University of California, Irvine

Life Events, Perceived Stress and Breast Cancer Risk in the Hereditary Breast and Ovarian Cancer Study of Orange County, CA

Dissertation

Submitted in partial satisfaction of the requirements
for the degree of

Doctor of Philosophy

in Epidemiology

by

Avital Sabrina Fischer

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Dedication

To my mother Brenda Geiger, who has always inspired me, loved me and nourished my curiosity. To my father, Michael Fischer, who supported me and encouraged my success. To my sisters Adina Fischer, who paved the way toward becoming a women physician scientist, Danielle Fischer, who encouraged my artistic inclination, Arielle Fischer, who taught me patiently how to think and question the world while gently pushing me forward and Eliana Fischer, who has shown me how to face life with a smile and enchanting optimism through love and friendship.

I would like to also dedicate this work to Wadie Najm, M.D., who has been for an amazing and caring mentor and to all the people I know struggling with treatments and uncertainty, to survivors who are courageously continuing their lives and to their families and friends who have supported, cared and loved them during a shared struggle toward the triumph over cancer.

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Presentations:

- *Antidepressant Use, Duration of Use and Breast Cancer Risk,* Fischer, A., Ziogas, A., Jiang, L., Anton-Culver, H., Oral Presentation, 76th American Psychosomatic Society Annual Meeting, *March, 2018,* Louisville, KY

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- *The Importance of Stress Perception in the Relationship Between Life Events and Breast Cancer Risk,* Oral Presentation, UCI Medical Scientist Training Program Conference, *October, 2017,* Lake Arrowhead, CA

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- *Perceived Stressfulness of Life Events Impacts Breast Cancer Risk.* Fischer, A., Ziogas, A., Anton-Culver, H. Poster presentation, American Association of Cancer Research Annual Meeting, *April, 2017,* Washington, DC

- *The Promoting Effect of Negative Valence Life Events in Breast Cancer Risk* Fischer, A., Ziogas, A., Anton-Culver, H. Poster presentation, American Psychosomatic Society Annual Meeting, *March, 2017,* Seville, Spain.

- *The Story of a Third Year Medical Student,* Fischer, A., Shapiro, J., Poster presentation, Medical Humanities Symposium, *January, 2017,* UC Irvine School of Medicine.

- *Life Event Valence and Breast Cancer Risk*. Fischer, A., Ziogas, A., Anton-Culver, H. Oral and poster presentations at West Regional Conference of American Association of Physician Scientists *December, 2016,* Keck School of Medicine, USC.
- *Life Events and Breast Cancer Risk in the Hereditary Breast and Ovarian Cancer (HBOC) Study.* Fischer, A., Anton-Culver, H. Oral presentation, Annual UCI Medical Scientist Training Program Conference, *October, 2016*
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Dissertation Abstract

Life Events, Perceived Stress and Breast Cancer Risk in the Hereditary Breast and Ovarian Cancer Study of Orange County, CA

By

Avital Sabrina Fischer

Doctor of Philosophy in Epidemiology

University of California, Irvine, 2018

Professor Hoda Anton-Culver, Chair

The presented doctoral work explores the relationship between psychosocial stress in the form of life events (LEs) and breast cancer risk. Life event valence and perception of life event stress were examined in an attempt to better quantify the effects of life events on breast cancer risk. Antidepressant medication use following LEs was used to estimate perturbations in stress physiology resulting from LEs. The population studied comprises 664 population-based incident, invasive primary breast cancer cases, 203 population-based controls and 156 sister controls that were part of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860). A case-control design was utilized to examine the distribution of life events among cases and controls. Unconditional and conditional logistic regression was used to calculate ORs and 95% CIs for breast cancer risk estimation associated with

LEs. This investigation identified stressful, negative valence later life events as important risk factors in future breast cancer risk. Pervious personal illness specifically stood out as a breast cancer risk factor. Early life exposures and genetics seem to influence susceptibility to the effects of later life events on breast cancer risk. Antidepressant medication could possibly attenuate the effects of life event stress on breast cancer risk. This research provides insight into the complex psycho-neuro-immunological interactions at play contributing to breast cancer onset. A more fine-tuned quantification of stress perception and physiological alterations are recommended. Future research is needed to explore the effects of major life stressors on breast cancer risk according to molecular subtype.

