Title
Cytokine Gene Variations are Associated with Levels of Exercise in Women Prior to Breast Cancer Surgery

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CYTOKINE GENE VARIATIONS ARE ASSOCIATED WITH LEVELS OF EXERCISE IN WOMEN PRIOR TO BREAST CANCER SURGERY

by

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THESIS

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Dedication and Acknowledgements

This work is dedicated to the memory Dr. William Lyon, MD, a UCSF alumni, accomplished surgeon, and loving father. I also want to acknowledge the mentorship, support, and hard work of my thesis advisor and committee chair, Dr. Kord Kober, and my committee members Dr. Christine Miaskowski and Carol Viele for their essential contributions to this thesis.

Abstract Cytokine Gene Variations are Associated with Levels of Exercise in Women Prior to Breast Cancer Surgery

By Nadia Haas

Background - Over 90% of women with breast cancer undergo surgery. Although the benefits of exercise prior to and following surgery have been demonstrated, many breast cancer patients exercise below the recommended level. Genetic variation may account for 30-70% of a persons regular level of exercise. Genes associated with exercise include ones involved inflammatory and anti-inflammatory processes.

Purpose - The purposes of this study is, in a sample of women evaluated prior to breast cancer surgery were to: evaluate for differences in demographic and clinical characteristics between patients who did (n=78) and those who did not (n=120) exercise on a regular basis and evaluate for associations between exercise group membership and cytokine gene variations.

Methods - This study draws its data from a larger longitudinal study on lymphedema and neuropathic pain in women follow breast cancer surgery. Patients completed enrollment questionnaires, and levels of exercise, demographic and clinical characteristics were obtained. Genotype data was obtained DNA isolated from peripheral blood. Three exercise groups were created using guidelines from Healthy People 2020: patients who did not exercise (NoEX, n=120), patients who exercised less than 150 minutes/week (LessEX, n=134), and patients who exercised for the recommended 150 minutes/week (RecEX, n=78). Differences in demographic and clinical characteristics between the NoEX and RecEx groups. Genetic variations of pro-inflammatory, anti-inflammatory and transcriptional regulators were tested for associations with membership in the NoEX and RecEx groups.

Results - As compared to the RecEX group, patients in the NoEX group had fewer years of education, were less likely to be White or Asia/Pacific Islander, more like to be Hispanic or of mixed ethnic background, and more likely to report a lower annual household income. Clinically, as compared to the RecEX group, patients in the NoEX group had a higher Body Mass Index

(BMI), a lower KPS score, a higher SCQ score, were more likely to self-report a diagnosis of high blood pressure, and were more likely to have received neoadjuvant chemotherapy. In the final multivariate regression models, variations in two SNPs were associated with RecEX or NoEX group membership: *IFNGR1* rs9376268 (p-value = 0.021) and *NFKB1* rs4648135 (p-value = 0.006). Both loci are in high linkage with missense SNPs located in putative regulatory regions.

Conclusions - This study is the first to identify non-modifiable demographic risk factors and modifiable clinical characteristics associated with lack of exercise in patients prior to breast cancer surgery. This exploratory candidate gene study is also the first to identify variations in a cytokine gene and a regulatory gene that are associated with lack of exercise in patients prior to breast cancer surgery. Given the anti-inflammatory effects of exercise can protect against the development of chronic conditions and improve overall health, our findings on the association between exercise and cytokine gene variations may explain some of the beneficial effects of exercise found in studies of patients with and survivors of breast cancer. In addition, given that exercise improves the quality of life of patients with breast cancer, clinicians can use the characteristics identified in this study (e.g. higher BMI, co-morbidities) to identify high risk patients. Furthermore, clinicians should identify barriers to regular exercise and counsel patients on the benefits of regular exercise during and following cancer treatment.

Table of Contents

List of Figures

List of Tables

INTRODUCTION

Over 90% of women with breast cancer undergo surgery.[1] Regular exercise is a modifiable lifestyle factor[2] that is associated with numerous health benefits prior to and following surgery, as well as during and after additional cancer treatments.[3] As noted in a recent review,[3] some of these benefits include: improved tolerance to chemotherapy and radiation therapy, reduction in symptom burden, maintenance of bone density and muscle strength, and improvements in quality of life and survival.

Despite the well-established evidence that demonstrates the overall health benefits of regular exercise,[4] many patients with breast cancer do not exercise or exercise below recommended levels.[5, 6] For example, in study of breast cancer survivors in Sweden,[6] 32.4% of these women were categorized as inactive (i.e., $\leq$30 minutes of exercise/week) prior to surgery. In terms of the general population of the United States, <5% of adults participate in 30 minutes of physical activity each day and only one in three meet the recommended amount of physical activity each week.[7] A similar percentage is observed globally, with 27.5% of adults not meeting recommended physical activity guidelines (i.e., $\geq$150 minutes of exercise per week).[8]

Numerous barriers to regular exercise have been reported in patients with breast cancer. For example, in one qualitative study of breast cancer survivors,[9] women reported a wide range of psychosocial (e.g., lack of motivation, lack of support), physical (e.g., fatigue), and environmental (e.g., bad weather, time constraints) barriers. While knowledge of these barriers is important, a growing body of evidence suggests that genetic variation is a possible determinant of an individual's level of exercise or physical activity. In fact, as noted in one review,[2] twin and family studies found that genetic factors contribute to variations in daily physical activity levels with heritability ranging from approximately 30% to 70%. The authors noted that this wide range in heredity estimates may be related to significant variability in the ages of the study participants and inconsistencies in the methods used to assess physical activity.

Findings from studies on the associations between exercise and genetic variations have been the subject of several reviews.[10-14] While some of these genetic association studies were focused on exercise intolerance; cardiorespiratory endurance; skeletal muscle adaptation during exercise; and physiologic responses to exercise (for review see [10]), the ones that evaluated for associations between genomic markers of physical activity and physical inactivity are most relevant to this paper. In the most recent systematic review,[14] of the 54 studies included, six were genome wide association studies (GWAS) and 48 were candidate gene studies. Findings from the GWASs discovered ten loci and findings from the candidate gene studies identified 30 different genes associated with physical activity or sedentariness. However, only nine candidate genes were found to be associated with physical activity or sedentary behavior in more than one study (i.e., *ACE, CSR, CYP19A, FTO, DRD2, CNR1, LEPR, MC4R, NPC1*). As noted by Bauman and colleagues,[2] several of these candidate genes were chosen because they were known to be associated with acute aversive (e.g., pain, fatigue) and reward (e.g., individuals who feel rewarded by accomplishing an activity) effects of physical activity.

