Examining the Effect of Tumor Features, BDNF, and 5-HTT Genotypes on Depressive Symptoms in Breast Cancer

by

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The development of depressive symptoms in women with breast cancer is multifactorial and may be impacted by both genetics and pathologic tumor features. The purpose of this study is to examine the contributions of the serotonin transporter (SERT) and brain-derived neurotrophic factor (BDNF) genes to the development of depressive symptoms in postmenopausal women with early-stage breast cancer. Another aim is to investigate the impact of pathologic tumor features on the development of depressive symptoms. N=258 women (n=162 women with breast cancer and n=96 matched healthy controls) were included in the genetic aims of this study, and N=329 women with breast cancer were included in the pathologic tumor features analysis. Depressive symptomology was measured using the Beck Depression Inventory (BDI – II) at baseline, and six months and 12 months post-baseline. Linear and logistic regression models were built both with and without control for treatment group as a predictor. Participants with a SERT genotype of L_A/L_A had significantly higher mean BDI-II scores across time compared to all other SERT genotypes. No significant associations were found between BDNF genotype and depressive symptoms. A high Ki67 classification was associated with decreased depressive symptoms, while multifocal tumors and increased HER2 classification were associated with increased depressive symptoms. Our results support a previous study conducted at the University of Pittsburgh School of Nursing suggesting that the L_A allele is a risk factor for depressive symptoms in women with breast cancer. In addition, pathologic tumor features that have previously been associated with poorer cognitive performance were similarly implicated in depressive symptom development.

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1.0 Background

1.1 **Depression**

Depression is a mood state characterized by sadness, despair, and hopelessness (1). Depressive symptoms interfere with quality of life, are associated with negative outcomes such as suicide (2), and include loss of pleasure in activities, sleep disturbances, fatigue, and cognitive deficits in concentration and decision-making. Depression can be a component of more serious mood disorders including major depressive disorder (MDD). Between 2005 and 2010 in the United States, the prevalence of MDD increased from 13.8 to 15.4 million adults, and healthcare costs due to MDD increased to $210.5 billion per year (3). The Center for Behavioral Health Statistics and Quality found that 20.7% of adults experience a major depressive episode in their lifetime (4).

The development of depression is complex and involves genetic, environmental, and physiological factors. Recent research has focused on contributions from risk genes, epigenetic regulation of gene expression, and shorter telomere length in DNA (5-7). Environmental factors such as low birth weight, prematurity, trauma, stress, and decreased social support are associated with depression, as well (8-9). Biological factors associated with depression include increased inflammation and decreased activity of certain neurotransmitters (10). Females experience single and recurrent depressive disorders at a rate almost twice that of males (11) and the risk of developing both short-term and long-term depression increases following the experience of a stressful life event. In this study, subjects have experienced a stressful life event, which we have identified as the diagnosis and treatment of breast cancer (12).

1.2 **Breast Cancer**

Breast cancer is the most common cancer occurring in women (13). In the United States this year, about 330,000 women will receive a new diagnosis of breast cancer and about 80% of these cases will be an invasive type (13). Additionally, there is a large number of breast cancer survivors (over 3.1 million) who are still undergoing or have completed treatment (13). A recent study followed women recently diagnosed with breast cancer and found that 16.6% exhibited symptoms indicating a major depressive episode at least once over a period of 12 months (14). This incidence is disproportionately high when compared to the general female population, which has a 12-month prevalence of depression of 8.4% (15). One study of older women with breast cancer found that depressive symptoms increased the most within the first six months after diagnosis (16). Depressive symptoms in women with breast cancer should be seriously considered as they are strongly correlated with increased mortality (17). In survivors, depression can affect quality of life by contributing to poor health outcomes, such as decreased screening for other cancers (18) and barriers to physical activity and healthy eating (19).

1.3 **Serotonin Transporter**

Serotonin is a monoamine neurotransmitter responsible for various functions due to the widespread distribution of serotonin receptors in the body. Most of the body's serotonin is concentrated in the gastrointestinal tract, where serotonin is involved in liver regeneration, gastrointestinal motility, and appetite (20). Serotonin also has important cardiovascular effects, contributing to blood vessel constriction and dilation, blood pressure, and heart rate (20).

Furthermore, serotonin activity in the central nervous system has a role in aggression, anxiety, memory, mood, sleep, learning, and addictive behaviors (20). Deficiency of serotonin is implicated in the monoamine hypothesis of depression, which explains the development of depression as a result of neurotransmitter deficiency. The monoamine hypothesis is the basis for antidepressant medications that inhibit the reuptake of serotonin and therefore potentiate its effects in the neuronal synapse (21).

The *SLC6A4* gene expresses the serotonin transporter protein (5-HTT), which is responsible for the return of serotonin from the synapse to the presynaptic neuron during reuptake (22). A 44 base-pair insertion/deletion functional polymorphism in the promoter region of *SLC6A4* is known as the serotonin-transporter-linked polymorphic region (5-HTTLPR) and has been a major subject of focus regarding the role of serotonin transporter in depression. Two common versions of the 5-HTTLPR polymorphism consist of the long "L"-allele (insertion) and the short "S"-allele (deletion). The L-allele is associated with increased serotonin transporter expression (and, thus, serotonin reuptake), while the S-allele is associated with decreased serotonin transporter expression and serotonin reuptake (23). In other words, the L-allele results in higher serotonin transporter activity, while the S-allele results in lower serotonin transporter activity. In addition, *SLC6A4* displays a single nucleotide polymorphism called rs25531 (SNP, rs25531 A>G) that is found exclusively within the L-allele. The L-allele has an adenine (A) base which is substituted for guanine (G) in the polymorphism, resulting in the variant L_G-allele functioning more like the S-allele than the original L_A-allele (24).

