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Genetic associations of depressive symptoms in breast cancer patients

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Genetic associations of depressive symptoms in breast cancer patients

by

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THESIS

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I would like to thank the DiFrancisco, Ogbebor, Parker and Saad family for their patience and dedication to my growth.

The text of this thesis is a reprint of the material as it appears in “Cytokine gene variation is associated with depressive symptom trajectories in oncology patients and family caregivers.” The coauthor listed in this publication directed and supervised the research that forms the basis for the thesis.

ABSTRACT

Background – This study sought to replicate the findings from our previous candidate gene analyses of pro- and anti-inflammatory cytokines in a sample of patients who were assessed prior to and for six months following breast cancer surgery. Specifically phenotypic differences between the Resilient (n=155) and Subsyndromal (n=180) depressive symptom classes were evaluated as well as variations in cytokine genes between the two latent classes.

Methods – Among 398 breast cancer patients following surgery, growth mixture modeling was used to identify latent classes based on Center for Epidemiological Studies Depression (CES-D) Scale scores. The CES-D was completed prior to surgery and monthly for a total of six months following breast cancer surgery. A total of 103 single nucleotide polymorphisms and 35 haplotypes among 15 candidate cytokine genes were included in the genetic association analyses.

Results – Patients in the Subsyndromal class were significantly younger, more likely to be married or partnered, and reported a significantly lower KPS score than patients in the Resilient class. Variation in three cytokine genes (i.e., tumor necrosis factor alpha (TNFA), interferon gamma receptor 1 (IFNGR1), interleukin 6 (IL6)), as well as age and functional status predicted membership in the Subsyndromal versus the Resilient class.

Conclusion – Growth mixture modeling identified two distinct groups of patients who differ in their experience with depressive symptoms. Variations in cytokine genes may influence the trajectory of depressive symptoms in high risk patients.

Key words: depression, breast cancer, cytokines, genetics

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INTRODUCTION

Oncology patients experience a number of psychiatric symptoms throughout treatment, with depression being the most common (1). Of note, women in the general population are twice as likely to develop depression compared to men (2). In patients with breast cancer, depressive symptoms can occur from the time of diagnosis into survivorship. The prevalence of depressive symptoms ranges from 5% to 20% depending on when the symptoms were assessed during the course of breast cancer treatment and the methods used to evaluate depressive symptoms (3). Depressive symptoms can have negative effects on patients' functional status (4), quality of life (QOL) (5), and survival (6).

Recent evidence suggests that some of the heterogeneity in depressive symptoms may be mediated through neuroendocrine and immune mechanisms (7, 8). For example, serum levels of a number of pro-inflammatory cytokines (e.g., tumor necrosis factor alpha (TNFA), interleukin 1 beta (ILB), IL-6) are elevated in depression (7). In addition, administration of IL2 to humans causes depression and agitation (8). Administration of cytokines to animals induces behaviors associated with malaise, weakness, and sleepiness that are different from their usual behaviors (9). Finally, the administration of antidepressants to humans reduces serum cytokine levels (8).

Despite evidence that phenotypic and genotypic characteristics are associated with depression (10, 11), most studies of depressive symptoms in women following breast cancer surgery evaluated phenotypic predictors. In a longitudinal study that measured psychological distress and QOL in patients following breast cancer surgery (5), decreases in QOL were associated with a higher incidence of mood disturbance. Findings from more recent longitudinal studies suggest that pessimism (12), financial

difficulty, neuroticism, ethnicity (13), and fatigue (14) are the strongest predictors of depression one year following breast cancer surgery.

Most of the longitudinal studies cited above that attempted to identify predictors of depression in breast cancer patients assessed the symptom only at the time of diagnosis and again at 12 months after surgery (12, 14). The majority of the patients in these studies were White. In addition, most studies reported mean depression scores for the entire sample or used variable cutoff scores to define cases. Taken together, these limitations may partially explain the wide range of prevalence rates for depression in breast cancer patients.

To overcome some of these limitations, we recently completed a study, using a newer method of longitudinal data analysis (i.e., growth mixture modeling (GMM)) that identified four subgroups of women with distinct depressive symptoms trajectories from prior to, to six months after breast cancer surgery (15). In brief, patients completed the Center for Epidemiological Studies Depression (CES-D) scale prior to surgery and monthly for a total of six months. Based on the GMM analysis of CES-D scores, the latent classes identified were named Resilient (n=155, 38.9%), Subsyndromal (n=180, 45.2%), Delayed (n= 45, 11.3%), and Peak (n=18, 4.5%). Patients in the Subsyndromal class were more likely to have had an axillary lymph node dissection (ALND) and were significantly younger than patients in the Resilient class. Because of the small numbers of patients in the Peak and Delayed classes, only data from patients in the Subsyndromal and Resilient classes will be used in the candidate gene analyses described in this paper.

In a subsequent study of oncology patients and their family caregivers (FCs) (15), we confirmed the same four latent classes of distinct depressive symptoms trajectories. In addition, because emerging evidence suggests that depressive symptoms are associated with inflammation (7), we evaluated for variations in a number

of pro- and anti-inflammatory cytokine genes between the Resilient and Subsyndromal classes. Participants who were younger, female, non-White, and who had higher baseline State and Trait anxiety scores were more likely to be in the Subsyndromal class compared to the Resilient class. Variation in three cytokine genes (i.e., IL1 receptor 2 (IL1R2), IL10, and TNFA), as well as younger age and poorer functional status predicted membership in the Subsyndromal versus the Resilient class.