Chapter 1:

Introduction to The Stress-Breast Cancer Relationship

Specific Aims:

Breast cancer is the most common cancer in women. Despite advances in understanding breast cancer etiology, 60 percent of variability in breast cancer risk remains unexplained. Even with strong evidence from animal and cellular experiments indicating that stress signaling increases breast tumorigenesis, epidemiological evidence in human populations is inconclusive. Studies investigating the relationship between stress in the form of life events (LEs) and breast cancer risk are conflicted. Using epidemiological data from 664 population-based breast cancer cases, 156 unaffected sister controls and 203 population-based controls from the UCI based Hereditary Breast and Ovarian Cancer (HBOC) case-control study, I will delve into the relationship between stress and breast cancer risk. Understanding if there is a relationship between stress and breast cancer and the nature of this relationship will allow development of improved prevention efforts, both in the clinic through identifying women who experienced distressing LEs and pharmacologically by determining additional chemotherapeutic targets.

Aim 1: Determine the effect of specific and summary LE parameters on breast cancer risk.

- *Hypothesis 1: Salient negative valence undesirable LEs increase breast cancer risk in a dose-response fashion.*
- *Hypothesis 2: Positive valence, desirable LEs moderate increased breast cancer risk resulting from negative valence LEs.*

Possible mechanism: Stress resulting from negative LEs like death of a spouse will overwhelm the body's ability to manage the stress response and increase breast cancer risk, while positive valence LEs allow restoration of the HPA axis and immune system back to baseline.

Aim 2: Investigate if perceived 'stressfulness' of LEs influences the effect of LEs on breast cancer risk.

- *Hypothesis: Negative valence life events subjectively perceived as stressful (SLEs) will increase breast cancer risk, while negative valence LEs not perceived as stressful (NSLEs) will not influence breast cancer risk.*

Possible mechanism: The subjective experience of stress, as assessed by the individual, is hypothesized to be an informative indicator of disruptions of cortisol signaling and hence breast cancer risk.

Aim 3: Determine whether shared genetics and environment influence the relationship between LEs and breast cancer risk.

- *Hypothesis: The odds of breast cancer in cases compared to sister controls experiencing LEs will be different than compared to population-based controls* (as quantified in Aim 1).

Possible mechanism: Because of an average 50% shared genetics and common early life experience, adulthood LEs are hypothesized to have a different impact on sisters than on the general population that is not matched for early life experiences and genetic risk.

Aim 4: Determine if breast cancer cases are more likely to have initiated anti-depressant medication use prior to breast cancer diagnosis.

- *Hypothesis 1: Breast cancer cases are more likely to have initiated anti-depressant medication use prior to breast cancer diagnosis.*
- *Hypothesis 2: Cases will be more likely to initiate anti-depressant medication use in response to negative valence LEs.*

Possible mechanism: Because stress signaling is hypothesized to increase breast cancer risk, women who developed breast cancer are more likely to have a history of higher stress resulting in anxiety and/or depression treated with anti-depressant medication. Further, cases are hypothesized to have a more reactive stress response and therefore more likely to initiate anti-depressant medication (as a metric of stress quantification) in response to LEs/SLEs.

Answers to the above aims are expected to further our understanding of the complex mechanisms behind breast cancer pathogenesis. These efforts have the prospect of improving prevention by increasing our understanding of risk factors and enhancing identification of women at risk for breast cancer.

Background

The influence of psychological stress on breast cancer etiology has been studied for many years yet no firm conclusion has been reached. Animal and cellular research suggests a strong relationship between stress signaling and breast tumorigenesis. However epidemiological investigation into the relationship between life event (LE) stress and breast cancer has shown conflicting results. Some studies show a clear positive relationship between experiencing severe LEs and breast cancer risk and others have shown no association. Breast cancer is the most common female malignancy and carries considerable morbidity and mortality. There is a need to expand our understanding of breast cancer risk factors to better identify women at risk and improve prevention efforts and treatment options.

The motivation of this study is to investigate whether stress in the form of LEs influences breast cancer risk. Within the scope of this project, I will investigate if desirability/valence and perception of stressfulness of LEs influences their impact on breast cancer risk. Further, I will use 2 control groups (population-based controls and sister controls) to evaluate how shared genetics and environment influence the risk of breast cancer associated with LEs. Finally, I will evaluate the use of antidepressant medication in light of adverse LEs among breast cancer cases and population-based controls.