Equally important in the evaluation of associations between exercise and genetic variations is the identification of potential mechanisms for the physiologic benefits of exercise. As noted in one review,[10] the physiologic responses and adaptations to regular exercise may be associated with genes that are involved in calcium transport, skeletal muscle performance and insulin sensitivity, as well as inflammation and metabolic fitness. While initial findings from these association studies are promising,[10] additional candidate gene studies are warranted to confirm or refute their findings. Therefore, given that several lines of evidence suggest that the benefits of exercise in oncology patients are associated with reductions in inflammatory processes;[15] that associations between physiologic responses to regular exercise and variations in inflammatory genes have been demonstrated;[10] and that no studies have evaluated for associations between regular exercise and genetic variations in cytokine genes in patients undergoing breast cancer surgery,[15] the purposes of this study, in a sample of women who were evaluated prior to breast

cancer surgery were to: evaluate for differences in demographic and clinical characteristics between patients who did (n=78) and those who did not (n=120) exercise on a regular basis and evaluate for associations between exercise group membership and cytokine gene variations. Patients were categorized into these exercise groups using the recommendations from the Office of Disease Prevention and Health Promotion Healthy People 2020 report (i.e., ≥150 minutes of exercise per week).[8]

MATERIALS AND METHODS

Patients and Settings

This exploratory genomic analysis draws its data from a larger, longitudinal study that evaluated neuropathic pain and lymphedema in women following breast cancer surgery. Details of the parent study's methods are published elsewhere.[16-19] Briefly, patients were recruited from Breast Care Centers located in a Comprehensive Cancer Center, two public hospitals, and four community practices. Eligibility criteria were as follows: adult women (≥18 years) scheduled for unilateral breast cancer surgery; able to read, write, and speak English; agreed to participate; and provided written informed consent. Exclusionary criteria included: previous or planned bilateral breast cancer surgery or 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 enrollment assessment. Commonly cited reasons for refusal to participate were: too busy; overwhelmed with the diagnosis; or insufficient time to complete enrollment assessment prior to surgery. For this analysis, responses from the 198 women who did and did not exercise on a regular basis were included in this analysis.

Instruments

Patients completed a demographic questionnaire, the Karnofsky Performance Status (KPS) scale,[20] and the Self-Administered Comorbidity Questionnaire (SCQ).[21]

Exercise Assessment

Patients completed a 6-item investigator-developed questionnaire that asked them whether they exercised on a regular basis; what types of physical activities they engaged in at present (e.g., walk, swim); how may days per week they exercised; how many times per day they exercised; and the duration and intensity of each session. Based on the responses to this questionnaire, three exercise groups were created: patients who did not exercise on a regular basis (NoEx); patients who exercised less than 150 minutes/week (LessEx); and patients who exercised for the recommended 150 minutes/week (RecEx).[8] Of the 332 patients who completed the exercise questionnaire, 120 were in the NoEx, 134 were in the LessEx, and 78 were in the RecEx groups. For the genetic analysis reported in this paper, we used an extreme phenotype approach (i.e., compared patients in the NoEx group to patients in the RecEx group).

Study Procedures

This study was approved by the Committee on Human Research at the University of California, San Francisco and the Institutional Review Boards at each study site. During the patient's preoperative visit, a clinician explained the study to the patient and determined her willingness to participate. For those women who were willing to participate, the clinician introduced the patient to the research nurse who determine eligibility and obtained written informed consent. After obtaining informed consent, patients completed the enrollment questionnaire an average of four days prior to surgery and a blood sample was obtained. Patient medical records were reviewed for disease and treatment information.

Statistical Analyses for the Phenotypic Data

Statistical analyses were performed using SPSS version 27 (IBM Corporation Armonk, New York) and STATA Version 15.[22] Differences in demographic and clinical characteristics between the two exercise groups were determined using Independent sample t-tests, Mann-Whitney U tests, Chi square analyses, and Fisher's Exact tests. A p-value of <0.05 was considered statistically significant.

Candidate Gene Selection and Genotyping

Analyses of the Genomic Data

Gene selection – The pro-inflammatory genes evaluated in this study were as follows: chemokine (C-C-C motif) ligand 8 (*CXCL8*, previous gene symbol interleukin 8 (*IL8*)), interferon gamma (*IFNG*), *IFNG* receptor 1 (*IFNGR1*), IL1 receptor 1 (*IL1R1*), *IL2*, *IL17A*, and members of the tumor necrosis factor (TNF) family (i.e., lymphotoxin alpha (*LTA*), *TNF*). The anti-inflammatory genes were as follows: *IL1R2*, *IL4*, *IL10*, and *IL13*. In addition, *IFNG1*, *IL1B*, and *IL6* that possess pro- and anti-inflammatory functions and nuclear factor kappa beta 1 (*NFKB1*) and *NFKB2* that regulate transcription of cytokine genes were evaluated.[23] Genes were identified and matched with the appropriate symbol stored in the Human Genome Organization (HUGO) Gene Nomenclature Committee (HGNC) database (http://www.genenames.org).

Blood collection and genotyping - Of the 398 patients who completed the enrollment assessment, 310 provided a blood sample for genomic analysis. Genomic DNA was extracted from peripheral blood mononuclear cells using the PUREGene DNA Isolation System (Invitrogen, Carlsbad, CA). Genotyping was performed blinded to clinical status and positive and negative controls were included. 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). Samples were genotyped using the Golden Gate genotyping platform (Illumina, San Diego, CA) and processed according to the standard protocol using GenomeStudio (Illumina, San Diego, CA). Signal intensity profiles and resulting genotype calls for each single nucleotide polymorphism (SNP) were visually inspected by two blinded reviewers. Disagreements were adjudicated by a third reviewer.

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 ≥.05) in public databases (e.g., HapMap). In order to ensure robust genetic

association analyses, quality control filtering of SNPs was performed. SNPs with call rates of <95% or a Hardy-Weinberg p-value of <.001 were excluded.