Given the confirmation of the depressive symptom phenotypes, in two independent samples (i.e. breast cancer patients (10), oncology patients and their FCs (15)), using GMM, as well as the identification of cytokines genes that differentiated between the Subsyndromal and Resilient classes, this study sought to replicate the findings from our previous candidate gene analyses of pro- and anti-inflammatory cytokines in a sample of patients who were assessed prior to and for six months following breast cancer surgery. Specifically, we evaluated for phenotypic differences between the Resilient and Subsyndromal depressive symptom classes as well as for variations in cytokine genes between the two latent classes.

MATERIALS AND METHODS

Patients and Settings

This analysis is part of a larger study that evaluated neuropathic pain and lymphedema in a sample of women who underwent breast cancer surgery. 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 women $\geq$18 years of age who underwent 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 to participate, 410 were enrolled in the study (response rate 79.4%), 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, education, ethnicity, marital status, employment status, living situation, and menopausal status. Medical records were reviewed for information on stage of disease, surgical procedure, neoadjuvant treatment, and reconstructive surgery.

Karnofsky Performance Status (KPS) scale is widely used to evaluate functional status in patients with cancer and has well established validity and reliability (16, 17). 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).

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 (18). 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 using a "yes/no" format. If they indicated that they had a condition, they were asked if they received treatment for it (yes/no; proxy for disease severity) and did it limit their activities (yes/no; indication of functional limitations). Patients were given the option to add two additional conditions not listed on the instrument. For each condition, a patient can receive a maximum of 3 points. Because the SCQ contains 13 defined medical

conditions and 2 optional conditions, the maximum score totals 45 points if the open-ended items are used and 39 points if only the closed-ended items are used. The SCQ has well-established validity and reliability and has been used in studies of patients with a variety of chronic conditions (19, 20).

The CES-D scale consists of 20 items that represents the major symptoms in the clinical syndrome of depression. Scores can range from 0 to 60, with scores ≥16 indicating the need for clinical evaluation for major depression. The CES-D has well established concurrent and construct validity (21). For this study, the Cronbach's alpha for the CES-D ranged from .85 to .90.

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 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. The research nurse met with the women, determined eligibility, and obtained written informed consent prior to surgery. After obtaining consent, patients completed the enrollment questionnaires on average four days prior to surgery and again at one, two, three, four, five, and six months after surgery. Medical records were reviewed for disease and treatment information.

Genomic analyses

Gene selection - Cytokines and their receptors are classes of polypeptides that mediate inflammatory processes (22). Cytokine dysregulation is associated with an increase in depressive symptoms (8, 23). These polypeptides are divided into pro- and anti-inflammatory cytokines. Pro-inflammatory cytokines promote systemic inflammation and include: interferon gamma 1 (IFNG1), IFNG receptor 1 (IFNGR1), IL1R1, IL2, IL8, IL17A,

nuclear factor kappa beta (NFKB1), NFKB2, and TNFA (24, 22). Anti-inflammatory cytokines suppress the activity of pro-inflammatory cytokines and include: IL1R2, IL4, IL10, and IL13 (24, 22). Of note, IFNG1, IL1B, and IL6 possess pro- and anti-inflammatory functions (24).

Blood collection and genotyping - Of the 398 patients who completed the baseline assessment, 302 provided a blood sample for genomic analysis. No differences were found in any demographic and clinical characteristics between patients who did and did not choose to participate in the study or in those patients who did and did not provide a blood sample for genomic analyses.

Genomic DNA was extracted from peripheral blood mononuclear cells 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 blinded to clinical status and positive and negative controls were included. 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). Two blinded reviewers visually inspected signal intensity profiles and resulting genotype calls for each SNP. Disagreements were adjudicated by a third reviewer. If consensus could not be reached, the SNP was excluded.

SNP selection - A combination of tagging SNPs and literature driven SNPs (i.e., reported as being associated with altered function and/or symptoms) were selected for analysis. Tagging SNPs were required to be common (defined as having a minor allele frequency ≥.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 <95%, or Hardy-Weinberg p<.001 were excluded. As shown in Table 1, a total of 103 SNPs among the 15 candidate genes (IFNG1: 6 SNPs, IFNGR1: 1 SNP; IL1B: 12

SNPs; IL1R1: 5 SNPs; IL1R2: 3 SNPs; IL2: 5 SNPs; IL4: 9 SNPs; IL6: 12 SNPs; IL8: 3 SNPs; IL10: 8 SNPs; IL13: 5 SNPs; IL17A: 6 SNPs; NFKB1: 14 SNPs; NFKB2: 4 SNPs; TNFA: 10 SNPs) passed all quality control filters and were included in the genetic association analyses. Potential functional roles of SNPs associated with depression were examined using PUPASuite 2.0 (25), a comprehensive search engine that tests a series of functional effects (i.e., non-synonymous changes, altered transcription factor binding sites, exonic splicing enhancing or silencing, splice site alterations, microRNA target alterations).

Statistical Analyses for the Phenotypic Data

Data were analyzed using SPSS version 19 (SPSS, Chicago, IL) and STATA Version 9 (26). 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. 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. A p-value of $<.05$ was considered statistically significant.

Unconditional GMM with robust maximum likelihood estimation was carried out to identify latent classes with distinct depressive symptom trajectories. These methods are described in detail elsewhere (10). 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 that best fit the data was identified using guidelines recommended by a number of experts (27, 28, 29).

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 (i.e., D' and r^2) were computed from the participants' genotypes with Haploview 4.2. Linkage disequilibrium (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. 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).

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 race/ethnicity, was used to evaluate the association between genotype and depression class membership. Only those genetic associations identified as significant from the univariate analyses were evaluated in the multivariate analyses.

A backwards stepwise approach was used to create the most 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 9.