Results in the literature are inconsistent regarding the relationship of serotonin transporter genotype and severity of depressive symptoms. Recently, a study of women with advanced breast cancer demonstrated that the S-allele was associated with greater depressive symptoms (25). In

another study, anxious and depressive symptoms were shown to persist longer in women with breast cancer who have one or two S-alleles compared to two L_A alleles, suggesting that the L_A/L_A genotype has a beneficial role in coping with mental distress (26). In women with breast cancer who experienced altered body image and reduced sexual function, the S-allele was associated with severity of depressive symptoms (27). On the other hand, the results of a study of women in the early postoperative period after breast cancer surgery suggest that some patients with the L_A/L_A genotype may be at greater risk for depressive symptoms and a sense of hopelessness (28). In a study by Rawson et al. (2015) of older adults who experienced a recent hip fracture (which was deemed a stressful event), participants with the L_A/L_A genotype had increased depressive symptoms compared to all other participants (29).

Recent studies of women with breast cancer (Table 1) have not included the rs25531 single-nucleotide polymorphism in analysis or have not had available subjects with the variant L_G-allele. In addition, none of the studies included comparison with healthy controls. A study (N=125) by University of Pittsburgh School of Nursing faculty included *n*=19 subjects with breast cancer with the L_G-allele as well as *n*=45 healthy controls. This study demonstrated that the L_A/L_A genotype was associated with increased depressive symptoms among the overall sample, though there was no statistically significant relationship between genotype and depressive symptoms among only women with breast cancer (30).

Table 1 Literature Regarding SERT Risk Polymorphisms In Women with Breast Cancer

	Schillani, G., Martinis, E., Capozzo, M.A.,… (2010)	Schillani, G., Era, D., Cristante, T.,… (2012)	Kim, K.R., Chung, H.C., Lee, E.,… (2012)	Kim, Y., Carver, C.S., Hallmayer, J.F,… (2018)	Wang, J., Bender, C.M., Conley, Y.P.,… (2018)

L_G allele	Not studied	No available subjects	Not studied	Not studied	N=19
Sample size?	53	48	186	95	125
Healthy controls	no	no	no	no	N= 45
Depression measure	Hospital Anxiety and Depression Scale	Hospital Anxiety and Depression Scale	Hospital Anxiety and Depression Scale, Hamilton Depression Rating Scale	Center for Epidemiological Studies Depression Scale	Beck Depression Inventory-II
Risk allele	L_A	S	S	S	L_A

1.4 **Brain-Derived Neurotrophic Factor**

Brain-derived neurotrophic factor (BDNF) is a protein involved in neuroplasticity, neurogenesis, and memorization (31). Abnormal serum levels of BDNF are associated with several neurological conditions, including depression, epilepsy, Alzheimer's disease, Parkinson's disease, and Huntington's disease (31). A single-nucleotide polymorphism in the BDNF gene (rs6265, or val66met) causes the amino acid methionine to be substituted for valine in the BDNF protein. This polymorphism has two alleles, the Met (A) variant allele and the Val (G) wild-type allele (32).

The Met allele has been associated with higher serum BDNF levels in both healthy subjects (32) and subjects who meet criteria for major depression (33). In a study of patients with small-cell lung cancer, lower serum BDNF levels were associated with more severe depression and shorter overall survival. In contrast, higher serum BDNF levels were associated with more mild depression and longer overall survival (34). However, once again, studies are inconsistent regarding the relationship between rs6265 genotype and depression as both the Val and Met alleles

have been implicated as risk factors for the development of depression (35). The study by Rawson et al. (2015) examined an interaction between the SERT polymorphism rs25531 and the BDNF polymorphism rs6265 and showed an association of the Met/Met genotype with increased depressive symptoms, which was only significant in participants with the L_A/L_A genotype (30).

1.5 **Pathologic Tumor Features**

A study by Koleck et al. (2017) examining pathologic tumor features of women with breast cancer showed that certain tumor characteristics were associated with decreased cognitive function (36). Another study demonstrated that having high psychoneurological symptoms (anxiety, depression, fatigue, etc.) at the time of breast cancer diagnosis predicted clinically relevant declines in cognitive function over time (37). Furthermore, cognitive impairment, such as difficulty thinking or concentrating, can be a diagnostic symptom of depression (38).

In this study, tumor stage was an important pathologic tumor feature categorized by early-stage tumor size: T1a (1-5mm), T1b (5-10mm), T1c (10-20mm), and T2 (20-50mm) (39). Ki67 classification was another pathologic tumor feature that is a marker for tumor growth and classification. Ki67 is an antigen in cell nuclei and its association with cellular proliferation is well-established (40). Lastly, HER2 (human epidermal growth factor receptor 2) is a receptor protein that is a product of the HER2, or ERBB2, gene. Its overexpression is associated with more aggressive disease and poorer prognosis (41-42).

2.0 Purpose

Analysis of the relationship between serotonin transporter and depression in the population of women with breast cancer has produced inconsistent results in the literature. In addition, there is limited research that examines the serotonin transporter and BDNF genes concurrently and, therefore, limited knowledge on the interaction between these genes. Lastly, prior research in women with breast cancer has found that pathologic tumor characteristics impact cognitive function; however, their impact on depressive symptoms has not been explored. The purpose of this study is to investigate an interaction between two biologically relevant genes as well as the impact of pathologic tumor characteristics on depressive symptoms in women with breast cancer.