As shown in Supplementary Table 1, 82 SNPs from a total of 104 SNPs among 15 candidate genes passed all of the quality control filters and were included in the genetic association analyses. Localization of SNPs on the human genome was performed using the GRCh38 human reference assembly. Regional annotations were identified using the University of California Santa Cruz (UCSC) Human Genome Browser NCBI36/hg18 (http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg18). Potential regulatory involvement of SNPs was investigated using a number of ENCODE data tracks.[24-27] Linkage disequilibrium (LD) between SNPs was calculated with Plink v1.90b4.6[28] using 1000 Genomes Phase 3 release v20130502 variants called from all populations.[29]

Statistical Analyses - Allele and genotype frequencies were determined by gene counting. Hardy-Weinberg equilibrium was assessed by the Chi-square or Fisher Exact tests. For the haplotype determinations, measures of LD (i.e., D' and r^2) were computed from the patients' genotypes with Haploview 4.2. LD-based haplotype block definition was based on D' confidence interval.[30]

For SNPs that were members of the same haploblock, haplotype analyses were conducted in order to localize the association signal 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.[31] In order to improve the stability of haplotype inference, the haplotype construction procedure was repeated five 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. Haplotypes were evaluated assuming a dosage model (i.e., analogous to the additive model).

Ancestry informative markers (AIMs) were used to minimize confounding due to population stratification.[32-34] Homogeneity in ancestry among patients was verified by principal

component analysis,[35] using Helix Tree (Golden Helix, 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 discernible clustering of patients by their self-reported race/ethnicity was possible (data not shown). One hundred and six AIMs were included in the analysis. The first three PCs were selected to adjust for potential confounding due to population substructure by including the three covariates in all of the logistic regression models.

For association tests, three genetic models were assessed for each SNP: additive, dominant, and recessive. Barring trivial improvements (i.e., delta of <10%), for significant SNPs, the genetic model that best fit the data, by maximizing the significance of the p-value was selected for that 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 association between each genotype and exercise group membership. A backwards stepwise approach was used to create the most parsimonious model. Except for genomic estimates of and self-reported 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 15.[22]

As was done in our previous studies,[16, 18, 36-49] based on recommendations in the literature,[50, 51] 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 regression analyses that controlled for differences in phenotypic characteristics, potential confounding due to population stratification, and variation 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 (bivariate) associations are reported for all SNPs passing quality control criteria in Supplementary Table 1 to allow for subsequent comparisons and meta-analyses.

RESULTS

Differences in Demographic and Clinical Characteristics

As shown in Table 1, compared to the RecEx group, patients in the NoEx group had fewer years of education, were less likely to be White or Asia/Pacific Islander, more likely to be Hispanic or of mixed ethnic background, and more likely to report a lower annual household income. In terms of clinical characteristics, compared to the RecEx group, patients in the NoEx group had a higher Body Mass Index (BMI), a lower KPS score, a higher SCQ score, were more likely to self-report a diagnosis of high blood pressure, and were more likely to have received neoadjuvant chemotherapy.

Candidate Gene Analyses for Exercise

As summarized in Supplementary Table 1, genotype frequencies were significantly different between the two exercise groups for 10 SNPs and three haplotypes spanning seven genes: *IFNG1* rs1861493, *IFNG1* rs1861494, *IFNGR1* rs9376268, *IL1B* rs1143643, *IL1B* rs1143633, *IL1B* rs3917356, *IL1B* s13032029, *IL1B* HapA4, *IL1B* HapB8, *IL4* HapA3, *IL6* rs2066992, *CXCL8* rs4073, and *NFKB1* rs4648135.

Regression Analyses for Significant Genotypes and Exercise Group

To understand the magnitude (i.e., odds ratio) and precision (95% confidence intervals (CI)) of genotype on the odds of belonging to the NoEx group compared to the RecEX group, multivariate logistic regression models were fit. In these regression analyses that included genomic estimates of and self-reported race/ethnicity, the only phenotypic characteristics that remained significant in the multivariable model were BMI and KPS score (in 10 unit increments).

Two SNPs spanning two different genes remained significant in the multivariate logistic regression analyses (Table 2, Figures 1a and 1b) For *IFNGR1* rs9376268 (G>A), a dominant

model fit the data best. Carrying one or two doses of the rare A allele (i.e., GG versus GA+AA) was associated with a 60% decrease in the odds of belonging to the NoEx group. For *NFKB1* rs4648135 (A>G), a dominant model fit the data best. Carrying one or two doses of the rare G allele (i.e., AA versus AG+GG) was associated with a 90% decrease in the odds of belonging to the NoEx group.

DISCUSSION

This exploratory candidate gene study is the first to evaluate for associations between lack of regular exercise and variations of cytokine genes in a sample of women prior to breast cancer surgery. Consistent with previous findings on associations between exercise and inflammatory genes,[10, 52] variations in *IFNGR1* and *NFKB1* were identified in this study. Of the total sample of 332 patients who completed the exercise questionnaire, only 23.5% met the recommendation for ≥150 minutes of exercise per week, which is lower than the 30% reported for the general population in the United States.[7] Similarly, 36.1% of our total sample were categorized in the NoEx group, which is slightly higher than the 34.2% found in the Swedish study of women prior to breast cancer surgery.[6] Given the fact that over 75% of our total sample did not meet the recommendations for regular exercise, clinicians need to assess patients prior to surgery and evaluate potential barriers to not meeting physical activity guidelines.[8]

Consistent with previous reports of patients with breast cancer,[53, 54] patients with other chronic conditions,[55, 56] as well as individuals in the general poulation,[56, 57] a higher percentage of non-white patients were in the NoEx group. In addition, and consistent with previous reports in oncology patients,[58-60] and the general population, [61, 62 63 64] patients in our NoEx group reported fewer years of education and had a lower annual household income. Barriers to regular exercise in individuals who are socioeconomically disadvantaged may include: neighborhood safety concerns; lack of recreation centers, parks, and gyms;[65] as well as lack of social support and time for exercise.[66]