Based on the recommendations of Rothman (32), adjustments were not made for multiple testing. However, rigorous controls were imposed on the analysis of the SNPs with p-values of <.05. As described above, each of these SNPs was evaluated using logistic regression analyses that controlled for differences in phenotypic characteristics, as well as potential confounding due to population stratification. Only those SNPs that remained significant were included in the final presentation of the results. In addition, the actual number of independent tests is more appropriately considered in relationship to the total number of cytokine genes evaluated (n=15), because the majority of the SNPs within each gene locus were in linkage disequilibrium. Therefore, the finding of three significant independent associations is unlikely to be due solely to chance. Findings are reported in Table 1 for all of the SNPs that were evaluated to have these data available in the literature for subsequent comparisons.

Ancestry informative markers (AIMs) can be used as a tool to minimize confounding due to population stratification in case-control association studies (33, 34, 35). Homogeneity in ancestry among participants was verified by principal component analysis (36), using Helix Tree (Golden Helix, Bozeman, MT). Briefly, the number of principal components (PCs) was sought which 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). The first three PCs were selected to adjust for potential confounding due to population substructure (i.e., race/ethnicity) by including them in all logistic regression models (described in the preceding paragraph). One hundred and six AIMs were included in the analysis.

RESULTS

Differences in Demographic and Clinical Characteristics Between Resilient and Subsyndromal Classes

As shown in Figure 1, patients in the Resilient class (n=155) had relatively low CES-D scores prior to surgery (mean= 6.8) which decreased slightly over the six months of the study. Patients in the Subsyndromal class (n=180) had a mean CES-D score prior to surgery that was just above the clinically meaningful CES-D cut-point of 16 (mean=17.1), that increased slightly and then decreased slightly over the course of the study.

As shown in Table 2, no differences were found between the two classes for the majority of the demographic and clinical characteristics. However, compared to the Resilient class, patients in the Subsyndromal class were younger (p=.001), more likely to be married/ partnered (p=.03), and reported a significantly lower KPS score (p<.0001). In terms of clinical characteristics, compared to the Resilient class, patients in the Subsyndromal class were more likely to have had an ALND (p=.03), had a higher number of lymph nodes removed (p=.01), and had received chemotherapy (CTX) during the first six months after surgery (p=.01).

Candidate Gene Analysis of the Two GMM Classes- As summarized in Table 1, the minor allele frequency was significantly different between the two latent classes for four SNPs: IFNGR1 rs9376268, IL6 rs2069840, and TNFA rs1799964 and 1800750 and two haplotypes: IL6 HapA5 (p=.037), and TNFA HapA5 (p=.010). For IFNGR1 rs9376268, a dominant model fit the data best (p=.047). For IL6 rs2069840 (p=.023) and TNFA rs1799964 (p=.005), a recessive model fit the data best. For TNFA rs1800750, an additive model fit the data best (p=.032).

Regression Analyses of IFNGR1, IL6, and TNFA Genotypes and Resilient versus Subsyndromal 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 Subsyndromal class as compared to the Resilient class, multivariate logistic regression models were fit. In addition to genotype, the phenotypic variables evaluated in the model were; age (5 year increments), being married or partnered, functional status (estimated by the KPS score, in 10 point increments), having undergone an ALND, number of lymph nodes removed, and having received CTX at any time during the six month follow-up period. After adjusting for age and functional status, none of the other predictors listed above were retained in the final models.

The only genetic associations that remained significant in the multivariate logistic regression analyses were for IFNGR1 rs9376268, IL6 rs2069840, and TNFA rs1800750 (Table 3, Figures 2A, 2B, and 2C). In the regression analysis for IFNGR1 rs9376268, after controlling for race/ethnicity, genotype, age, and functional status were the only predictors retained in the final model (p=.0011). The overall model explained 7.7% of the variance in the odds of belonging in the Subsyndromal class compared to the Resilient class. Controlling for age and functional status, carrying one or two doses of the minor allele (i.e., GG versus GA + AA) was associated with a 1.87-fold increase in the odds of belonging to the Subsyndromal class (p=.022).

In the regression analysis for IL6 rs2069840, after controlling for race/ethnicity, genotype, age, and functional status were the only predictors retained in the final model (p=.001). The overall model explained 7.8% of the variance in the odds of belonging to the Subsyndromal class compared to the Resilient class. Controlling for age and functional status, being homozygous for minor allele (i.e., CC + CG versus GG) was

associated with a 3.06-fold increase in the odds of belonging to the Subsyndromal class (p=.023).

In the regression analysis for TNFA rs1800750, after controlling for race/ethnicity, genotype, age, and functional status were the only predictors retained in the final model (p=.0003). The overall model explained 8.7% of the variance in the odds of belonging to the Subsyndromal class compared to the Resilient class. Controlling for age and functional status, each additional dose of the minor allele (i.e., GG versus GA versus AA) was associated with a 93% decrease in the odds of belonging to the Subsyndromal class (p=.018). While the regression analysis for TNFA rs1799964 was significant (p<.0001), the results are not presented because this SNP is collinear with rs1800750 (i.e., it is a surrogate marker).

DISCUSSION

This study is the first to attempt to replicate associations between pro- and anti-inflammatory cytokine genes and distinct depressive symptom trajectories in a relatively large sample of women who underwent surgery for breast cancer. An evaluation of differences in phenotypic characteristics between the Resilient and Subsyndromal classes is described in detail in our previous report (15). In brief, in both groups of patients the trajectory of depressive symptoms remained relatively stable across the six months of the study. This stability within each latent class may indicate a predisposition for a better or worse mental health status. While younger age, having poorer functional status, being married/partnered, and having more extensive treatment was associated with being in the Subsyndromal class, additional phenotypic predictors of class membership like personality need to be evaluated in future studies.