3.0 Aims

The first aim is to characterize the relationship between serotonin transporter (SERT) genotype and depressive symptoms in women with breast cancer. The second aim is to examine the relationship between BDNF genotype and depressive symptoms, and to analyze the effect of any interaction between SERT and BDNF. Lastly, the third aim is to investigate pathologic tumor characteristics and their impact on the development of depressive symptoms.

4.0 Hypotheses

Based on results from a University of Pittsburgh School of Nursing study (30), our hypothesis for the first aim is that the L_A/L_A genotype will be associated with increased depressive symptoms in the overall sample. For the second aim, our hypothesis is that the Met (A) variant allele will be associated with increased depressive symptoms. Lastly, our hypothesis for the third aim is that tumor characteristics associated with worse cognitive function will also be associated with increased depressive symptoms in women with breast cancer.

5.0 Methods

This study is ancillary from a longitudinal study conducted by University of Pittsburgh School of Nursing faculty. In the longitudinal study, depressive symptoms were measured at baseline after the breast cancer diagnosis and primary surgery but before the initiation of chemotherapy and/or hormonal therapy (anastrozole) for breast cancer participants. They were measured again at six months and 12 months post-baseline.

5.1 **Participants**

The longitudinal study was approved by the University of Pittsburgh Institutional Review Board and informed consent was obtained from all study participants. Data from a total of N=258 participants provided samples for genomic evaluation and were included in the analysis for the genetic-based objectives of this study. Postmenopausal women with early stage breast cancer (n=162) were recruited from the Comprehensive Breast Care Program of the University of Pittsburgh Cancer Institute and the University of Pittsburgh Medical Center Cancer Centers. Participants were characterized by treatment group, with n=60 subjects receiving both chemotherapy and anastrozole and n=102 patients receiving anastrozole only. Due to a small sample size of subjects receiving chemotherapy only (n=15), we did not include these subjects in statistical analysis. Control group participants (n=96) were healthy postmenopausal women who were matched with breast cancer participants on age, IQ, and years of education.

For analysis of pathologic tumor feature data, the sample was comprised of n=329 postmenopausal women with early stage breast cancer recruited from the Comprehensive Breast Care Program of the University of Pittsburgh Cancer Institute. Data were collected from surgical pathology reports in participants' medical records.

All participants were between the ages of 18 and 75 years, could speak and read English, and had at least 8 years of education. Demographic information was collected by self-report. Exclusion criteria included hospitalization for psychiatric illness within 2 years of study enrollment or a history of neurologic disease or cancer.

5.2 **Depressive Symptom Data Collection**

Depressive symptoms, the main dependent variable of interest for this study, was measured using the second edition of the Beck Depression Inventory (BDI – II). The BDI – II is a 21-item self-report measure in which participants rate depressive symptoms and attitudes on a scale from 0 (absence of symptom) to 3 (persistent expression of symptom in the past 2 weeks). The overall score is a measure of the severity of depressive symptoms, with a score of 14-28 indicating mild to moderate depression and a score of 29-63 indicating severe depression. The BDI – II has been found to have high internal consistency ranging from $\alpha = 0.88$ to $\alpha = 0.9420$.

5.3 **Genetic Data Collection**

DNA was extracted from either blood or saliva using standard techniques and then analyzed for the two *SLC6A4* polymorphisms, 5-HTTLPR and rs25531. The two polymorphisms of interest were genotyped using a polymerase chain reaction (PCR) restriction fragment length polymorphism assay with the following composition from Qiagen Multiplex PCR Kit: 63% Master Mix, 6.3% each of the forward and reverse primers, 25% 5Q solution. PCR proceeded with initial denaturation at 95°C for 15 min., 35 cycles consisting of 94°C 30 s., 57°C 1 min. 30 s., 72°C 1 min. 30 s.; final extension step on 72°C 10 min. The sequence of the forward primer was CTCCCTGTACCCCTCCTAGG, and the sequence of the reverse primer was TGCAAGGAGAATGCTGGAG. The PCR products were then analyzed in 2% agarose gels stained with ethidium bromide to genotype 5-HTTLPR. To genotype rs25531, the PCR products were digested with 3U of MspI (Fermentas, Canada) according to manufacturer's recommendations and resolved in 2% agarose gels. The digested product for the S-allele was 270 base pairs, and the digested product for the L_A-allele was 300 base pairs. The enzyme cut at the G for A substitution in the L allele, indicating the L_G-allele if the substitution was present.

The plan for genotyping the BDNF rs6265 polymorphism originally utilized a TaqMan experiment that uses quantitative real-time PCR methodology. This methodology was adapted since the assay did not work after multiple attempts despite substantial trouble-shooting. Analysis for the BDNF rs6265 polymorphism proceeded with a PCR assay with the following composition: 56% H_2O, 10% buffer (magnesium chloride), 12% DMSO, 16% dNTP, 5% each of the forward and reverse primers, and 0.5% Denville Taq polymerase. The assay proceeded as follows: 35 cycles of 95°C 30 s., 54°C 36 s., 72°C 40 s.; then, 72°C 10 min. The sequence of the forward primer was AAACATCCGAGGACAAGGTG and the sequence of the reverse primer was

AGAAGAGGAGGCTCCAAAGG. The PCR products were analyzed in 1% agarose gels stained with ethidium bromide to check for amplification. To genotype rs6265, enzymatic digestion was carried out with 1U of BsaAI according to manufacturer's recommendations and resolved in 2% agarose gels. The enzyme cut at the 124th base pair (G) in the 249-base pair polymorphism. Genotype was determined by bands created by migration of DNA fragments in the gel. Met/Met (A/A) genotype was indicated by a longer fragment (negative digestion), Val/Val (G/G) genotype was indicated by a shorter fragment (positive digestion), and Met/Val (A/G) genotype was indicated by two fragments.