As noted in previous studies of patients with breast cancer[67, 68] and individuals in the general population,[69, 70] patients in the NoEx group had a higher BMI, a higher comorbidity burden, lower levels of function, were more likely to self-report a diagnosis of high blood pressure, and had received neoadjuvant chemotherapy. These associations may be partially explained by the presence of chronic, low-grade inflammation. As noted in one review, [71] obesity and the development of chronic conditions like diabetes, heart disease, and cancer are associated with chronic inflammation. In contrast, regular exercise can decrease chronic inflammation.[72] The health benefits of exercise across a number of chronic conditions are related to the fact that exercise reduces levels of circulating pro-inflammatory cytokines released by adipocytes, and promotes the release of regulatory and anti-inflammatory cytokines.[52, 73]

Cytokine Gene Variations and Levels of Exercise

IFNGR1 is a gene that codes the for a protein subunit of the receptor for interferon gamma (*IFNG*). It is associated with immunologic activity as well as apoptosis, atherogenesis, and inflammation.[74] As noted in a recent review,[75] the *IFNG* family of genes is involved in a wide range of physiologic processes from protective immunological activity to paradoxical upregulation of inhibitory molecules on tumor cells. Patients with one or two doses of the rare 'A' allele in *IFNGR1* rs9376268 are less likely to belong to the NoEx group. This SNP is located in an intron region of the *IFNGR1* gene and is a non-coding variant.

While no putative regulatory patterns were identified at this locus using the ENCODE database, this locus is in high LD with a SNP in the first codon of IFNGR1 (i.e. rs11575936, R^2 = 0.012, D' = 1.0, $p<0.0001$). In terms of a functional association, this linked loci is a missense variant (i.e., NM_000416.3(IFNGR1):c.40G>A, p.Val14Met). While in a cell culture study[76] this variant was associated with lower expression of the receptor in the cell membrane, it did not affect the membrane's responsiveness to *IFNG* stimulation. In addition, multiple lines of evidence suggest that this linked variant resides in a genomic region involved in regulatory

activity (Figure 2A). First, it is located in a CpG island that is implicated in transcriptional regulation.[77] In addition, it is located in a region associated with enhancers and promoters (i.e., H3K4Me1 promoter-associated histone marks and DNase hypersensitive sites). Finally, it is located in a region where transcription factor binding was identified using chromatin immunoprecipitation. Therefore, polymorphisms at *IFNGR1* rs9376268 may be related to missense variation and gene regulatory activities at the linked rs11575936 loci. Additional studies are needed to determine if variations at these linked sites are associated with both decreases in *IFNGR1* function and decreases in the inflammatory effects of *IFNG* and increases in levels of exercise.

The *NFKB1* gene codes for a regulatory transcription factor protein that directs innate and adaptive immunity, stress responses, and cell proliferation and apoptosis. [78] Of note, the group of *NFKB1* genes generally regulates the release of inflammatory mediators following exercise[10, 71] and may be involved in muscle remodeling processes.[10] Therefore, *NFKB1* may confer a protective function as a regulator of inflammatory processes and apoptosis.[79] In this study, patients with one or two doses of the rare G allele in *NFKB1* rs4648135 were less likely to belong to the NoEx group. This SNP is located in an intron region of the *NFKB1* gene and is a non-coding variant.

While no putative regulatory patterns were identified at this locus using the ENCODE database, this locus is in high LD with a SNP in the first codon of *NFKB1* (i.e. rs4648072, R^2 = 0.013, D' = 0.95, $p<0.0001$). In terms of a functional variation, this linked loci is a missense variant (i.e., NM_003998.4(*NFKB1*):c.1519A>, p.Met507Val) and is located in a region associated with enhancers and promoters (i.e., H3K4Me1 promoter-associated histone marks; Figure 2B). Given that no studies were found that evaluated the functional effects of these variants, additional research is needed to determine if variations at these linked sites are associated with changes in *NFKB1* function and inflammatory responses and if these changes are associated with levels of exercise.

Our findings on the association between exercise and cytokine gene variations may explain some of the beneficial effects of exercise found in studies of patients with[3] and survivors of breast cancer.[73, 80] Regular exercise is hypothesized to improve treatment outcomes in these patients through the mitigation of chronic low-grade inflammation from adiposity and pro-inflammatory states associated with the cancer and its treatments.[23, 81] Exercise improves metabolic health; decreases visceral fat;[82] increases the release of inflammatory mediators that decrease the severity of symptoms (e.g., fatigue, depression) associated with cancer treatments;[72, 82] and may increase survival.[15]

More specifically, the anti-inflammatory effects of exercise can protect against the development of chronic conditions and improve overall health.[83] Acute exercise (i.e., 30[84] to 45[85] minutes) initiates a cascade of biochemical responses including the release of pro- and anti-inflammatory cytokines (reviewed in [71]). The anti-inflammatory benefits of exercise depend on a well-timed, intricate inflammatory cascade that is initiated by pro-inflammatory responses.[71] In terms of the pro-inflammatory response, exercise induces a cytokine cascade that is unique as compared to that induced by an endotoxin.[86-88] The anti-inflammatory benefits of exercise occur through the regulation of these initial pro-inflammatory processes and the subsequent stimulation of anti-inflammatory processes.[71]

However, exercise alone does not confer immediate benefits. The intensity, duration, and timing of activity over a lifetime evoke different inflammatory responses.[71] For example, exhaustive exercise causes the activation of pro-inflammatory cytokines in skeletal muscle and the induction of reactive oxygen species (ROS). If not resolved, this pro-inflammatory activation can lead to immunosuppression.[83] In contrast, cross-sectional[89] and intervention[90] studies found that regular (>3 months), moderate (e.g., between 40% of maximal heart rate for 16 minutes per session), and at 60% to 70% of maximal heart rate for 45 minutes[90, 91, 92] exercise training was associated with a reduction in inflammatory biomarkers (e.g., C-reactive protein, fibrinogen[89]). In addition, repeated physical exercise directs the immune response towards an anti-inflammatory

state.[91] The mechanisms that regulate adaptation and recovery from the pro-inflammatory responses associated with acute exercise and the sustained reduction in systemic inflammatory states following moderate exercise are areas of active research.[71]

Limitations

Several limitations warrant acknowledgment. First, because the exercise questionnaire was administered only at enrollment, no causal relationship can be made between levels of exercise and demographic and clinical characteristics or cytokine gene variations. Given the relatively small sample size, our findings warrant confirmation with a larger, independent sample. In addition, levels of exercise were evaluated by self-report that is susceptible to recall and social desirability bias.[93] Future studies should incorporate both objective and subjective measures of exercise. Given that the entire sample was women, our results may not generalize to men with breast or other types of cancer. Finally, this study evaluated women prior to breast cancer surgery. Therefore, our results may not be generalizable to associations between exercise and cytokine gene variations prior to a cancer diagnosis or into survivorship.