The main focus of this paper was to determine if findings from our previous study that tested associations between similar depressive symptom trajectories and cytokine

candidate genes (15), could be replicated in a different sample. In both of our studies, the trajectories of the Resilient and Subsyndromal classes were identical. However, in the previous study (15), the mean CES-D scores for the Resilient and Subsyndromal classes on enrollment were 4.6 and 14.7 respectively. Compared to the breast cancer patients, the lower CES-D scores for both classes may be related to the heterogeneity of the sample that included patients with a variety of cancer diagnoses (i.e., breast, prostate, lung, brain), both genders, as well as family caregivers.

When comparisons were made between the genetic associations identified in our previous study (15) and this study, the only cytokine gene in common was TNFA. In the previous study, two SNPs in TNFA (i.e., rs2229094 and rs1800629) were associated with the Subsyndromal phenotype. In the current study, the p-values for these two SNPS were p=.176 and p=.092, respectively, and the genetic models were not consistent. In contrast in the present study, variations in TNFA rs1800750 explained 2.4% of the variance in latent class membership. Each dose of the minor “A” allele was associated with an increased odd of belonging to the Subsyndromal class. In the previous study by Dunn and colleagues (15), this SNP was not significant (p=.384). These inconsistent findings may be attributed to differences in sample characteristics, particularly gender.

That said, findings across both studies suggest that genetic variations in TNFA partially explain differences in our depression phenotypes. Of note, TNFA rs1800750 is a functional SNP located in the promoter region of the TNFA gene. In a previous study (36), carriers of the TNFA rs1800629 minor “A” allele reported lower levels of sleep disturbance and morning fatigue. Taken together, these findings suggest that being heterozygous (GA) or homozygous (AA) for the rare allele is associated with a decreased risk for depressive symptoms, sleep disturbance, and morning fatigue.

Two new candidate genes (i.e., IFNGR1, IL6) were identified in the current study. Variation in IFNGR1 rs9376268 explained 1.5% of variance in the latent class association, such that carrying the minor allele “A” was associated with an increased odds of belonging to the Subsyndromal class. In our previous study (15), this SNP was not significant (p=.888). One reason for the inconsistent findings may be differences in a number of demographic and clinical characteristics between the two samples. This SNP is located in the intronic region of the gene and its function is unknown. While no studies were found that evaluated the role of the IFNGR1 in depression, it is known that this receptor modulates the effects of other pro- and anti-inflammatory genes (38).

Individuals homozygous for the rare “G” allele for IL6 rs2069840 had a 1.6% increased odds of belonging to the Subsyndromal class. In our previous study (15), the p-value for this SNP was .130. The smaller sample size in the previous study may account for the lack of statistical significance. However, findings from several studies suggest that IL6 is involved in depression. Through its effects on the central nervous system, IL6 may affect emotional behavior (39). For example, compared to healthy controls, depressed patients had higher levels of IL6 (40). In addition, higher levels of IL6 were found in cerebrospinal fluid of patients who attempted suicide (41) and increased levels of IL6 were found after a stress test (42). Taken together, these findings suggest that IL6 is associated with the severity of depressive symptoms. Although rs2069840 is located in the intronic region of IL6 and has no known function, it may be in linkage disequilibrium with a functional SNP.

Lastly, in our previous study (15), two different candidate genes (i.e., IL10, IL1R2) were associated with the Subsyndromal phenotype. IL10 rs1518111 explained 1.6% of the latent class membership and increased the odds by four fold of belonging to the Subsyndromal class. In the current study, this SNP was not significant (p=.764). In our previous study (15), variation in IL1R2 Haplotype A1 was associated with a two-fold

increase in the odds of belonging to the Subsyndromal class. In the current study, this haplotype was not significant (p=.985). The reasons for these inconsistent findings may be attributed to differences in sample characteristics in particular gender and variation in cancer diagnoses. Additional research is warranted to confirm or refute these findings.

Individuals who are categorized as having Subsyndromal depression have depressive symptoms but do not meet the criteria for a depressive disorder. Studies of older adults (6) classified Subsyndromal depression as a less severe condition with greater cumulative morbidity. Patients in this class are at risk because they are under-diagnosed and may not be receiving proper treatment for their symptoms. Studies in the elderly show that in comparison to major depression, Subsyndromal depression results in functional disability (4).

Recent studies suggest that patients with Subsyndromal depression are at greater risk for transitioning into major depression (43). Individuals with subclinical depressive symptoms have an increased symptom burden, and over time, this can develop into a major psychiatric illness. Therefore, it is important to study the genetic risk factors for this group of individuals.

LIMITATIONS

Limitations of this study must be acknowledged. While the information collected about depressive symptoms was obtained using valid and reliable tools, self-reported data is a limiting factor. Other characteristics of the sample such as personality traits and preexisting life situations could mediate latent class membership. With that in mind, future studies need to include a clinical evaluation of preexisting mental health conditions. Finally, the single diagnosis of breast cancer limits the generalizability of the study's findings.

Despite these limitations, GMM identified two distinct groups of patients who differ in their experience with depressive symptoms. Latent class associations can help identify breast cancer patients who are at a greater risk for more severe depressive symptoms. However, additional research in independent samples and other cancer populations of women and men is needed to validate these associations.

Given the increased incidence of depressive symptoms in women (2) and oncology patients (1) and evidence of associations found among pro- and anti-inflammatory cytokines and depression (7, 8), research with a focus on genotype screening may help better identify those patients at a greater risk for severe depressive symptoms but are clinically missed.

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Figure legends

Figure 1 - Observed and estimated Center for Epidemiological Studies Depression Scale (CES-D) trajectories for the participants in each of the latent classes, as well as the mean CES-D scores for the total sample.

Figure 2A – Differences between the latent classes in the percentages of participants who were homozygous for the common allele (GG) or heterozygous of homozygous for the minor allele (GA+AA) for rs9376268 in interferon gamma receptor 1 (IFNGR1).

