SLC6A4	rs2066713	25575791	17	.345	C>T	0.999	.607	A	4.337	.114	A
SLC6A4	HapA01					0.386	.825		2.173	.337	
SLC6A4	HapA11					0.432	.806		2.324	.313	
SLC6A4	HapB01					0.041	.980		2.573	.276	

SLC6A4	HapB04					0.535	.765		6.867	.032	
TACHYKININ PRECURSOR 1											
TAC1	rs7793277	97197521	7	.267	C>G	0.788	.674	A	2.257	.323	A
TAC1	rs2072100	97199720	7	.476	A>G	FE	<.0001	R	FE	.019	R
TAC1	rs1229434	97203778	7	.429	A>G	FE	.004	R	3.535	.171	A
TAC1	rs4526299	97205565	7	.195	C>T	1.723	.422	A	3.950	.139	A
TAC1	HapA01					9.317	.009		3.141	.208	
TAC1	HapA05					1.700	.427		4.030	.133	
TAC1	HapA06					0.857	.651		2.232	.328	
TACHYKININ RECEPTOR 1											
TACR1	rs1106855	75131495	2	.243	G>A	3.306	.191	A	0.598	.741	A
TACR1	rs4439987	75140614	2	.385	A>G	0.259	.879	A	0.461	.794	A
TACR1	rs11688000	75146665	2	.390	A>G	0.135	.935	A	0.174	.917	A
TACR1	rs6546952	75155271	2	.399	T>C	0.374	.829	A	0.362	.834	A
TACR1	rs17564182	75155814	2	.224	C>G	1.544	.462	A	3.227	.199	A
TACR1	rs3771810	75161161	2	.167	T>C	0.303	.860	A	1.636	.441	A
TACR1	rs34242711	75174688	2	.199	G>A	3.557	.169	A	1.303	.521	A
TACR1	rs2111378	75208112	2	.315	C>T	1.493	.474	A	2.217	.330	A
TACR1	rs3771825	75208988	2	.197	C>T	0.748	.688	A	1.295	.523	A
TACR1	rs3771827	75215372	2	.453	T>C	n/a	n/a	n/a	n/a	n/a	n/a
TACR1	rs741418	75216694	2	.440	A>G	5.424	.066	A	2.041	.360	A
TACR1	rs9808455	75223077	2	.479	T>C	1.683	.431	A	2.945	.229	A
TACR1	rs3771836	75234460	2	.484	T>G	0.595	.743	A	3.431	.180	A
TACR1	rs759588	75238057	2	.378	C>T	1.174	.556	A	3.500	.174	A
TACR1	rs3821318	75240819	2	.458	C>T	2.240	.326	A	1.267	.531	A
TACR1	rs6733933	75241342	2	.189	A>G	0.007	.996	A	0.040	.980	A
TACR1	rs13428269	75249287	2	.169	C>T	0.333	.847	A	FE	.018	R
TACR1	rs3771853	75255122	2	.407	C>T	0.808	.668	A	0.092	.955	A
TACR1	rs12477554	75255573	2	.462	G>A	2.724	.256	A	0.624	.732	A
TACR1	rs4853116	75264786	2	.334	A>G	1.126	.570	A	0.301	.860	A
TACR1	rs3821320	75267600	2	.410	A>G	1.363	.506	A	0.755	.685	A
TACR1	rs4853119	75269804	2	.229	T>C	1.598	.450	A	1.375	.503	A
TACR1	rs3771863	75273222	2	.195	C>T	2.710	.258	A	2.490	.288	A