Recommendations for Clinical Practice

Despite these limitations, findings from this study support previous associations between exercise and a number of demographic and clinical characteristics.[61, 94] In addition, our findings suggest associations between exercise and cytokine gene variations. Given that exercise improves the quality of life of patients with breast cancer,[72, 82, 95] clinicians can use the characteristics identified in this study (e.g. higher BMI, co-morbidities) to identify high risk patients. Clinicians need to identify barriers to regular exercise[9] and counsel patients on the benefits of regular exercise during and following cancer treatment.[3] Patients may warrant referrals to dieticians for weight management and cancer rehabilitation programs[96] to improve their levels of physical activity.

Recommendations for Research

Given the beneficial effects of exercise in patients with breast cancer,[3, 6] future studies need to focus on the mechanisms that underlie these benefits. While our analysis was focused on cytokine genes, future studies need to focus on the genes identified in previous studies that were associated with physical activity or sedentary behavior.[14] In addition, studies of changes in gene expression may provide insights into the mechanisms that foster or inhibit an individual's level of regular exercise.[10]

Figures

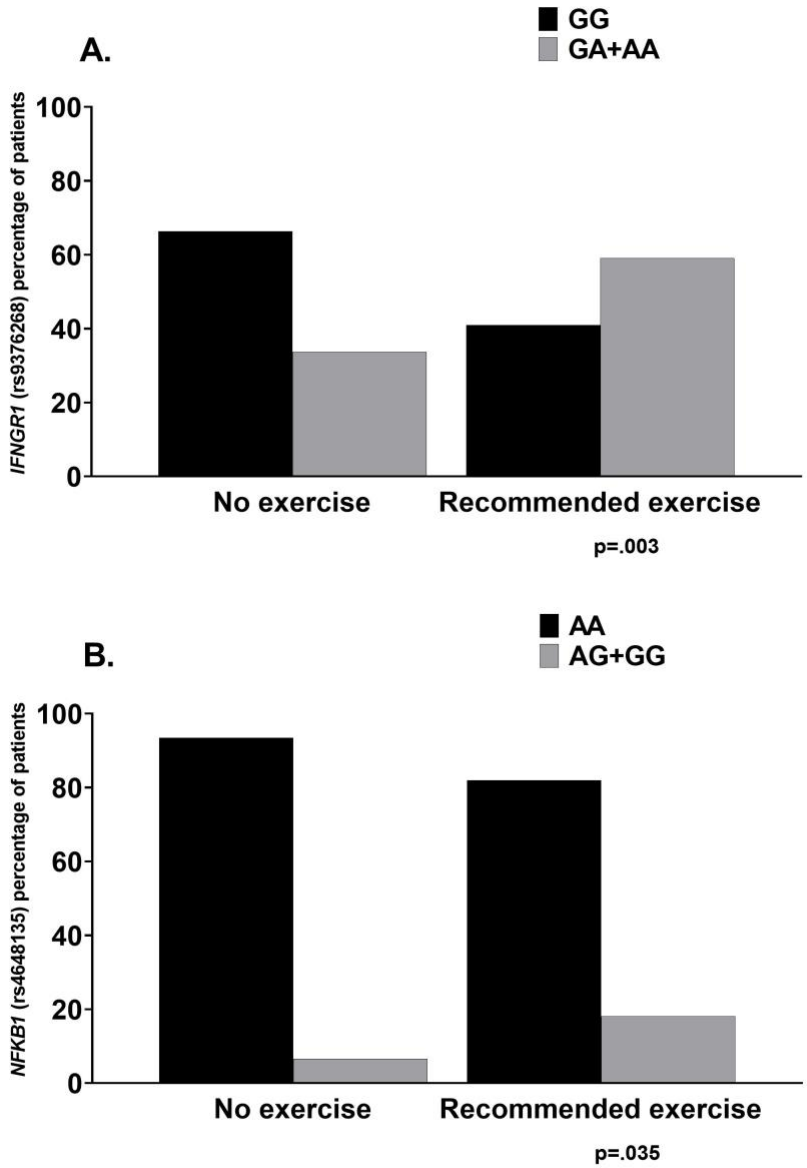

Figure 1. Differences between the no exercise and recommended exercise groups in the percentage of patients who were: (A) homozygous for the common allele (GG) or heterozygous or homozygous for the rare allele (GA + AA) at the rs9376269 loci in *IFNGR1* and (B) homozygous for the common allele (AA) or heterozygous or homozygous for the rare allele (AG + GG) at the rs4648135 loci in NFKB1. Values are plotted as unadjusted proportions with corresponding *p*-value.