Figure 2B - Differences between the latent classes in the percentages of participants who were homozygous or heterozygous for the common allele (CC+CG) or homozygous for the minor allele (GG) for rs2069840) in interleukin 6 (IL6).

Figure 2C - Differences between the latent classes in the percentages of participants who were homozygous for the common allele (GG) or heterozygous for the common allele (GA) for rs1800750) in tumor necrosis factor alpha (TNF-A).

Table 1 - Summary of Single Nucleotide Polymorphisms Analyzed for Pro- and Anti-Inflammatory Cytokine Genes and the Growth Mixture Model Analysis for Depression

Gene	SNP	Position	Chr	MAF	Alleles	Chi Square	p-value	Model
IFNG1	rs2069728	66834051	12	.079	G>A	.041	.980	A
IFNG1	rs2069727	66834490	12	.411	A>G	2.150	.341	A
IFNG1	rs2069718	66836429	12	.442	C>T	.745	.689	A
IFNG1	rs1861493	66837463	12	.264	A>G	.500	.779	A
IFNG1	rs1861494	66837676	12	.279	T>C	.104	.949	A
IFNG1	rs2069709	66839970	12	.008	G>T	FE	1.000	A
INFG1	HapA3					.500	.779	
INFG1	HapA5					2.150	.341	
IFNGR1	**rs9376268**	**137574444**	**6**	**.246**	**G>A**	**FE**	**.047**	**D**
IL1B	rs1071676	106042060	2	.189	G>C	.954	.621	A
IL1B	rs1143643	106042929	2	.383	G>A	1.927	.381	A
IL1B	rs1143642	106043180	2	.082	C>T	.001	.999	A
IL1B	rs1143634	106045017	2	.187	C>T	.710	.701	A
IL1B	rs1143633	106045094	2	.392	G>A	2.456	.293	A
IL1B	rs1143630	106046282	2	.115	C>A	.513	.774	A
IL1B	rs3917356	106046990	2	.450	A>G	.058	.971	A
IL1B	rs1143629	106048145	2	.389	T>C	1.209	.546	A
IL1B	rs1143627	106049014	2	.397	T>C	.989	.610	A
IL1B	rs16944	106049494	2	.386	G>A	.652	.722	A
IL1B	rs1143623	106050452	2	.277	G>C	1.067	.587	A
IL1B	rs13032029	106055022	2	.448	C>T	.058	.971	A
IL1B	HapA1					.957	.620	
IL1B	HapA4					1.925	.382	
IL1B	HapA6					.791	.673	
IL1B	HapB1					1.579	.454	
IL1B	HapB6					1.303	.521	
IL1B	HapB8					.033	.984	
IL1R1	rs949963	96533648	2	.223	G>A	.192	.909	A
IL1R1	rs2228139	96545511	2	.053	C>G	4.344	.114	A
IL1R1	rs3917320	96556738	2	.047	A>C	1.065	.587	A
IL1R1	rs2110726	96558145	2	.317	C>T	2.691	.260	A
IL1R1	rs3917332	96560387	2	.187	T>A	1.499	.473	A
IL1R1	HapA1					.030	.985	
IL1R1	HapA2					.386	.825	
IL1R1	HapA3					1.499	.473	
IL1R2	rs4141134	96370336	2	.362	T>C	2.047	.359	A
IL1R2	rs11674595	96374804	2	.247	T>C	.262	.877	A
IL1R2	rs7570441	96380807	2	.408	G>A	3.099	.212	A
IL1R2	HapA1					2.845	.241	
IL1R2	HapA2					FE	.162	
IL1R2	HapA4					.823	.663	
IL2	rs1479923	119096993	4	.308	C>T	1.094	.579	A
IL2	rs2069776	119098582	4	*.184*	T>C	N/A	N/A	N/A
IL2	rs2069772	119099739	4	.241	A>G	3.873	.144	A
IL2	rs2069777	119103043	4	.047	C>T	2.406	.300	A
IL2	rs2069763	119104088	4	.277	T>G	.643	.725	A

IL2	HapA1					3.564	.168	
IL2	HapA2					.643	.725	
IL2	HapA3					3.873	.144	
IL4	rs2243248	127200946	5	.086	T>G	2.499	.287	A
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	G>C	3.095	.213	A
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					3.492	.174	
IL4	HapA3					.989	.610	
IL4	Hapx1					.444	.801	
IL6	rs4719714	22643793	7	.255	A>T	.475	.789	A
IL6	rs2069827	22648536	7	.069	G>T	3.415	.181	A
IL6	rs1800796	22649326	7	*.134*	G>C	N/A	N/A	N/A
IL6	rs1800795	22649725	7	.285	C>G	1.410	.494	A
IL6	rs2069835	22650951	7	*.130*	T>C	N/A	N/A	N/A
IL6	rs2066992	22651329	7	.091	G>T	.164	.921	A
IL6	**rs2069840**	**22651652**	**7**	**.333**	**C>G**	**FE**	**.023**	**R**
IL6	rs1554606	22651787	7	.319	T>G	.353	.838	A
IL6	rs2069845	22653229	7	.319	G>A	.668	.716	A
IL6	rs2069849	22654236	7	.024	C>T	1.100	.577	A
IL6	rs2069861	22654734	7	.056	C>T	.311	.856	A
IL6	rs35610689	22656903	7	.259	A>G	1.744	.418	A
IL6	HapA1					.958	.619	
IL6	**HapA5**					**6.608**	**.037**	
IL6	HapA8					2.042	.360	
IL8	rs4073	70417508	4	.455	T>A	3.347	.188	A
IL8	rs2227306	70418539	4	.366	C>T	.376	.829	A
IL8	rs2227543	70419394	4	.368	C>T	.552	.759	A
IL8	HapA1					3.347	.188	
IL8	HapA4					.399	.819	
IL10	rs3024505	177638230	1	.129	C>T	1.696	.428	A
IL10	rs3024498	177639855	1	.204	A>G	1.169	.557	A
IL10	rs3024496	177640190	1	.421	T>C	4.470	.107	A
IL10	rs1878672	177642039	1	.416	G>C	3.050	.218	A
IL10	rs3024492	177642438	1	*.161*	A>T	N/A	N/A	N/A
IL10	rs1518111	177642971	1	.303	G>A	.537	.764	A
IL10	rs1518110	177643187	1	.301	G>T	.346	.841	A
IL10	rs3024491	177643372	1	.408	T>G	4.103	.129	A
IL10	HapA1					.282	.869	
IL10	HapA2					.130	.937	
IL10	HapA8					1.169	.557	
IL13	rs1881457	127184713	5	.210	A>C	2.913	.233	A
IL13	rs1800925	127185113	5	.233	C>T	1.185	.553	A
IL13	rs2069743	127185579	5	.019	A>G	3.084	.214	A
IL13	rs1295686	127188147	5	.265	G>A	.024	.988	A
IL13	rs20541	127188268	5	.212	C>T	1.882	.390	A