TACR1	HapA01					3.414	.181		0.394	.821	
TACR1	HapA04					0.342	.843		0.668	.716	
TACR1	HapB01					1.493	.474		2.217	.330	
TACR1	HapB02					1.846	.397		0.081	.961	
TACR1	HapB03					0.748	.688		1.295	.523	
TACR1	HapC01					5.149	.076		1.896	.388	
TACR1	HapC04					1.691	.429		2.971	.226	
TACR1	HapD03					0.416	.812		4.246	.120	
TACR1	HapD05					1.174	.556		3.500	.174	
TACR1	HapE01					2.217	.330		0.617	.735	
TACR1	HapE04					1.250	.535		0.124	.940	
TYROSINE HYDROXYLASE											
TH	rs2070762	2142911	11	.500	T>C	0.021	.990	A	0.738	.691	A
TH	rs6357	2144814	11	.243	G>A	0.755	.686	A	1.481	.477	A
TH	rs6356	2147527	11	.403	G>A	1.687	.430	A	1.292	.524	A
TH	HapA01					1.354	.508		1.319	.517	
TH	HapA02					0.733	.693		0.005	.998	
TH	HapA04					FE	.555		FE	.538	
TRYTOPHAN HYDROXYLASE 2											
TPH2	rs11179000	70624895	12	.268	A>T	0.383	.826	A	3.130	.209	A
TPH2	rs7955501	70636293	12	.357	A>T	0.108	.948	A	2.892	.236	A
TPH2	rs1487275	70696559	12	.259	T>G	0.097	.952	A	0.154	.926	A

A = additive model, ABCB1 = ATP-binding cassette, subfamily B (MDR/TAP) member 1, ADRA1D = adrenergic, alpha-1D receptor, ADRA2A = adrenergic, alpha-2A receptor, ADRB2 = adrenergic, beta-2 receptor, surface, ADRB3 = adrenergic, beta 3 receptor, ADRBK2 = adrenergic, beta, receptor kinase 2, BDNF = brain derived neurotrophic factor, Chr = chromosome, COMT = catechol-O-methyltransferase, CYP3A4 = cytochrome P450, family 3, subfamily A, polypeptide 4, D = dominant model, FE = Fisher's Exact, GAL = galanin, GALR1 = galanin receptor 1, GALR2 = galanin receptor 2, GCH1 = GTP cyclohydrolase 1, Hap = haplotype, HTR1A = 5-hydroxytryptamine receptor 1A, G protein coupled, HTR1B = 5-hydroxytryptamine receptor 1B, G protein coupled, HTR2A = 5-hydroxytryptamine receptor 2A, G protein coupled, HTR3A = 5-hydroxytryptamine receptor 3A, ionotropic, MAF = minor allele frequency, n/a = not assayed because the SNP violated Hardy-Weinberg expectations (p<.001) or because its MAF was <.05, NOS1 = nitric oxide synthase 1, NOS2A = nitric oxide synthase 2, inducible, NPY = neuropeptide Y, NPYR1 = neuropeptide Y receptor Y1, PDYN = prodynorphin; R = recessive model, SLC6A1 = solute carrier family 6 (neurotransmitter transporter, GABA) member 1, SLC6A2 = solute carrier family 6 (neurotransmitter transporter, noradrenaline) member 2, SLC6A3 = solute carrier family 6 (neurotransmitter transporter, dopamine) member 3, SLC6A4 = solute carrier family 6 (neurotransmitter transporter, serotonin) member 4, SNP= single nucleotide polymorphism, TAC = tachykinin, precursor 1, TACR1 = tachykinin receptor 1, TH = tyrosine hydroxylase, TPH2 = tryptophan hydroxylase 2

Table 2 - Differences in Demographic and Clinical Characteristics Between the Lower Fatigue (n= 153) and Higher Fatigue (n= 244) Classes