Breast cancer statistics

Other than skin cancer, breast cancer is the most common form of cancer in women. Breast cancer comprises approximately 30% of newly diagnosed female cancers. Approximately 266,120 new cases of invasive breast cancer, 63,960 cases of non-invasive (in situ) breast cancer diagnoses and 40,920 breast cancer deaths are estimated to occur in 2018.[1] Over the course of a woman's life, around 12% (1 in 8) will develop invasive breast cancer in the United States. Breast cancer is the leading cause of cancer mortality among women in developing countries and the second leading cause of cancer mortality among women in the United States.[2] Overall, breast cancer death rates have decreased since 1989. This decrease has been attributed to improvements in screening leading to early detection and advanced treatment options. Nevertheless, the high morbidity and mortality associated with breast cancer warrants further investigation.

Despite advances in understanding breast cancer risk factors such as reproductive variables, obesity, alcohol consumption and genetics, it is currently estimated that up to 60 percent of the variability in a women's breast cancer risk cannot be explained by these factors.[3] Approximately 85% of women develop breast cancer sporadically, with no family history of breast cancer.[4,5] Therefore the investigation of additional factors associated with breast cancer risk will expand our understanding of breast cancer etiology and is expected to lead to better methods of prevention, diagnostic tools and treatment options.

Estrogens and breast cancer

The relationship between estrogen exposure and breast cancer has been known for more than a century since bilateral oophorectomy caused remission of breast cancer in premenopausal woman.[6] The carcinogenic role of estrogens was further demonstrated by the use of hormone replacement therapy increasing breast cancer risk in the Women's Health Initiative Study.[7] Experiments have demonstrated the carcinogenic role of estrogen on breast tissue both via the estrogen receptor and through the action of estrogen metabolites. Estrogen causes proliferation of breast tissue and may promote the progression from normal cellular proliferation to hyperplasia and neoplasia.[8] By increasing the number of cell divisions estrogen increases the possibilities for DNA mutation leading to higher chances of tumorigenesis.[9] Further, estrogen metabolites are directly genotoxic.[10,11]

Established breast cancer risk factors

The most important risk factor for breast cancer is female sex. There is a 100:1 ratio between female to male breast malignancies. The second most important risk factor for breast cancer is age. With increasing age, breast cancer incidence rises and peaks around 75-80 years of age. Breast cancer is very rare among women aged <25 years of age. Mean age at diagnosis is slightly older for White women (61 years) as compared to Hispanic women (56 years) and African American women (46 years). The incidence of breast cancer is highest among non-Hispanic White women, followed by African Americans, Hispanics and the lowest among Asian/Pacific Islanders.[12]

Other than female sex and increasing age, among the most well established risk factors for breast cancer pertain to a women's cumulative exposure to estrogens manifesting in her reproductive history and exposure to exogenous hormones.[13] Earlier age at menarche, nulliparity, later age at first full-term pregnancy, and earlier menopause have been repeatedly associated with increased breast cancer risk.[14,15] Reproductive risk factors are particularly important when determining risk of developing hormone responsive (HR+) breast cancer, expressing either the progesterone receptor (PR) or estrogen receptor (ER).[16]

Women who have a first-degree relative (sister, mother, daughter) affected with breast cancer have increased risk of developing breast cancer, especially if the family member had early-onset breast cancer. However, among women with a family history of breast cancer, the majority will not develop breast cancer. It is therefore hypothesized that family history contributes to increased breast cancer risk because of an interaction between low-risk susceptibility genes and non-inherited factors.[17] Hereditary breast cancers occur in approximately 12% of the cases. Mutations in the *BRCA1* and *BRCA2* genes are responsible for 3% of breast cancers, the highest proportion attributable to a single genetic mutation. Other hereditary syndromes increasing breast cancer risk are Li-Fraumeni syndrome and Li-Fraumeni variant syndrome, which are caused by mutations in the tumor suppressor genes *p53* and *CHEK2* respectively. These genetic alterations together comprise approximately 2% of breast cancers.[12]

Other factors that increase breast cancer risk include atypical hyperplasia of the breast, higher breast density and chest radiation exposure.[12,17] Positive lactation history and longer duration of breast-feeding reduce breast cancer risk.[16,18] Premenopausal obesity decreases breast cancer risk, while post-menopausal obesity increases breast cancer risk. Women who are physically active likely have a minor reduction in breast cancer risk whereas heavy alcohol consumption may increase breast cancer risk. The influence of other dietary factors, tobacco and environmental toxins on breast cancer risk is less well established.[12]