IL13	HapA1					.024	.988	
IL13	HapA4					1.882	.390	
IL17A	rs4711998	51881422	6	.346	G>A	2.236	.327	A
IL17A	rs8193036	51881562	6	.327	T>C	.050	.975	A
IL17A	rs3819024	51881855	6	.372	A>G	2.217	.330	A
IL17A	rs2275913	51882102	6	.361	G>A	2.172	.338	A
IL17A	rs3804513	51884266	6	.023	A>T	FE	.160	A
IL17A	rs7747909	51885318	6	.217	G>A	1.728	.421	A
NFKB1	rs3774933	103645369	4	.409	T>C	.298	.862	A
NFKB1	rs170731	103667933	4	.397	T>A	5.136	.077	A
NFKB1	rs17032779	103685279	4	.023	T>C	FE	.685	A
NFKB1	rs230510	103695201	4	.366	T>A	.984	.612	A
NFKB1	rs230494	103706005	4	.477	A>G	.928	.629	A
NFKB1	rs4648016	103708706	4	.017	C>T	FE	.371	A
NFKB1	rs4648018	103709236	4	.025	G>C	FE	.532	A
NFKB1	rs3774956	103727564	4	.479	C>T	1.279	.528	A
NFKB1	rs10489114	103730426	4	.025	A>G	FE	.532	A
NFKB1	rs4648068	103737343	4	.366	A>G	2.188	.335	A
NFKB1	rs4648095	103746914	4	.052	T>C	FE	1.000	A
NFKB1	rs4648110	103752867	4	.205	T>A	.678	.713	A
NFKB1	rs4648135	103755716	4	.060	A>G	FE	.463	A
NFKB1	rs4648141	103755947	4	.188	G>A	.227	.893	A
NFKB1	rs1609798	103756488	4	.337	C>T	3.841	.147	A
NFKB1	HapA1					.673	.714	
NFKB1	HapA9					5.148	.076	
NFKB2	rs12772374	104146901	10	.157	A>G	5.831	.054	A
NFKB2	rs7897947	104147701	10	.229	T>G	1.217	.544	A
NFKB2	rs11574849	104149686	10	.085	G>A	1.578	.454	A
NFKB2	rs1056890	104152760	10	.317	C>T	.986	.611	A
TNFA	rs2857602	31533378	6	.341	T>C	.024	.988	A
TNFA	rs1800683	31540071	6	.390	G>A	1.385	.500	A
TNFA	rs2239704	31540141	6	.335	G>T	.028	.986	A
TNFA	rs2229094	31540556	6	.278	T>C	3.480	.176	A
TNFA	rs1041981	31540784	6	.386	C>A	1.223	.543	A
TNFA	**rs1799964**	**31542308**	**6**	**.224**	**T>C**	**FE**	**.005**	**R**
TNFA	**rs1800750**	**31542963**	**6**	**.016**	**G>A**	**FE**	**.032**	**A**
TNFA	rs1800629	31543031	6	.149	G>A	4.766	.092	A
TNFA	rs1800610	31543827	6	.100	C>T	.119	.942	A
TNFA	rs3093662	31544189	6	.074	A>G	1.602	.449	A
TNFA	HapA1					.213	.899	
TNFA	**HapA5**					**9.188**	**.010**	
TNFA	HapA6					1.507	.471	

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

Single nucleotide polymorphisms (SNPs) that violated Hardy-Weinberg expectations are denoted in italics in the MAF column.

Table 2 - Differences in Demographic and Clinical Characteristics Between Resilient (n=155) and Subsyndromal (n=180) Classes