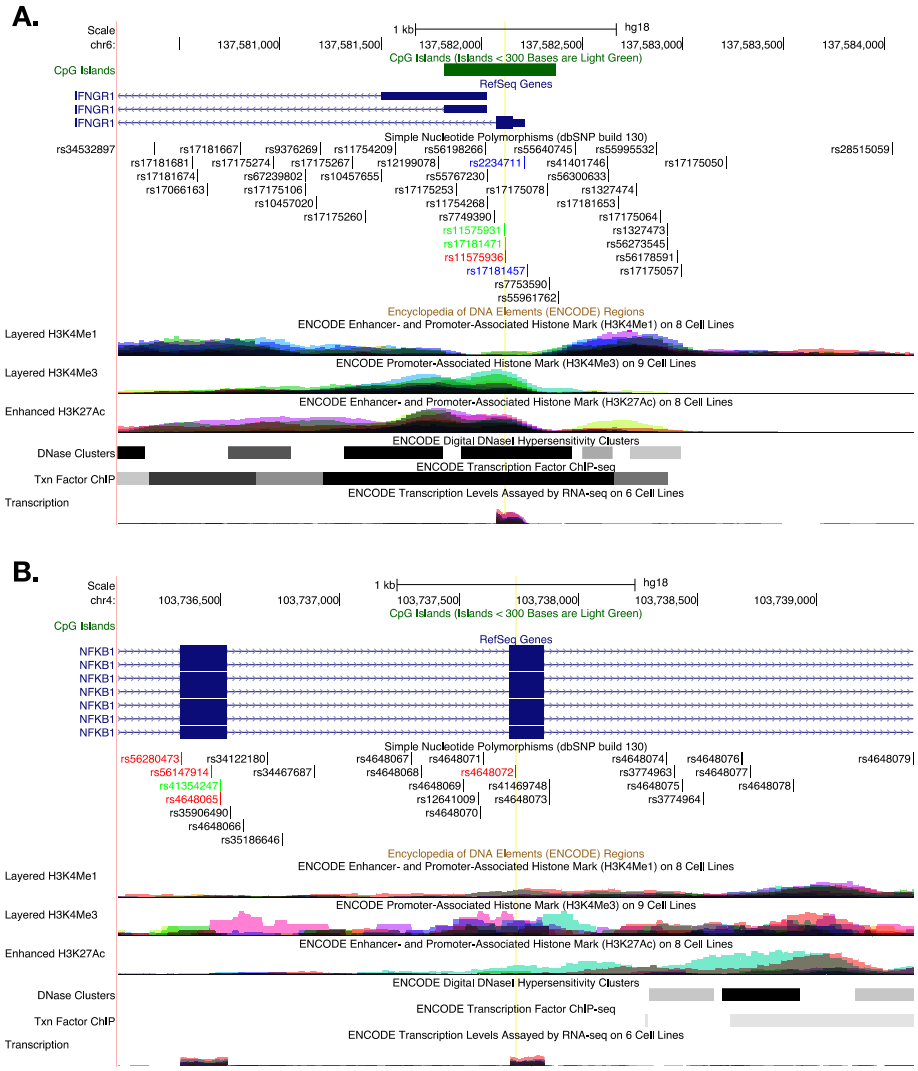

Figure 2. Visualization from the University of California Santa Cruz Human Genome Browser (hg18) of genomic regions in (A) *IFGR1* (rs11575936) and (B) *NFKB1* (rs4648072) that were in high linkage disequilibrium with single nucleotide polymorphisms (SNPs) found in this study to be associated with levels of exercise in patients with breast cancer. The gene models are provided by the "RefSeq" track. The CpG islands are identified in light green when present. The linked SNPs are labeled and identified by a vertical yellow line. SNPs are annotated by dbSNP release 130 and colored by variant attributes: unknown (black), coding – synonymous (green), coding – non-synonymous (red). Putative regulatory regions are identified by the ENCODE tracks

Table 1 - Differences in Demographic and Clinical Characteristics Between the No Exercise and Recommended Exercise Groups

Characteristic	NoEx Group (0) n=120 (60.6%)	RecEx Group (1) n= 78 (39.4%)	Statistic and p-value
	Mean (SD)	Mean (SD)	
Age (years)	56.9 (11.7)	55.3 (10.8)	t=0.97, p=.333
Education (years)	14.8 (2.5)	16.0 (2.5)	t=-3.05, p=.003
Body mass index (kilograms/meter squared)	28.9 (7.7)	25.4 (5.5)	t=3.65, p<.001
Karnofsky Performance Status score	90.8 (12.6)	95.9 (6.3)	t=-3.78, p<.001
Self-administered Comorbidity Questionnaire score	5.2 (3.0)	4.1 (3.0)	t=2.57, p=.011
Number of breast biopsies in past year	1.5 (0.9)	1.4 (0.6)	U, p=.779
Number of positive lymph nodes	1.2 (3.1)	0.9 (2.0)	t=0.87, p=.386
Number of lymph nodes removed	6.1 (7.5)	5.2 (5.7)	t=0.80, p=.423
	n (%)	n (%)	
Ethnicity			X^2=14.75, p=.002
White	70 (58.8)	56 (72.7)	0 < 1
Black	18 (15.1)	5 (6.5)	NS
Asian/Pacific Islander	8 (6.7)	12 (15.6)	0 < 1
Hispanic/Mixed ethnic background/Other	23 (19.3)	4 (5.2)	0 > 1
Married/partnered (% yes)	58 (48.3)	38 (49.4)	FE, p=1.000
Lives alone (% yes)	35 (29.4)	22 (28.6)	FE, p=1.000
Work for pay (% yes)	54 (45.0)	34 (44.2)	FE, p=1.000
Annual household income			
<$30,000	25 (26.3)	12 (18.2)	U, p=.010
$30,000 - $99,000	50 (52.6)	26 (39.4)	
≥$100,000	20 (21.1)	28 (42.4)	
Gone through menopause (% yes)	85 (72.0)	52 (67.5)	FE, p=.525
On hormone replacement therapy prior to diagnosis (% yes)	23 (19.2)	22 (28.2)	FE, p=.166
Comorbid conditions (% yes)			
Heart disease	9 (7.5)	2 (2.6)	FE, p=.206
High blood pressure	50 (41.7)	20 (25.6)	FE, p=.023
Lung disease	4 (3.3)	3 (3.8)	FE, p=1.000
Diabetes	10 (8.3)	5 (6.4)	FE, p=.785
Ulcer	6 (5.0)	2 (2.6)	FE, p=.484
Kidney disease	1 (0.8)	1 (1.3)	FE, p=1.000
Liver disease	2 (1.7)	2 (2.6)	FE, p=.647
Anemia	14 (11.7)	4 (5.1)	FE, p=.136
Depression	36 (30.0)	16 (20.5)	FE, p=.186
Osteoarthritis	25 (20.8)	18 (23.1)	FE, p=.727
Back pain	45 (37.5)	23 (29.5)	FE, p=.285
Rheumatoid arthritis	6 (5.0)	4 (5.1)	FE, p=1.000
Stage of disease			
0	22 (18.3)	18 (23.1)	U, p=.110
I	40 (33.3)	32 (41.0)	
IIA and IIB	45 (37.5)	22 (28.2)	
IIIA, IIIB, IIIC, and IV	13 (10.8)	6 (7.7)	
Surgical treatment			
Breast conservation	97 (80.8)	64 (82.1)	FE, p=.855
Mastectomy	23 (19.2)	14 (17.9)	
Sentinel node biopsy (% yes)	98 (81.7)	62 (79.5)	FE, p=.715
Axillary lymph node dissection (% yes)	47 (39.2)	25 (32.5)	FE, p=.366
Neoadjuvant chemotherapy (% yes)	29 (24.2)	8 (10.4)	FE, p=.016