Breast cancer molecular subtypes

Breast cancer is a heterogeneous disease with different molecular subtypes. Patient characteristics, risk factors, prognosis and treatment are somewhat different according to molecular subtype.[16] ER positive breast carcinomas are the most common. These tumors are characterized by a gene expression profile under control of the hormone estrogen. Over 95% of breast malignancies are adenocarcinomas, originating from glandular epithelial structures. Adenocarcinomas are further divided based on evasion of the basement membrane to in situ and invasive.[12] According to histological gene expression profiling, adenomcarcinomas of the breast are further divided into four main subtypes: luminal A, luminal B, HER-2 (human epidermal growth factor receptor) positive and triple-negative breast cancer (basal-like and normal breast-like).[19,20]

Luminal A breast cancers are hormone receptor positive and express the ER and/or PR and are also HER-2 receptor negative. Luminal B breast cancers are also hormone receptor positive and express the ER+ and/or PR+ with often expression of the HER-2

receptor.[21] Luminal A breast cancers are the most prevalent type of invasive carcinoma. These tumors are most commonly found in post-menopausal women, are usually moderately to highly differentiated and respond well to hormone-blocking treatments such as Tamoxifen and Raloxifene.[12] Luminal B breast cancers are less differentiated (higher grade) than luminal A breast cancer and have a worse prognosis. Because these tumors express HER-2, they can be treated with Herceptin, a monoclonal antibody targeting the HER-2 receptor. Tumors that lack all three receptors: ER, PR and HER-2 are classified as triple-negative breast cancers (TNBC).[22] These tumors are treated non-selectively with chemotherapy. However, the response to chemotherapy is often limited and these tumors have higher relapse rates than tumors expressing the ER or the HER-2 receptor.[23]

The physiological stress response

Psychological stress comes about when an individual is no longer able to cope with environmental demands.[24] Stressful life events influence biological and psychological processes and have the potential to increase disease risk and cancer risk in particular.[24,25] Perturbations in physical and psychological homeostasis lead to a state of stress culminating in activation of the HPA (hypothalamic pituitary adrenal axis) and SAM (sympathetic adrenal medullary) regulatory systems. The hypothalamus releases corticotropin-releasing hormone (CRH) that stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), subsequently triggering the adrenal cortex to secrete glucocorticoids (GCs). Cortisol, the main glucocorticoid in humans, elicits the physiological stress response by binding to the glucocorticoid receptor (GR).[26] Intricate

regulation of the stress response is important to reduce the potential pathological effects of unbalanced stress that could lead to physical or psychological illness.[27]

The response to stress is variable and depends on genetic and physiological factors as well as the individual's vulnerability and resiliency to stress, his/her coping skills and social support. There is strong evidence linking genetic predisposition to stress reactivity and depression onset.[28] From twin studies, it is estimated that depression risk is around 40% heritable.[29] First-degree relatives of those affected with major depressive disorder are at 2.84 times the risk of depression compared to first degree relatives of healthy controls.[30] Research has shown that there are shared genetics contributing to both depression and cancer risk. Specifically, aberrant regulation of the Ras proto-oncogene has been observed to decrease seratonergic and dopaminergic signaling, which are important for mood regulation and motivation.[31]

The glucocorticoid receptor in mammary epithelial cells

In response to emotional and physiological stressors GCs are secreted from the adrenal gland and are responsible for physiological homeostasis by regulating metabolic, cardiovascular and immune function.[32] Cortisol plays an important role in normal mammary development, differentiation,[33] and proliferation during pregnancy.[34] The GR is a nuclear receptor transcription factor that mediates the effects of glucocorticoids on homeostasis and gene expression. Consequently, alterations in the GR have the potential to alter cellular proliferation and differentiation, which may influence tumorigenesis of mammary epithlium.[35]

A recent study identified GR nuclear expression in approximately 62% of breast malignancies.[36] The importance of the GR in normal breast biology and expression in breast tumors supports the epidemiologic investigation into the role of altered stress signaling in breast cancer etiology.[37] In the mammary gland, glucocorticoids and estrogens are thought to have opposing influences on cellular proliferation. In general, estrogens increase cellular proliferation, whereas, glucocorticoids inhibit cellular proliferation.[38] However, glucocorticoids have also been observed to mediate mammary epithelial tumor cell survival via inhibition of apoptosis.[39,40] This is in contrast to the pro-apoptotic effects of glucocorticoids on lymphocytes.[41] Further, cortisol interferes with P53 and thus cell cycle surveillance.[42,43] Therefore, genetic variants of the GR gene (*NR3C1/GRL)* may contribute to cellular transformation. Consistently, breast cancer risk was observed to increase with increasing numbers of *NR3C1/GRL* repeats.[44]