Characteristic	Resilient Class n=155 (46.3%) Mean (SD)	Subsyndromal Class n=180 (53.7%) Mean (SD)	Statistic and p-value
Age (years)	57. 3 (11.0)	53.0 (11.9)	t=3.50, p=.001
Education (years)	15.8 (2.5)	15.9 (2.8)	t=-0.16, p=.87
Karnofsky Performance Status score	95.5 (8.7)	91.1 (11.1)	t=3.93, p<.0001
Self-administered Comorbidity Questionnaire score	4.0 (2.5)	4.6 (3.1)	t=-1.84, p=.07
Center for Epidemiological Studies Depression score	6.8 (4.7)	17.1 (8.6)	t=-13.6, p<.0001
Number of breast biopsies in past year	1.5 (0.8)	1.6 (0.9)	U, p=.29
Number of positive lymph nodes	0.9 (2.6)	1.0 (2.0)	t=-0.34, p=.74
Number of lymph nodes removed	5.0 (5.9)	7.0 (7.8)	t=-2.64, p=.01
	n (%)	n (%)	
Ethnicity			
White	107 (69.5)	112 (62.6)	
Black	16 (10.4)	16 (8.9)	x^2=4.02, p=.26
Asian/Pacific Islander	18 (11.7)	24 (13.4)	
Hispanic/Mixed ethnic background/Other	13 (8.4)	27 (15.1)	
Married/partnered (% yes)	54 (35.1)	84 (46.9)	FE, p=.03
Work for pay (% yes)	78 (50.3)	83 (46.6)	FE, p=.51
Lives alone (% yes)	34 (22.1)	41 (23.0)	FE, p=.90
Gone through menopause (% yes)	104 (68.0)	104 (60.1)	FE, p=.17
Stage of disease			
0	26 (16.8)	34 (18.9)	
I	68 (43.9)	54 (30.0)	
IIA	36 (23.2)	49 (27.2)	
IIB	14 (9.0)	24 (13.3)	U, p=.12
IIIA	5 (3.2)	14 (7.8)	
IIIB	1 (0.6)	1 (0.6)	
IIIC	4 (2.6)	4 (2.2)	
IV	1 (0.6)	0 (0.0)	
Surgical treatment			
Breast conservation	127 (81.9)	142 (78.9)	FE, p=.50
Mastectomy	28 (18.1)	38 (21.1)	
Sentinel node biopsy (% yes)	133 (85.8)	144 (80.0)	FE, p=.19
Axillary lymph node dissection (% yes)	52 (33.8)	82 (45.6)	FE, p=.03
Breast reconstruction at the time of surgery (% yes)	28 (18.2)	41 (22.8)	FE, p=.34
Neoadjuvant chemotherapy (% yes)	26 (16.9)	44 (24.4)	FE, p=.11
Radiation therapy during the first 6 months (% yes)	93 (60.0)	95 (52.8)	FE, p=.19
Chemotherapy during the first 6 months (% yes)	42 (27.1)	73 (40.6)	FE, p=.01

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

Table 3 - Multiple Logistic Regression Analyses for Interferon Gamma Receptor 1(IFNGR1) rs9376268, Interleukin 6 (L6) rs2069840, and Tumor Necrosis Factor Alpha (TNFA) rs1800750 to Predict Subsyndromal Latent Class Membership

Predictor	Odds Ratio	Standard Error	95% CI	Z	p-value
IFNGR1 Genotype	1.87	0.512	1.097, 3.201	2.30	0.022
Age	0.83	0.050	0.740, 0.938	-3.02	0.003
KPS score	0.71	0.102	0.539, 0.943	-2.37	0.018
Overall model fit: $\chi^2 = 27.60$, p =0.0011, $R^2 = 0.0772$					
IL6 Genotype	3.06	1.511	1.165, 8.054	2.27	0.023
Age	0.83	0.050	0.734, 0.932	-3.11	0.002
KPS score	0.73	0.103	0.553, 0.963	-2.23	0.026
Overall model fit: $\chi^2 = 27.84$, p = 0.0010, $R^2 = 0.0779$					
TNFA Genotype	0.07	0.079	0.008, 0.633	-2.37	0.018
Age	0.83	0.051	0.740, 0.939	-2.99	0.003
KPS score	0.69	0.101	0.514, 0.915	-2.56	0.010
Overall model fit: $\chi^2 = 30.94$, p = 0.0003, $R^2 = 0.0865$					

Multiple logistic regression analysis of candidate gene associations with resilient versus subsyndromal classes. For each model, the first three principal components identified from the analysis of ancestry informative markers as well as self-report race/ethnicity were retained in all models to adjust for potential confounding due to race or ethnicity (data not shown). Predictors evaluated in each model included genotype (IFNGR1 rs9376268: GG versus GA + AA; IL6 rs2069840: CC + CG versus GG; TNFA rs1800750: GG versus GA versus AA), age (in 5 year increments), and functional status at baseline (estimated by the KPS score, 10 point increments).

Abbreviations; CI =confidence interval; KPS = Karnofsky Performance Status.