Characteristic	Lower Fatigue Class n=153 (38.4%) Mean (SD)	Higher Fatigue Class n= 244 (61.3%) Mean (SD)	Statistic and p-value
Age (years)	57.8 (11.9)	53.1 (11.0)	t=4.09, p<.0001
Education (years)	15.3 (2.5)	15.9 (2.8)	t=-2.02, p=.04
Karnofsky Performance Status score	96.6 (7.0)	91.1 (11.4)	t=5.86, p<.0001
Self-administered Comorbidity Questionnaire score	3.8 (2.6)	4.6 (3.0)	t=-2.64, p=.009
Fatigue severity score at enrollment	1.6 (1.6)	4.1 (2.2)	t=-12.55, p<.0001
Number of breast biopsies in past year	1.5 (0.8)	1.5 (0.8)	U, p=.47
Number of positive lymph nodes	0.8 (1.9)	1.0 (2.4)	t=-0.88, p=.38
Number of lymph nodes removed	4.8 (5.1)	6.4 (7.5)	t=-2.43, p=.016
	n (%)	n (%)	
Ethnicity			
White	100 (65.8)	155 (63.8)	
Black	19 (12.5)	21 (8.6)	X^2=2.82, p=.42
Asian/Pacific Islander	17 (11.2)	32 (13.2)	
Hispanic/Mixed ethnic background/Other	16 (10.5)	35 (14.4)	
Married/partnered (% yes)	64 (42.1)	100 (41.5)	FE, p=.92
Work for pay (% yes)	71 (46.4)	118 (49.0)	FE, p=.68
Lives alone (% yes)	40 (26.5)	54 (22.4)	FE, p=.40
Gone through menopause (% yes)	96 (63.6)	151 (64.3)	FE, p=.91
Stage of disease			
0	29 (19.0)	44 (18.0)	
I	66 (43.1)	85 (34.8)	U, p=.13
IIA and IIB	48 (31.4)	92 (37.7)	
IIIA, IIIB, IIIC, and IV	10 (6.5)	23 (9.4)	
Surgical treatment			
Breast conservation	123 (80.4)	195 (79.9)	FE, p=1.00
Mastectomy	30 (19.6)	49 (20.1)	
Sentinel node biopsy (% yes)	130 (85.0)	197 (80.7)	FE, p=.34
Axillary lymph node dissection (% yes)	50 (32.7)	98 (40.3)	FE, p=.14
Breast reconstruction at the time of surgery (% yes)	33 (21.7)	53 (21.7)	FE, p=1.00
Neoadjuvant chemotherapy (% yes)	21 (13.7)	58 (23.9)	FE, p=.014
Radiation therapy during the first 6 months (% yes)	87 (56.9)	137 (56.1)	FE, p=.92
Chemotherapy during the first 6 months (% yes)	36 (23.5)	97 (39.8)	FE, p=.001

Abbreviations: FE=Fisher Exact test, SD = standard deviation, U=Mann Whitney U test

Table 3 - Differences in Demographic and Clinical Characteristics Between the Higher Energy (n=127) and Lower Energy (n=270) Classes

Characteristic	Higher Energy Class n=127 (31.9%) Mean(SD)	Lower Energy Class n= 270 (67.8%) Mean (SD)	Statistic and p-value
Age (years)	56.5 (10.8)	54.2 (11.8)	t=1.88, p=.061
Education (years)	15.7 (2.2)	15.7 (2.8)	t=0.01, p=.994
Karnofsky Performance Status score	95.4 (9.4)	92.2 (10.6)	t=3.06, p=.002
Self-administered Comorbidity Questionnaire score	3.6 (2.3)	4.6 (3.0)	t=-3.47, p=.001
Mean energy score at enrollment	6.1 (2.7)	4.4 (2.2)	t=-6.26, p<.0001
Number of breast biopsies in past year	1.5 (0.8)	1.5 (0.8)	U, p=.604
Number of positive lymph nodes	0.8 (2.0)	1.0 (2.3)	t=0.76, p=.450
Number of lymph nodes removed	5.0 (6.3)	6.1 (6.9)	t=-1.51, p=.132
	n (%)	n (%)	
Ethnicity			
White	86 (68.3)	169 (62.8)	
Black	10 (7.9)	30 (11.2)	X^2=1.75, p=.627
Asian/Pacific Islander	16 (12.7)	33 (12.3)	
Hispanic/Mixed ethnic background/Other	14 (11.1)	37 (13.8)	
Married/partnered (% yes)	50 (39.7)	114 (42.7)	FE, p=.586
Work for pay (% yes)	66 (52.4)	123 (45.9)	FE, p=.236
Lives alone (% yes)	29 (23.0)	65 (24.4)	FE, p=.801
Gone through menopause (% yes)	84 (68.3)	163 (62.0)	FE, p=.256
Stage of disease			
0	29 (22.8)	44 (16.3)	
I	51 (40.2)	100 (37.0)	U, p=.040
IIA and IIB	39 (30.7)	101 (37.4)	
IIIA, IIIB, IIIC, and IV	8 (6.3)	25 (9.3)	
Surgical treatment			
Breast conservation	100 (78.7)	218 (80.7)	FE, p=.686
Mastectomy	27 (21.3)	52 (19.3)	
Sentinel node biopsy (% yes)	103 (81.1)	224 (83.0)	FE, p=.673
Axillary lymph node dissection (% yes)	40 (31.7)	108 (40.0)	FE, p=.120
Breast reconstruction at the time of surgery (% yes)	28 (22.2)	58 (21.5)	FE, p=.896
Neoadjuvant chemotherapy (% yes)	22 (17.5)	57 (21.1)	FE, p=.421
Radiation therapy during the first 6 months (% yes)	75 (59.1)	149 (55.2)	FE, p=.515
Chemotherapy during the first 6 months (% yes)	34 (26.8)	99 (36.7)	FE, p=.054