Epidemiology of stress and breast cancer risk

Results from epidemiological studies focusing on the stress-breast cancer relationship are conflicting. I will go over some specific studies to illuminate differences in study design, stress quantification methodology and results. A Swedish prospective cohort study published in 2003 found a positive association between everyday stress and breast cancer. In this study, 1462 women aged 38-69 were followed for 24 years. A two-fold increase in breast cancer risk was observed for women experiencing more than occasional everyday stress defined as: irritability, tension, nervousness, etc., compared to women who had occasional or no stress in the 5 years preceding. This increase in

breast cancer risk was observed for breast cancer but not for total cancer, eluding to a possible specific effect of stress on breast cancer.[45]

Conversely, in a prospective cohort study of 6689 Danish women published in 2005 the relationship between self-reported everyday stress frequency and intensity and breast cancer risk showed a protective effect of stress on breast cancer. A decrease in breast cancer risk was observed for women in the high stress group compared to the low stress group (HR=0.60, 95% CI: 0.37-0.97). Additionally, an 8% reduction in breast cancer risk was observed for each point increase on a 6-point stress scale taking into account stress intensity and frequency.[46] These discrepancies in findings characterize the literature examining the stress-breast cancer relationship.

Life events and breast cancer epidemiology

Epidemiological studies focusing on life events for quantification of stress exposure have shown a stronger and more consistent association with breast cancer risk compared to studies that have focused on everyday stress.[47] However here too, epidemiological studies have come to varied conclusions regarding the role of life events in breast cancer pathogenesis (Table 1.1). Life events are comprised of an accumulation of ordinary life events such as pregnancy and marriage and severe life events such as death of a spouse.[48] The accompanying psychological and physiological stress and behavioral reactions to life events are believed to increase susceptibility to disease.

A 2012 case-control study found a significant positive association between severe/moderate life events and breast cancer risk. This study included 858 cases of invasive breast cancer and 1085 controls matched for age (ranging from 28-79) and location of residence in Poland. The cumulative effect of lifetime life events on breast cancer risk was explored. Severe/moderate life events included in the analysis were a subset of the Holmes and Rahe social readjustment scale.[49] Overall, significant severe/moderate life events individually increased the odds of breast cancer by approximately 2-fold. Women with a cumulative total of 4-6 major life events were at a 5.33-fold (95% CI: 4.01-8.21) increased risk of breast cancer compared to women reporting no major life events. Women with a total lifetime life event score of 210 or higher were at approximately 5 times the risk (OR=5.09; 95% CI: 3.41-8.50) of breast cancer compared to women having scores ranging from 0-70. A dose response relationship between number and cumulative score of lifetime life events and breast cancer risk was observed.[50]

In the Finnish Twin Cohort study 10,808 women were followed prospectively for 15 years between 1982-1996. An emphasis was placed on life events occurring in the 5 years preceding completion of the life event risk factor questionnaire. The multivariate adjusted HR for breast cancer per one life event was 1.07 (95% CI: 1.00-1.15). This risk was increased to 1.35 when including only major life events based on the Holmes and Rahe life event scale (death of a husband, divorce/separation, illness, death of a friend or relative and loss of job) and adjusting for daily stress, life satisfaction and neuroticism in addition to the commonly adjusted for covariates such as age, BMI, alcohol use, etc.

(HR=1.35; 95% CI: 1.09-1.67). Further, the following specific life events were individually significantly associated with elevated breast cancer risk: divorce/separation (HR=2.26; 95% CI: 1.25-4.07), death of a husband (HR=2.00, 95% CI: 1.03-3.88), and death of a close relative or friend (HR=1.36, 95% CI: 1.00-1.86).[48]

A case-control study performed in the Breast Cancer Screening Assessment Unit and surgical outpatient clinics at King's College Hospital in London examined life events and breast cancer risk among 41 malignant breast cancer cases and 78 controls with benign breast disease. The life events and difficulties schedule, a semi-structured interview, was used to evaluate life events. Subsequently, event severity was categorized on a 1-4 point scale. Breast cancer risk increased substantially after severe life events in the 5 years before diagnosis with an OR of 11.64 (95% CI: 3.10-43.66) after adjusting for breast cancer covariates.[51]