Figure 1

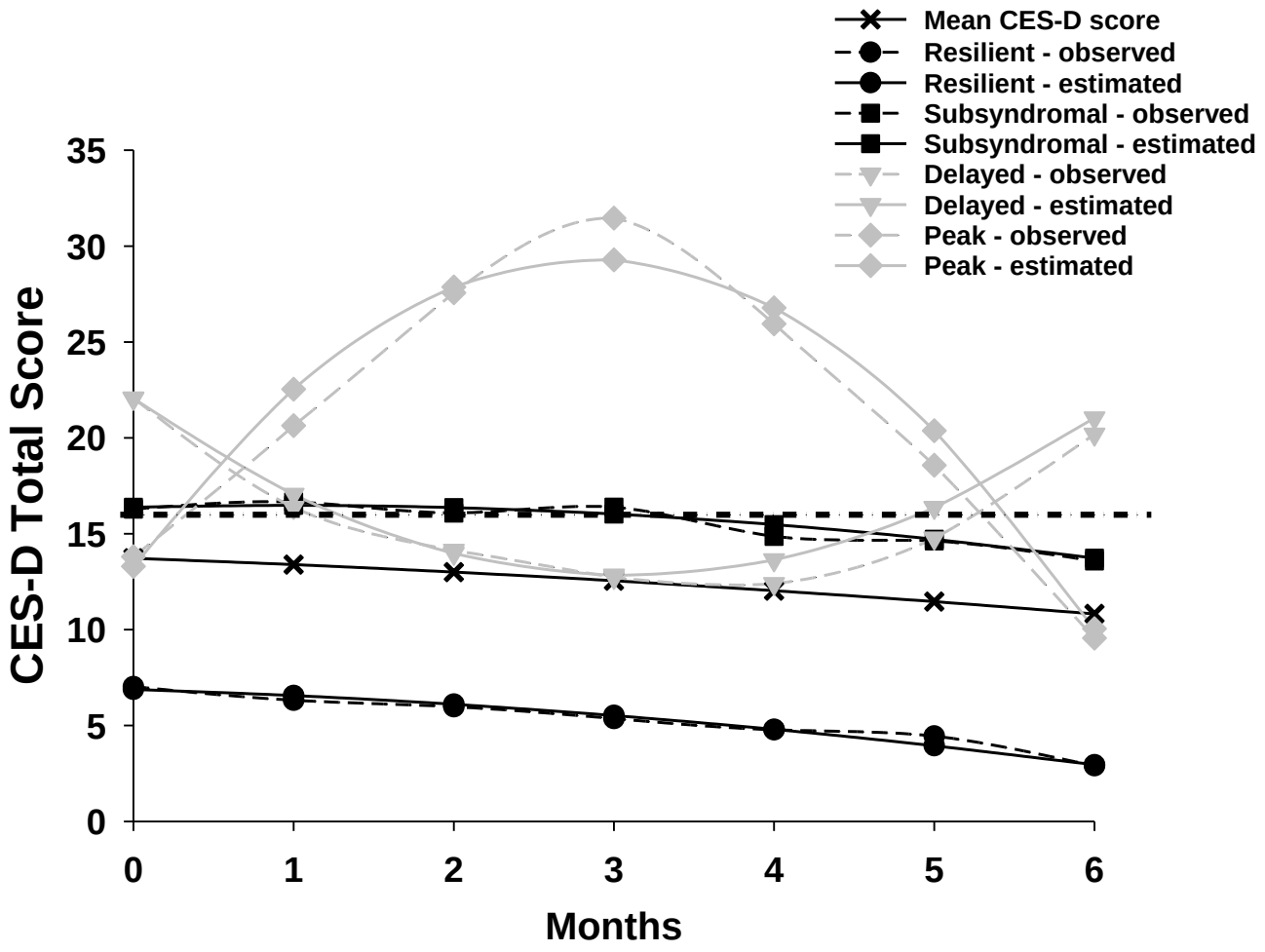
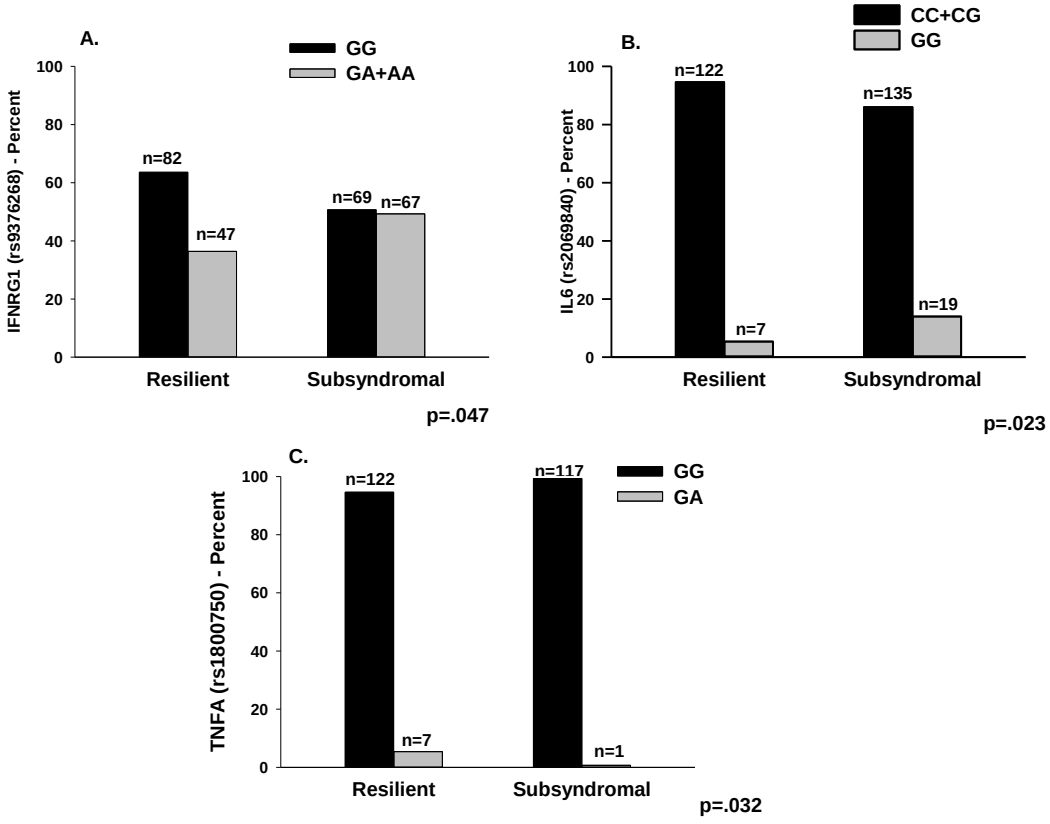

Mean CES-D score
Resilient - observed
Resilient - estimated
Subsyndromal - observed
Subsyndromal - estimated
Delayed - observed
Delayed - estimated
Peak - observed
Peak - estimated
CES-D Total Score
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
Months

Figure 2


A.
GG
GA+AA
IFNRG1 (rs9376268) - Percent
100
80
60
40
20
0
n=82
n=47
n=69
n=67
Resilient
Subsyndromal
p=.047
B.
CC+CG
GG
IL6 (rs2069840) - Percent
n=122
n=7
n=135
n=19
Resilient
Subsyndromal
p=.023
C.
GG
GA
TNFA (rs1800750) - Percent
n=122
n=7
n=117
n=1
Resilient
Subsyndromal
p=.032

32

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