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

Table 2 - Multiple Logistic Regression Analyses for Cytokine Genes and Recommended Exercise and No Exercise Groups

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
IFNGR1 rs9376268	0.40	0.159	0.186, 0.872	-2.31	.021
BMI	1.11	0.037	1.039, 1.184	3.11	.002
KPS score	0.94	0.022	0.90, 0.988	-2.48	.013
Overall model fit: $\chi^2 = 38.30$, $p < .001$					
NFKB1 rs4648135	0.10	0.086	0.021, 0.523	-2.75	.006
BMI	1.11	0.038	1.035, 1.184	2.97	.003
KPS score	0.94	0.022	0.895, 0.982	-2.73	.006
Overall model fit: $\chi^2 = 42.27$, $p < 0.001$					

Multiple logistic regression analyses of candidate gene associations with recommended exercise (n=78) versus no exercise (n=120) groups. 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 population structure (data not shown). For the regression analyses, predictors evaluated in each model included genotype (*IFNGR1* rs9376268 (G>A): GG vs GA+AA *NFKB1* rs4648135 (A>G): AA vs AG+GG), BMI, and functional status (KPS score in 10 unit increments).

Abbreviations: BMI = body mass index, CI = confidence interval, IFNGR1 = interferon gamma receptor 1, KPS = Karnofsky Performance Status, NFKB = nuclear factor kappa beta

Supplementary Table 1 - Summary of Single Nucleotide Polymorphisms Analyzed for Pro- and Anti-Inflammatory Cytokine Genes and No Exercise Versus Recommended Exercise Groups

Gene	SNP	Position	Chr	MAF	Alleles			
						Chi Square	p-value	Model
IFNG1	rs2069728	66834051	12	.110	G>A	2.72	0.10	R
IFNG1	rs2069727	66834490	12	.384	A>G	1.06	0.30	R
IFNG1	rs2069718	66836429	12	.494	C>T	2.48	0.12	D
IFNG1	rs1861493	66837463	12	.266	A>G	5.11	0.02	R
IFNG1	rs1861494	66837676	12	.273	T>C	5.11	0.02	R
IFNG1	rs2069709	66839970	12	.003	G>T	n/a	n/a	n/a
IFNG1	HapA3					5.15	0.08	
IFNG1	HapA5					1.09	0.58	
IFNGR1	rs9376268	137574444	6	.254	G>A	9.55	<0.01	D
IL1B	rs1071676	106042060	2	.189	G>C	0.38	0.54	R
IL1B	rs1143643	106042929	2	.383	G>A	6.73	0.03	A
IL1B	rs1143642	106043180	2	.082	C>T	1.34	0.25	R
IL1B	rs1143634	106045017	2	.187	C>T	0.38	0.54	R
IL1B	rs1143633	106045094	2	.392	G>A	6.97	0.03	A
IL1B	rs1143630	106046282	2	.115	C>A	2.42	0.12	D
IL1B	rs3917356	106046990	2	.450	G>A	8.03	0.02	A
IL1B	rs1143629	106048145	2	.389	T>C	3.50	0.06	R
IL1B	rs1143627	106049014	2	.397	T>C	2.82	0.09	R
IL1B	rs16944	106049494	2	.386	G>A	1.91	0.17	R
IL1B	rs1143623	106050452	2	.277	G>C	2.21	0.14	D
IL1B	rs13032029	106055022	2	.448	C>T	6.37	0.04	A
IL1B	HapA1					1.95	0.38	
IL1B	HapA4					6.39	0.04	
IL1B	HapA6					0.56	0.76	
IL1B	HapB1					3.57	0.17	
IL1B	HapB6					3.79	0.15	
IL1B	HapB8					6.07	0.05	
IL1R1	rs949963	96533648	2	.223	G>A	2.08	0.15	D
IL1R1	rs2228139	96545511	2	.053	C>G	0.01	0.93	D
IL1R1	rs3917320	96556738	2	.047	A>C	n/a	n/a	n/a
IL1R1	rs2110726	96558145	2	.317	C>T	3.98	0.05	R
IL1R1	rs3917332	96560387	2	.187	A>T	0.11	0.74	R
IL1R1	HapA1					3.71	0.16	
IL1R1	HapA2					0.22	0.90	
IL1R1	HapA3					0.13	0.94	
IL1R2	rs4141134	96370336	2	.362	T>C	3.14	0.08	D
IL1R2	rs11674595	96374804	2	.258	T>C	1.20	0.27	R
IL1R2	rs7570441	96380807	2	.408	G>A	3.68	0.06	R
IL1R2	HapA1					5.14	0.08	
IL1R2	HapA2					2.09	0.15	
IL1R2	HapA4					3.33	0.19	
IL2	rs1479923	119096993	4	.308	C>T	0.68	0.41	D
IL2	rs2069776	119098582	4	.184	T>C	n/a	n/a	n/a
IL2	rs2069772	119099739	4	.241	A>G	0.56	0.46	R
IL2	rs2069777	119103043	4	.047	C>T	n/a	n/a	n/a