Abbreviations: FE=Fisher Exact test, SD = standard deviation, U=Mann Whitney U test

Post-hoc contrasts of the difference in stage of disease between the Higher Energy and Lower Energy classes failed to identify the sub-groups that differed between the classes (p<.0083)

Table 4 - Multiple Logistic Regression Analyses for Neurotransmitter Genes and Lower Fatigue Versus Higher Fatigue Classes

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
ADRB2 rs1042718	0.13	0.100	0.030, 0.582	-2.67	.008
Age	0.80	0.052	0.707, 0.912	-3.39	.001
KPS score	0.56	0.097	0.396, 0.783	-3.36	.001
SCQ score	1.11	0.062	0.998, 1.243	1.92	.054
Any chemotherapy	2.31	0.669	1.307, 4.072	2.88	.004
Overall model fit: χ^2 = 59.87, p <.0001 R^2 = 0.1479					
BDNF rs6265	0.50	0.149	0.278, 0.897	-2.32	.020
Age	0.80	0.052	0.707, 0.910	-3.43	.001
KPS score	0.57	0.101	0.406, 0.810	-3.16	.002
SCQ score	1.13	0.063	1.010, 1.256	2.14	.032
Any chemotherapy	2.50	0.727	1.414, 4.420	3.15	.002
Overall model fit: χ^2 = 56.84, p <.0001 R^2 = 0.1404					
COMT rs9332377	0.48	0.158	0.256, 0.919	-2.22	.026
Age	0.82	0.052	0.723, 0.928	-3.13	.002
KPS score	0.55	0.095	0.389, 0.767	-3.49	<.0001
SCQ score	1.13	0.063	1.011, 1.260	2.15	.031
Any chemotherapy	2.41	0.697	1.370, 4.251	3.05	.002
Overall model fit: χ^2 = 56.34, p <.0001 R^2 = 0.1392					
CYP3A4 rs4646437	0.48	0.157	0.253, 0.914	-2.24	.025
Age	0.81	0.052	0.710, 0.914	-3.36	.001
KPS score	0.55	0.098	0.392, 0.783	-3.34	.001
SCQ score	1.12	0.063	1.005, 1.251	2.04	.041
Any chemotherapy	2.40	0.691	1.365, 4.221	3.04	.002
Overall model fit: χ^2 = 56.43, p <.0001 R^2 = 0.1394					
GALR1 rs949060	2.46	0.950	1.150, 5.244	2.32	.020
Age	0.81	0.053	0.713, 0.920	-3.25	.001
KPS score	0.58	0.100	0.413, 0.814	-3.15	.002
SCQ score	1.12	0.063	1.007, 1.253	2.09	.037
Any chemotherapy	2.55	0.738	1.444, 4.496	3.23	.001
Overall model fit: χ^2 = 56.98, p <.0001 R^2 = 0.1411					
GCH1 rs3783642	0.47	0.144	0.260, 0.859	-2.46	.014
Age	0.81	0.052	0.713, 0.917	-3.31	.001
KPS score	0.58	0.102	0.411, 0.818	-3.10	.002
SCQ score	1.12	0.064	1.006, 1.256	2.07	.039
Any chemotherapy	2.40	0.690	1.364, 4.216	3.04	.002
Overall model fit: χ^2 = 57.66, p <.0001 R^2 = 0.1424					
NOS1 rs9658498	0.45	0.164	0.223, 0.920	-2.19	.029
NOS1 rs2293052	4.58	2.429	1.621, 12.953	2.87	.004
Age	0.80	0.053	0.705, 0.913	-3.33	.001
KPS score	0.54	0.095	0.383, 0.762	-3.51	<.0001
SCQ score	1.11	0.063	0.991, 1.240	1.80	.072
Any chemotherapy	2.45	0.721	1.373, 4.361	3.04	.002
Overall model fit: χ^2 = 69.13, p <.0001 R^2 = 0.1708					
NPYR1 Haplotype A04	1.77	0.346	1.207, 2.595	2.92	.003
Age	0.81	0.052	0.711, 0.917	-3.31	.001
KPS score	0.55	0.099	0.388, 0.784	-3.32	.001
SCQ score	1.11	0.063	0.994, 1.241	1.85	.064