5.4 **Pathologic Tumor Feature Data Collection**

Pathologic tumor feature data were obtained from the Koleck et al. (2017) study, which was ancillary to another University of Pittsburgh School of Nursing study. Both studies were approved by the University of Pittsburgh Institutional Review Board. The sample of N=329 participants was comprised of postmenopausal women with early stage breast cancer recruited from the Comprehensive Breast Care Program of the University of Pittsburgh Cancer Institute. Inclusion and exclusion criteria were the same as discussed above. Data were collected from surgical pathology reports in participants' medical records.

5.5 **Statistical Analysis**

During exploratory analysis, we found that the BDI-II scores showed a right-skewed distribution (i.e., the majority of women reported minimal depressive symptoms) in both samples. We performed statistical analysis treating the BDI-II score both as a continuous variable and as a binary variable according to no depressive symptoms vs. some depressive symptoms, as well as minimal depression vs. moderate or greater depression. Score ranges for BDI-II categories were established as follows: minimal (0-13), mild (14-19), moderate (20-28), and severe (29-63).

To determine associations between genetic and tumor feature variables with depressive symptoms, ANOVA, correlation, and and chi-square/Fisher's exact test analyses were performed at each time point. Unadjusted and adjusted linear and logistic regression models were then generated with and without control for treatment group (i.e., chemotherapy/anastrozole or anastrozole only) as a predictor. Unstandardized beta coefficients and two-tailed significance tests were used to determine statistical significance. All analyses were performed using IBM SPSS Statistics 25.0 with a statistical significance level of $\alpha = 0.05$. The results that are ultimately presented are from linear regression models adjusted for treatment group with main effects only.

5.5.1 **Aim 1: SERT Genotype and BDI-II Score and Aim 2: BDNF Genotype and BDI-II Score**

Demographic characteristics for the N=258 participants included in genetic analysis consisted of means, standard deviations, medians, minima, and maxima for continuous variables, as well as frequencies for categorical variables. These characteristics were computed for the

overall sample and by cohort. Statistical significance to identify differences between treatment groups was determined by ANOVA or Fisher's exact tests.

SERT genotype was categorized into three groups for statistical analysis, with the L_G-allele being treated as functionally equivalent to the S-allele. Thus, SERT genotypes were compared according to L_A vs. L_A/L_G +S/L_A vs. S+S/L_G + L_G. The binary model compared L_A (high serotonin activity) vs. all other genotypes. An additive model was included to treat SERT genotype as a continuous predictor for depressive symptoms, both with and without treatment group predictors. Genotype frequencies were calculated by cohort and for the overall sample. Boxplots were constructed to compare BDI-II scores by SERT genotype across all timepoints, allowing BDI-II score outliers to be identified. In addition, we added interaction terms to regression models analyzing BDI-II score by treatment cohort and SERT genotype.

Genotype frequencies for BDNF were also calculated by cohort and for the overall sample. BDNF genotypes were compared individually according to Val/Val vs. Met/Val vs. Met/Met, and in two groups according to Val/Val vs. at least one Met allele. An additive model was created to examine BDNF genotype as a continuous predictor for depressive symptoms, both with and without treatment group predictors.

5.5.2 **Aim 3: Pathologic Tumor Features and BDI-II Score**

Demographic characteristics and pathologic tumor feature data for the N=329 participants consisted of means, standard deviations, medians, minima, and maxima for continuous variables, as well as frequencies for categorical variables. These characteristics were computed for the overall sample and by cohort. Statistical significance to identify differences between treatment

groups was determined by t-tests for equality of means (equal variances not assumed) and Fisher's exact tests.

We conducted additional analyses on tumor stage. Specifically, we performed ANOVA to compare age and years of education between participants with different tumor stages. We created spaghetti plots using R to visualize the trajectory of BDI-II scores over time by tumor stage and treatment group for each participant. In response, a follow-up sensitivity analysis was conducted to evaluate potential influential points.

6.0 Results

Table 2 Descriptive Statistics for BDI-II Score in the Genetic-Based Sample (N=258)

	Baseline			**6-months**			**12-months**		
	Overall			**Overall**			**Overall**		
Mean (SD)	4.83 (4.87)			5.72 (5.88)			5.28 (5.87)		
Median	4			4			4		
	Chemo + Anast	**Anast Only**	**Healthy Control**	**Chemo + Anast**	**Anast Only**	**Healthy Control**	**Chemo + Anast**	**Anast Only**	**Healthy Control**
Mean	5.08	4.65	4.85	8.02	5.37	4.63	5.63	5.83	4.47
Median	5	4	3	7	3	3	5	4	3

Table 3 Descriptive Statistics for BDI-II Score in the Pathologic Tumor Features Sample (N=329)

	Baseline		**6-months**		**12-months**	
	Overall		**Overall**		**Overall**	
Mean (SD)	5.33 (5.62)		6.08 (6.09)		5.79 (6.60)	
Median	4		5		4	
	Chemo + Anast	**Anast Only**	**Chemo + Anast**	**Anast Only**	**Chemo + Anast**	**Anast Only**
Mean	5.88	5.03	7.32	5.03	5.54	5.94
Median	4	4	6	4	5	4

Table 4 BDI-II Score Categories in the Genetic-Based Sample (N=258)

	N (%)		
	Baseline	6-months	12-months
Minimal depression	239 (93.4%)	235 (91.8%)	208 (92.4%)
Moderate or higher depression	17 (6.6%)	21 (8.2%)	17 (7.6%)

Table 5 BDI-II Score Categories in the Pathologic Tumor Features Sample (N=329)