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IL2	HapA1					2.35	0.31	
IL2	HapA2					0.04	0.98	
IL2	HapA3					0.98	0.61	
IL4	rs2243248	127200946	5	.086	T>G	0.19	0.67	D
IL4	rs2243250	127201455	5	.269	C>T	n/a	n/a	n/a
IL4	rs2070874	127202011	5	.245	C>T	n/a	n/a	n/a
IL4	rs2227284	127205027	5	.387	C>A	n/a	n/a	n/a
IL4	rs2227282	127205481	5	.390	C>G	n/a	n/a	n/a
IL4	rs2243263	127205601	5	.124	C>G	2.72	0.10	R
IL4	rs2243266	127206091	5	.237	G>A	n/a	n/a	n/a
IL4	rs2243267	127206188	5	.237	G>C	n/a	n/a	n/a
IL4	rs2243274	127207134	5	.261	G>A	n/a	n/a	n/a
IL4	HapA1					3.79	0.15	
IL4	HapA3					6.50	0.04	
IL4	HapX1					2.55	0.28	
IL6	rs4719714	22643793	7	.255	A>T	1.06	0.30	D
IL6	rs2069827	22648536	7	.069	G>T	0.67	0.41	R
IL6	rs1800796	22649326	7	.134	C>G	n/a	n/a	n/a
IL6	rs1800795	22649725	7	.285	C>G	3.99	0.14	A
IL6	rs2069835	22650951	7	.061	T>C	n/a	n/a	n/a
IL6	rs2066992	22651329	7	.049	G>T	7.95	0.02	A
IL6	rs2069840	22651652	7	.333	C>G	0.55	0.46	R
IL6	rs1554606	22651787	7	.319	G>T	4.08	0.13	A
IL6	rs2069845	22653229	7	.319	A>G	3.46	0.18	A
IL6	rs2069849	22654236	7	.024	C>T	n/a	n/a	n/a
IL6	rs2069861	22654734	7	.056	C>T	0.30	0.59	D
IL6	rs35610689	22656903	7	.259	A>G	0.03	0.87	D
IL6	HapA1					0.52	0.77	
IL6	HapA5					0.59	0.74	
IL6	HapA8					5.06	0.08	
IL8	rs4073	70417508	4	.455	T>A	4.52	0.03	R
IL8	rs2227306	70418539	4	.366	C>T	0.40	0.53	D
IL8	rs2227543	70419394	4	.368	C>T	0.28	0.60	D
IL8	HapA1					4.58	0.10	
IL8	HapA4					0.81	0.67	
IL10	rs3024505	177638230	1	.129	C>T	0.23	0.63	D
IL10	rs3024498	177639855	1	.204	A>G	1.70	0.19	D
IL10	rs3024496	177640190	1	.421	T>C	0.50	0.48	D
IL10	rs1878672	177642039	1	.416	G>C	0.71	0.40	D
IL10	rs3024492	177642438	1	.190	T>A	n/a	n/a	n/a
IL10	rs1518111	177642971	1	.303	G>A	2.25	0.13	R
IL10	rs1518110	177643187	1	.301	G>T	2.31	0.13	R
IL10	rs3024491	177643372	1	.408	G>T	1.25	0.26	D
IL10	HapA1					3.07	0.22	
IL10	HapA2					4.43	0.11	
IL10	HapA8					1.73	0.42	
IL13	rs1881457	127184713	5	.210	A>C	0.89	0.35	R

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IL13	rs1800925	127185113	5	.233	C>T	2.42	0.12	D
IL13	rs2069743	127185579	5	.019	A>G	n/a	n/a	n/a
IL13	rs1295686	127188147	5	.265	G>A	3.61	0.06	D
IL13	rs20541	127188268	5	.212	C>T	2.00	0.16	R
IL13	HapA1					4.82	0.09	
IL13	HapA4					3.10	0.21	
IL17A	rs4711998	51881422	6	.346	G>A	2.65	0.10	R
IL17A	rs8193036	51881562	6	.327	T>C	0.61	0.44	R
IL17A	rs3819024	51881855	6	.372	A>G	1.22	0.27	R
IL17A	rs2275913	51882102	6	.361	G>A	0.52	0.47	R
IL17A	rs3804513	51884266	6	.023	A>T	n/a	n/a	n/a
IL17A	rs7747909	51885318	6	.217	G>A	0.32	0.57	R
NFKB1	rs3774933	103645369	4	.409	T>C	1.30	0.25	D
NFKB1	rs170731	103667933	4	.358	A>T	0.94	0.33	R
NFKB1	rs17032779	103685279	4	.011	T>C	n/a	n/a	n/a
NFKB1	rs230510	103695201	4	.410	T>A	0.36	0.55	R
NFKB1	rs230494	103706005	4	.434	A>G	2.88	0.09	D
NFKB1	rs4648016	103708706	4	.010	C>T	n/a	n/a	n/a
NFKB1	rs4648018	103709236	4	.018	G>C	n/a	n/a	n/a
NFKB1	rs3774956	103727564	4	.435	C>T	2.72	0.10	D
NFKB1	rs10489114	103730426	4	.018	A>G	n/a	n/a	n/a
NFKB1	rs4648068	103737343	4	.363	A>G	0.60	0.44	D
NFKB1	rs4648095	103746914	4	.052	T>C	1.92	0.17	D
NFKB1	rs4648110	103752867	4	.170	T>A	0.37	0.55	D
NFKB1	rs4648135	103755716	4	.061	A>G	4.92	0.03	D
NFKB1	rs4648141	103755947	4	.180	G>A	0.22	0.64	D
NFKB1	rs1609798	103756488	4	.337	C>T	1.50	0.22	D
NFKB1	HapA1					0.16	0.92	
NFKB1	HapA9					2.31	0.32	
NFKB2	rs12772374	104146901	10	.168	A>G	2.03	0.15	R
NFKB2	rs7897947	104147701	10	.221	T>G	1.91	0.17	D
NFKB2	rs11574849	104149686	10	.070	G>A	1.68	0.20	D
NFKB2	rs1056890	104152760	10	.305	C>T	1.80	0.18	R
TNFA	rs2857602	31533378	6	.341	T>C	0.32	0.57	D
TNFA	rs1800683	31540071	6	.390	G>A	1.40	0.24	R
TNFA	rs2239704	31540141	6	.335	G>T	0.12	0.73	D
TNFA	rs2229094	31540556	6	.278	T>C	2.89	0.09	D
TNFA	rs1041981	31540784	6	.386	C>A	1.04	0.31	R
TNFA	rs1799964	31542308	6	.224	T>C	1.59	0.21	D
TNFA	rs1800750	31542963	6	.016	G>A	2.72	0.10	D
TNFA	rs1800629	31543031	6	.149	G>A	0.83	0.36	R
TNFA	rs1800610	31543827	6	.100	C>T	3.77	0.15	A
TNFA	rs3093662	31544189	6	.074	A>G	1.10	0.29	D
TNFA	HapA1					0.31	0.86	
TNFA	HapA5					1.29	0.53	
TNFA	HapA6					3.06	0.22	

A = additive model, Chr = chromosome, D = dominant model, Hap = haplotype, IFNG = interferon gamma, IFNGR = IFNG receptor, IL = interleukin, MAF = minor allele frequency, n/a = not assayed because SNP violated Hardy-Weinberg expectations ($p<0.001$) or because MAF was <.05, NFKB = nuclear factor kappa beta, R = recessive model, SNP= single nucleotide polymorphism, TNFA = tumor necrosis factor alpha

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