Any chemotherapy	2.58	0.756	1.454, 4.584	3.24	.001
Overall model fit: χ^2 = 60.22, p <.0001 R^2 = 0.1487					
SLC6A2 rs17841327	10.31	8.139	2.195, 48.439	2.96	.003
Age	0.81	0.053	0.717, 0.924	-3.18	.001
KPS score	0.56	0.101	0.395, 0.797	-3.23	.001
SCQ score	1.13	0.064	1.007, 1.257	2.08	.037
Any chemotherapy	2.68	0.784	1.514, 4.756	3.38	.001
Overall model fit: χ^2 = 65.01, p <.0001 R^2 = 0.1606					

Multiple logistic regression analyses of candidate gene associations with Lower Fatigue versus Higher Fatigue classes (n=301). For each model, the first three principal components identified from the analysis of ancestry informative markers, as well as self-reported race/ethnicity, were retained in all models to adjust for potential confounding due to race/ethnicity (data not shown). For the regression analyses, predictors evaluated in each model included genotype (ADRB2 rs1042718: CC+CA versus AA; BDNF rs6265: GG versus GA+AA; COMT rs9332377: TT versus TC+CC; CYP3A4 rs4646437: CC versus CT+TT; GALR1 rs949060: GG+GC versus CC; GCH1 rs3783642: TT versus TC+CC; NOS1 rs9658498: TT+TC versus CC; NOS1 rs2293052: CC+CT versus TT; NPYR1 HapA04: haplotype composed of the rs9764 common T allele and the rs7687423 common G allele; SLC6A2 rs17841327: CC+CA versus AA), age (5 years increments), functional status (KPS score in 10 unit increments), number of comorbid conditions, and receipt of chemotherapy within six months after surgery.

Abbreviations: ADRB2 = adrenergic, beta-2 receptor, surface; any chemotherapy = receipt of chemotherapy within six months after surgery; BDNF = brain derived neurotrophic factor; CI = confidence interval; COMT = catechol-O-methyltransferase; CYP3A4 = cytochrome P450, family 3, subfamily A, polypeptide 4; GALR1 = galanin receptor 1; GCH1 = GTP cyclohydrolase 1; Hap = haplotype; KPS, Karnofsky Performance Status; NOS1 = nitric oxide synthase 1; NPYR1 = neuropeptide Y receptor Y1; SCQ = Self-administered Comorbidity Questionnaire; SLC6A2 = solute carrier family 6 (neurotransmitter transporter, noradrenaline) member 2

Table 5 - Multiple Logistic Regression Analyses for Neurotransmitter Genes and Higher Energy Versus Lower Energy Classes