	N (%)		
	Baseline	6-months	12-months
Minimal depression	299 (90.9%)	243 (90.7%)	176 (91.2%)

Moderate or higher depression	30 (9.1%)	25 (9.3%)	17 (8.8%)

Table 6 Characteristics of Patients Included in Genetic Analysis, Overall Sample (N=258)

Characteristic	Mean (SD), Median or n (%)	Minimum	Maximum
Age (years)	60.16 (6.20), 60	43	75
Education (years)	15.20 (2.99), 15	9	29
Marital Status (currently married or living with significant other)	169 (65.5)	NA	NA
Number of Children	1.99 (1.37), 2	0	8
Race (Caucasian)	245 (95.0)	NA	NA

Table 7 Characteristics of Patients Included in Genetic Analysis By Cohort

	Chemotherapy/Anastrozole (n=60)			Anastrozole Only (n=102)			Healthy Controls (n=96)			p-value
Characteristic	Mean (SD), Median or n (%)	Min.	Max.	Mean (SD), Median or n (%)	Min.	Max.	Mean (SD), Median or n (%)	Min.	Max.	
Age (years)	59.00 (5.59), 59	47	71	62.35 (5.86), 62.5	51	75	58.54 (6.28), 59	43	74	<0.001
Education (years)	15.65 (2.76), 16	12	22	14.80 (2.93), 14	9	26	15.33 (3.18), 16	11	29	0.189
Marital Status (currently married or living with significant other)	42 (70.0)	NA	NA	70 (68.6)	NA	NA	57 (59.4)	NA	NA	0.295
Number of Children	1.70 (1.21), 2	0	5	2.08 (1.33), 2	0	7	2.08 (1.48), 2	0	8	0.168
Race (Caucasian)	57 (95.0)	NA	NA	99 (97.1)	NA	NA	89 (92.7)	NA	NA	0.39

Table 8 SERT Genotypes

	Chemotherapy/Anastrozole (n=60) *N* (%)	Anastrozole Only (n=102) *N* (%)	Healthy controls (n=96) *N* (%)	Total sample (n=258) *N* (%)
S/S	11 (18.3)	19 (18.6)	22 (22.9)	52 (20.1)
S/L_G	4 (6.7)	5 (4.9)	7 (7.2)	16 (6.2)
S/L_A	25 (41.6)	42 (41.1)	42 (43.7)	109 (42.2)
L_A/L_G	4 (6.7)	4 (3.9)	6 (6.2)	14 (5.4)
L_A/L_A	16 (26.7)	31 (30.3)	19 (19.7)	66 (25.6)
L_G/L_G	0 (0.0)	1 (0.9)	0 (0.0)	1 (0.3)

Table 9 BDNF Genotypes

	Chemotherapy/Anastrozole (n=60) *N* (%)	Anastrozole Only (n=102) *N* (%)	Healthy controls (n=96) *N* (%)	Total sample (n=258) *N* (%)
Met/Met (A/A)	3 (5.0)	8 (7.8)	7 (7.2)	18 (6.9)
Met/Val (A/G)	18 (30.0)	42 (41.1)	34 (35.4)	94 (36.4)
Val/Val (G/G)	39 (65.0)	52 (50.9)	55 (57.2)	146 (56.5)

Table 10 Characteristics of Patients Included in Pathologic Tumor Feature Analysis, Overall Sample (N=329)

Characteristic	**Mean (SD), Median** or **n (%)**	**Minimum**	**Maximum**
Age (years)	61.05 (5.98), 61	45	75
Education (years)	14.80 (2.81), 14	6	26
Marital Status (currently married or living with significant other)	223 (67.8)	NA	NA
Number of Children	1.89 (1.24), 2	0	7
Race (Caucasian)	317 (96.4)	NA	NA

Table 11 Characteristics of Patients Included in Pathologic Tumor Feature Analysis By Cohort

	Chemotherapy/Anastrozole (n=117)			Anastrozole Only (n=212)			p-value
Characteristic	Mean (SD), Median or n (%)	Min.	Max.	Mean (SD), Median or n (%)	Min.	Max.	
Age (years)	59.45 (5.20), 60	47	71	61.92 (6.20), 61	45	75	<0.001
Education (years)	14.89 (2.92), 14	6	23	14.75 (2.75), 14	9	26	0.663
Marital Status (currently married or living with significant other)	81 (69.2)	NA	NA	142 (67.0)	NA	NA	0.713
Number of Children	1.85 (1.23), 2	0	5	1.91 (1.25), 2	0	7	0.695
Race (Caucasian)	111 (94.9)	NA	NA	206 (97.2)	NA	NA	0.359

Table 12 Pathologic Tumor Features, Overall Sample

Tumor Characteristics	Mean (SD), Median or n (%)	Minimum	Maximum	*n*
AJCC Tumor Stage				329
Stage I	214 (65.0)	NA	NA	
Stage IIA	75 (22.8)	NA	NA	
Stage IIB	24 (7.3)	NA	NA	
Stage IIIA	16 (4.9)	NA	NA	
Tumor Size (cm)	1.66 (1.50), 1.30	0.1	14	328
Aggregate Tumor Size (cm)	1.80 (1.60), 1.40	0.1	14	328
Tumor Classification				329
T1a	37 (11.2)	NA	NA	
T1b	82 (24.9)	NA	NA	
T1c	133 (40.4)	NA	NA	
T2	65 (19.8)	NA	NA	
T3	12 (3.6)	NA	NA	
Lymph Node				325
Positive	73 (22.5)	NA	NA	
Negative	252 (77.5)	NA	NA	
Number of Positive Nodes	0.42 (1.05), 0	0	8	329
Tumor Focality/Centricity				329
Single	277 (84.2)	NA	NA	
Multiple	52 (15.8)	NA	NA	
Tumor Laterality				329

Right breast	149 (45.3)	NA	NA	
Left breast	180 (54.7)	NA	NA	
Tumor Location Octant				323
Upper outer	125 (38.7)	NA	NA	
Lower outer	28 (8.7)	NA	NA	
Lower inner	21 (6.5)	NA	NA	
Upper inner	42 (13.0)	NA	NA	
Upper junction	38 (11.8)	NA	NA	
Lower junction	17 (5.3)	NA	NA	
Outer junction	30 (9.3)	NA	NA	
Inner junction	9 (2.8)	NA	NA	
Retroareolar	13 (4.0)	NA	NA	
Tumor Location Quadrant				323
Upper outer	163 (50.5)	NA	NA	
Lower outer	58 (18.0)	NA	NA	
Lower inner	38 (11.8)	NA	NA	
Upper inner	51 (15.8)	NA	NA	
Retroareolar	13 (4.0)	NA	NA	
Invasive Type				328
Ductal	285 (86.9)	NA	NA	
Lobular	35 (10.7)	NA	NA	
Ductal & Lobular	8 (2.4)	NA	NA	
Nottingham Score	6.04 (1.31), 6	3	9	315
Nottingham Grade				316
Grade 1	95 (30.1)	NA	NA	
Grade 2	171 (54.1)	NA	NA	
Grade 3	50 (15.8)	NA	NA	
ER Status				328
Positive	324 (98.8)	NA	NA	
Negative	4 (1.2)	NA	NA	
ER H-Score	256.90 (59.98), 280	0	300	311
PR Status				328
Positive	288 (87.8)	NA	NA	
Negative	40 (12.2)	NA	NA	
PR H-Score	130.08 (101.30), 130	0	300	310
HER2 Status				318
Positive	28 (8.8)	NA	NA	
Negative	290 (91.2)	NA	NA	
HER2 IHC Score	1.21 (0.87), 1	0	3	291
LV Invasion				323
Present	68 (21.1)	NA	NA	
Absent	255 (78.9)	NA	NA	
KI67 Classification				169
Low	66 (39.1)	NA	NA	

Moderate High Very High	50 (29.6) 34 (20.1) 19 (11.2)	NA NA NA	NA NA NA	
KI67 Index	23.10 (21.52), 15	1	90	168
Oncotype DX Recurrence Score	18.26 (9.76), 18	0	63	160

Table 13 Pathologic Tumor Features By Cohort

	Chemotherapy/Anastrozole				Anastrozole Only (n=212)				p-value
Tumor Characteristics	Mean (SD), Median or n (%)	Min.	Max.	n	Mean (SD), Median or n (%)	Min.	Max.	n	
AJCC Tumor Stage				117				212	<0.001
Stage I	43 (36.8)	NA	NA		171 (80.7)	NA	NA		
Stage IIA	41 (35.0)	NA	NA		34 (16.0)	NA	NA		
Stage IIB	17 (14.5)	NA	NA		7 (3.3)	NA	NA		
Stage IIIA	16 (13.7)	NA	NA		0 (0)	NA	NA		
Tumor Size (cm)	2.32 (1.91), 1.80	0.4	14	117	1.30 (1.06), 1.10	0.1	10	211	<0.001
Aggregate Tumor Size (cm)	2.50 (2.00), 1.90	0.4	14	117	1.41 (1.17), 1.10	0.1	10	211	<0.001
Tumor Classification				117				212	<0.001
T1a	2 (1.7)	NA	NA		35 (16.5)	NA	NA		
T1b	19 (16.2)	NA	NA		63 (29.7)	NA	NA		
T1c	48 (41.0)	NA	NA		85 (40.1)	NA	NA		
T2	38 (32.5)	NA	NA		27 (12.7)	NA	NA		
T3	10 (8.5)	NA	NA		2 (0.9)	NA	NA		
Lymph Node				117				208	<0.001
Positive	62 (53.0)	NA	NA		18 (8.7)	NA	NA		
Negative	55 (47.0)	NA	NA		190 (91.3)	NA	NA		
Number of Positive Nodes	1.01 (1.55), 0	0	8	117	0.10 (0.344), 0	0	2	212	<0.001
Tumor Focality/Centricity				117				212	0.876
Single	98 (83.8)	NA	NA		179 (84.4)	NA	NA		
Multiple	19 (16.2)	NA	NA		33 (15.6)	NA	NA		
Tumor Laterality				117				212	0.203
Right breast	47 (40.2)	NA	NA		102 (48.1)	NA	NA		
Left breast	70 (59.8)	NA	NA		110 (51.9)	NA	NA		
Tumor Location Octant				115				208	0.915
Upper outer	44 (38.3)	NA	NA		81 (38.9)	NA	NA		
Lower outer	9 (7.8)	NA	NA		19 (9.1)	NA	NA		
Lower inner	6 (5.2)	NA	NA		15 (7.2)	NA	NA		
Upper inner	19 (16.5)	NA	NA		23 (11.1)	NA	NA		