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
NOS1 rs471871	0.28	0.138	0.103,0.736	-2.57	.010
KPS score	0.65	0.101	0.483, 0.884	-2.75	.006
Any chemotherapy	1.73	0.479	1.002, 2.972	1.97	.049
Overall model fit: χ^2 = 24.43, p =.0037 R^2 = 0.0638					
SLC6A1 rs2675163	1.85	0.507	1.082, 3.166	2.25	.025
SLC6A1 Haplotype D01	0.60	0.116	0.413, 0.880	-2.62	.009
KPS score	0.68	0.105	0.503, 0.921	-2.49	.013
Any chemotherapy	1.56	0.440	0.898, 2.714	1.58	.114
Overall model fit: χ^2 = 30.86, p =.0006 R^2 = 0.0810					
SLC6A2 rs36027	0.59	0.107	0.415, 0.844	-2.90	.004
KPS score	0.66	0.102	0.484, 0.889	-2.72	.007
Any chemotherapy	1.75	0.485	1.014, 3.010	2.01	.044
Overall model fit: χ^2 = 26.25, p =.0019 R^2 = 0.0686					
SLC6A3 rs37022	9.75	10.612	1.155, 82.302	2.09	.036
KPS score	0.66	0.103	0.484, 0.895	-2.67	.008
Any chemotherapy	1.75	0.487	1.017, 3.022	2.02	.043
Overall model fit: χ^2 = 24.77, p =.0032 R^2 = 0.0647					
SLC6A4 rs2020942	0.36	0.144	0.161, 0.787	-2.55	.011
KPS score	0.66	0.103	0.488, 0.898	-2.65	.008
Any chemotherapy	1.73	0.482	1.006, 2.991	1.98	.047
Overall model fit: χ^2 = 24.16, p =.0041 R^2 = 0.0631					
TAC1 rs2072100	2.11	0.718	1.083, 4.113	2.19	.028
KPS score	0.67	0.102	0.498, 0.905	-2.61	.009
Any chemotherapy	1.73	0.480	1.007, 2.983	1.98	.047
Overall model fit: χ^2 = 22.78, p =.0067 R^2 = 0.0595					

Multiple logistic regression analyses of candidate gene associations with Higher Energy versus Lower Energy classes (n=301). For each model, the first three principal components identified from the analysis of ancestry informative markers, as well as self-reported race/ethnicity, were retained in all models to adjust for potential confounding due to race/ethnicity. For the regression analyses, predictors evaluated in each model included genotype (NOS1 rs471871 genotype: AA +AT versus TT; SLC6A1 rs2675163 genotype: TT versus TC+CC; SLC6A1 HapD01 haplotype: composed of the rs10514669 common C allele, the rs2697138 common C allele, and the rs1062246 common A allele; SLC6A2 rs36027 genotype: AA versus AG versus GG; SLC6A3 rs37022 genotype: TT+TA versus AA; SLC6A4 rs2020942 genotype: GG+GA versus AA; TAC1 rs2072100 genotype: AA+AG versus GG), functional status (KPS score in 10 unit increments), and receipt of chemotherapy within six months after surgery.

Abbreviations: Any chemotherapy = receipt of chemotherapy within six months after surgery; CI = confidence interval; Hap = haplotype; KPS, Karnofsky Performance Status; NOS1 = nitric oxide synthase 1; SCQ = Self-administered Comorbidity Questionnaire; SLC6A1 = solute carrier family 6 (neurotransmitter transporter, GABA) member 1; SLC6A2 = solute carrier family 6 (neurotransmitter transporter, noradrenaline) member 2; SLC6A3 = solute carrier family 6 (neurotransmitter transporter, dopamine) member 3; SLC6A4 = solute carrier family 6 (neurotransmitter transporter, serotonin) member 4; TAC1 = tachykinin, precursor 1

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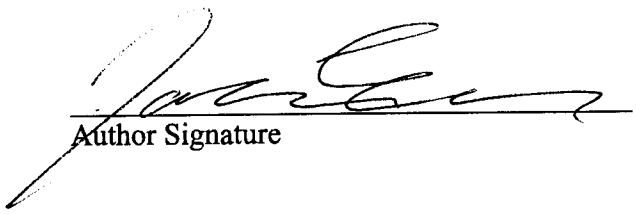

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