Upper junction	11 (9.6)	NA	NA		27 (13.0)	NA	NA		
Lower junction	6 (5.2)	NA	NA		11 (5.3)	NA	NA		
Outer junction	12 (10.4)	NA	NA		18 (8.7)	NA	NA		
Inner junction	3 (2.6)	NA	NA		6 (2.9)	NA	NA		
Retroareolar	5 (4.3)	NA	NA		8 (3.8)	NA	NA		
Tumor Location Quadrant				115				208	0.76
Upper outer	55 (47.8)	NA	NA		108 (51.9)	NA	NA		
Lower outer	21 (18.3)	NA	NA		37 (17.8)	NA	NA		
Lower inner	12 (10.4)	NA	NA		26 (12.5)	NA	NA		
Upper inner	22 (19.1)	NA	NA		29 (13.9)	NA	NA		
Retroareolar	5 (4.3)	NA	NA		8 (3.8)	NA	NA		
Invasive Type				117				211	<0.001
Ductal	106 (90.6)	NA	NA		179 (84.4)	NA	NA		
Lobular	11 (9.4)	NA	NA		24 (11.4)	NA	NA		
Ductal & Lobular	0	NA	NA		8 (3.8)	NA	NA		
Nottingham Score	6.71 (1.32), 6	4	9	117	5.66 (1.13), 6	3	9	199	0.075
Nottingham Grade				117				199	<0.001
Grade 1	18 (15.4)	NA	NA		77 (38.7)	NA	NA		
Grade 2	60 (51.3)	NA	NA		111 (55.8)	NA	NA		
Grade 3	39 (33.3)	NA	NA		11 (5.5)	NA	NA		
ER Status				117				211	0.016
Positive	113 (96.6)	NA	NA		211 (100)	NA	NA		
Negative	4 (3.4)	NA	NA		0 (0)	NA	NA		
ER H-Score	237.73 (79.13), 270	0	300	112	267.68 (42.40), 280	80	300	199	0.001
PR Status				117				211	0.001
Positive	93 (79.5)	NA	NA		195 (92.4)	NA	NA		
Negative	24 (20.5)	NA	NA		16 (7.6)	NA	NA		
PR H-Score	104.71 (101.17), 91	0	300	112	267.68 (42.40), 280	80	300	199	0.001
HER2 Status				113				205	<0.001
Positive	19 (16.2)	NA	NA		9 (4.4)	NA	NA		
Negative	94 (83.2)	NA	NA		196 (95.6)	NA	NA		
HER2 IHC Score	1.47 (0.89), 1	0	3	107	1.07 (0.82), 1	0	3	184	<0.001
LV Invasion				115				208	<0.001
Present	47 (40.9)	NA	NA		21 (10.1)	NA	NA		
Absent	68 (59.1)	NA	NA		187 (89.9)	NA	NA		
KI67 Classification				60				109	0.004
Low	19 (31.7)	NA	NA		47 (43.1)	NA	NA		
Moderate	15 (25.0)	NA	NA		35 (32.1)	NA	NA		

High Very High	12 (20.0) 14 (23.3)	NA NA	NA NA		22 (20.2) 5 (4.6)	NA NA	NA NA		
KI67 Index	31.62 (27.03), 22.5	2	90	60	18.37 (16.03), 15	1	80	18	0.001
Oncotype DX Recurrence Score	26.82 (10.09), 25	9	63	49	14.48 (6.79), 15	0	29	111	<0.001

Using the three-group comparison (L_A vs. L_A/L_G +S/L_A vs. S+S/L_G + L_G), we found that participants with one copy of the L_A-allele had lower BDI-II scores at baseline (b=-1.641, p=0.029; $\bar{x}$=4.27, SD=4.1, Median=3.5), six months (b=-1.84, p=0.038; $\bar{x}$=4.96, SD=4.72, Median=3.5), and 12 months (b=-2.276, p=0.017; $\bar{x}$=4.51, SD=4.49, Median=4) compared to participants homozygous for the L_A-allele (baseline: $\bar{x}$=5.88, SD=5.76, Median=4.5; 6-months: $\bar{x}$=6.89, SD=6.44, Median=5; 12-months: $\bar{x}$=6.93, SD=7.33, Median=5). A similar trend was noted for participants with no L_A-alleles at baseline (b=-1.129, p=0.182; $\bar{x}$=4.79, SD=5.08, Median=3), six months (b=-0.793, p=0.426; $\bar{x}$=5.93, SD=6.94, Median=4), and 12 months (b=-1.775, p=0.101; $\bar{x}$=5.01, SD=6.22, Median=3) compared to participants homozygous for the L_A-allele. Using the comparison of L_A vs. all other genotypes, we again found that participants homozygous for the L_A-allele had higher BDI-II scores at baseline (b=-1.459, p=0.037; $\bar{x}$=5.88, SD=5.76, Median=4.5) and at 12 months post-baseline (b=-2.098, p=0.019; $\bar{x}$=6.93, SD=7.33, Median=5) compared to all other genotypes (baseline: $\bar{x}$=4.46, SD=4.47, Median=3; 12 months: $\bar{x}$=4.69, SD=5.16, Median=3).

There were no statistically significant differences in mean BDI-II score between subjects with the different BDNF genotypes throughout the study. Subjects with at least one Met allele had consistently higher mean BDI-II scores at all timepoints (baseline: $\bar{x}$=4.91, SD=5.04, Median=4; 6-months: $\bar{x}$=5.9, SD=6.19, Median=4; 12-months: $\bar{x}$=5.48, SD=5.82, Median=4) compared to subjects with Val/Val (baseline: $\bar{x}$=4.76, SD=4.73, Median=3; 6-months: $\bar{x}$=5.57, SD=5.63,

Median=4; 12-months: $\bar{x}$=5.12, SD=5.93, Median=4) though these differences were not significant. In addition, the additive model for BDNF genotype as a predictor for depressive symptoms was not significant.

At baseline, a high Ki67 classification was associated with fewer depressive symptoms (*b*=-2.543, *p*=0.035; $\bar{x}$=3.88, SD=3.78, Median=3) compared to a low Ki67 classification ($\bar{x}$=6.36, SD=5.88, Median=5) At six months post-baseline, a multifocal tumor (*b*=2.479, *p*=0.012; $\bar{x}$=8.09, SD=9.36, Median=5) was associated with increased depressive symptoms compared to a single focus tumor ($\bar{x}$=5.68, SD=5.14, Median=5). Also at six months post-baseline, as HER2 classification score increased (*b*=1.079, *p*=0.02), BDI-II score increased. At 12 months post-baseline, tumor stage was a significant predictor of depressive symptoms. Subjects with tumor stages T1b (*b*=-3.669, *p*=0.029; $\bar{x}$=5.15, SD=5.07, Median=4), T1c (*b*=-3.292, *p*=0.041; $\bar{x}$=5.56, SD=5.66, Median=4), and T2 (*b*=-3.762, *p*=0.036; $\bar{x}$=5.12, SD=4.9, Median=3.5) had significantly lower BDI-II scores compared to subjects with T1a ($\bar{x}$=8.79, SD=12, Median=4). At face value, this suggests that patients with an earlier tumor stage experience increased depressive symptoms. HER2-positive subjects had higher BDI-II scores (baseline: $\bar{x}$=6.64, SD=6.89, Median=5; 6-months: $\bar{x}$=8.91, SD=9.48, Median=6.5; 12-months: $\bar{x}$=7.06, SD=10.77, Median=4.5) than HER2-negative subjects (baseline: $\bar{x}$=5.28, SD=5.53, Median=4; 6-months: $\bar{x}$=5.85, SD=5.64, Median=5; 12-months: $\bar{x}$=5.68, SD=6.17, Median=4) across all three timepoints, but these differences were not statistically significant.

7.0 Discussion

The significant association between the L_A-allele and increased depressive symptoms in this study is consistent with the previous study conducted by University of Pittsburgh School of Nursing faculty with a smaller sample size (n=125). Our results suggest that the L_A-allele may be a risk allele for depressive symptoms in women with breast cancer and healthy controls. However, the converse is not supported by the data; in other words, having no copies of the L_A-allele was not a protective factor. Interestingly, there was not a significant difference in depressive symptomology between the group with two copies of the L_A-allele and the group with no copies of the L_A-allele. This may suggest that an extreme in serotonin transporter activity in either direction (high or low) may be a risk factor for the development of depressive symptoms. Another possible explanation is that SERT genotype alone is not a reliable predictor of depressive symptoms and that there may be epigenetic influences involved. Lastly, mean BDI-II scores for all groups at all timepoints fall within the category of minimal depression, so differences may not be clinically significant.

Although there is evidence in the literature for associations between BDNF genotype and serum BDNF level, as well as between serum BDNF level and depressive symptoms, it seems more difficult to ascertain a relationship, if any exists, between BDNF genotype and depression. For example, in a study of patients receiving treatment for depression, there was a significant relationship between having at least one Met allele and having higher plasma BDNF levels. However, neither genotype nor plasma BDNF level were significantly associated with depressive symptoms or response to treatment, suggesting that these biological characteristics carry limited clinical implications for patients (43). Our study is consistent with this finding as well as with

another study of healthy older adults, in which no association was found between BDNF genotype and mood status, including depression (44). It is likely that the relationship between BDNF genotype and depression involves other factors that require further investigation, such as gender (35, 45), promoter methylation (46), other candidate genes, and negative life events (47).

There were no pathologic tumor features that were significantly associated with depressive symptoms across all three timepoints. We hypothesized that a possible difference in age among subjects with T1a compared to subjects with more advanced tumor stages could have contributed to the difference seen in severity of depressive symptoms. However, further testing showed that subjects of the different tumor stages did not differ significantly in age or years of education. Spaghetti plots allowed us to identify two subjects in the anastrozole-only group with stage T1a tumors and BDI-II scores at 12 months post-baseline categorized as severe (score > 28). Follow-up influential point regression analysis excluding these two subjects did not result in statistical significance, indicating that our original result was a direct effect of these two influential points. Thus, we cannot conclude any association between tumor stage and depressive symptoms in women with breast cancer.

However, our results are similar to those demonstrated in the Koleck et al. (2017) study even though they were not constant over time. Koleck et al. (2017) demonstrated that having a tumor with a moderate Ki67 classification contributed more favorably to cognitive function than a low classification. Similarly, we found that a high classification was associated with fewer depressive symptoms compared to a low classification. A study of the basolateral complex of the amygdala in rats found a negative relationship between cellular proliferation (measured by an assay for Ki67 expression) and depression-like behavior, modulated by anxiety level, which could support an association between increased Ki67 expression and reduced depressive symptoms (48).

Subjects with multifocal tumors and increased HER2 classification had higher depressive symptoms, which is consistent with associations between these features and poorer cognitive performance. In addition to HER2 receptor, the family of ERBB genes encodes for receptor tyrosine kinases, which are activated by proteins called neuregulins (NRG). The network of NRG-ERBB interactions has been implicated in nervous system development and has also been proposed to influence psychiatric disorders such as schizophrenia, bipolar disorder, and depression (41).

8.0 Limitations

A main limitation of this study was a lack of variability in BDI-II score, with the majority of participants having minimal depressive symptoms. After categorizing participants into clinically meaningful groups by BDI-II score to mitigate the effect of the skew, our groups were still unevenly distributed. In this way, we chose to treat BDI-II score as a continuous variable. Another limitation is that the chemotherapy-only cohort of participants with breast cancer was not included in analysis due to a small sample size (n=15). In addition, exclusion criteria for participation did not include pre-morbid depression and this may have contributed to the few influential points and high BDI-II scores identified during follow-up analysis.

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