This study also explored if stress coping strategies influence how life events impact breast cancer risk. Among women who experienced severe life events and a more proactive coping style characterized by confronting the stress had an elevated breast cancer risk (OR=3.11, 95% CI: 1.18-8.19). This result highlights the variability in the effect of stress based on the individual's personal coping style. There were no differences in mean scores among cases and controls on a general health questionnaire administered. Therefore the increase in breast cancer risk for highly stressful life events is most likely not due to other known breast cancer risk factors and overall health discrepancies between cases and controls.[51]

Based on the above studies there is evidence supporting a significant positive relationship between life events and breast cancer risk despite the magnitude of effect varying among studies. Nevertheless, other studies have reached null findings. In a population-based case control study in Wisconsin that also used the Holmes and Rahe Scale (1967), no significant associations between life events in the prior 5 years and breast cancer risk were observed.[52] Similarly, another hospital-based case control study found no significant associations between life events and breast cancer risk. This study examined women from 3 medical clinics in Leeds, United Kingdom that received a diagnostic biopsy for a breast lump. Life events queried about occurred in the 5-year interval prior to clinical presentation. Women who received a negative histological report for cancer and hence had benign breast disease (n=226) were categorized as controls and those with a positive biopsy for cancer cells were categorized as cases (n=106). This study attempted to reproduce the increased odds of breast cancer for life events observed in Chen *et. al*, 1995 while adjusting for additional risk factors in a larger sample but failed to do so.[53]

In a recent meta-analysis from 2013 analyzing studies published from 1995-2012, the pooled OR across the 7 studies included was 1.51 (95% CI=1.15-1.97, P=0.003) for striking life events and primary breast cancer onset.[54] In another meta-analysis examining 27 studies published from 1966-2002, a significant positive association was reported for total life events (OR=1.77; 95% CI: 1.31-2.4) and separately for death of a spouse (OR=1.37; 95% CI: 1.10-1.71) and death of a relative or friend (OR=1.35; 95%

CI: 1.09-1.68).[55] Despite there being an overall 35-77% increase in breast cancer risk for stressful life events, there was considerable heterogeneity among studies. Some of the potential sources of variation in study outcomes stemmed from differences in study design, population of study, control group selection, covariates adjusted for and stress quantification methodology.[55]

Author/year	Country	Design	Age	Tool	Sample	Time	RR (95% CI)
Chen, 1995	England	Case-control (suspicious lump)	20-69	LEDS	41/78	Previous 5 years	7.08 (2.31-21.65)
Roberts, 1996	U.S.	Case-control (population-based)	50-79	**Holmes & Rahe**	258/614	Previous 5 years	0.9 (0.78-1.05)
Protheroe, 1999	Australia	Case-control (suspicious lump)	40-79	LEDS	106/226	Previous 5 years	0.91 (0.47-1.81)
Kruk, 2012	Poland	Case-control	28-79	**Holmes & Rahe**	858/1085	**Lifetime**	5.09 (3.41-8.50)
Helgesson, 2003	Sweden	Prospective	38-60	Perceived stress (dichotomous)	1462	Previous 5 years	2.1 (1.2-3.7)
Lillberg, 2003	Finland	Prospective	>24	**Holmes & Rahe**	10,808	Previous 5 years	1.07 (1.00-1.15)
Michael, 2009	U.S.	Prospective	50-79	**Holmes & Rahe**	84,334	Previous 1 year	1.12 (1.01-1.25)

LEDS: Life Events and Difficulties Schedule: Semi structured interview
Holmes & Rahe: uni-directional scale quantifying amount of change

Table 1.1 Epidemiological studies examining the relationship between life events and breast cancer risk. Light green: case-control study design, Darker green: prospective cohort study design, Orange: significant positive associations between life events and breast cancer risk, Bolded: Use of the most common epidemiological life event scale, the Holmes and Rahe Scale (1967).

Epidemiological quantification of life event stress

When assessing the effect of LEs on breast cancer risk in epidemiological research studies have generally focused on severe LEs measured by the Holmes and Rahe Social Readjustment Rating Scale (1967)[48,50,52] or the Life Events and Difficulties Schedule (LEDS) (1978).[51,53] The Holmes and Rahe scale (1967), which is used in the majority of studies, uses a marriage-normalized life change scale to quantify life change and hence stress resulting from LEs.[49] The LEDS is a semi-structured interview where participants are asked about contextual information surrounding the reported life events. Life event dictionaries are then used to determine contextual threat and severity rating for each event.[56]

The LEDS attempts to account for the positive (desirable) or negative (undesirable) nature i.e. valence of LEs. Yet, a major limitation of the commonly used Holmes and Rahe (1967) Social Readjustment Rating Scale is that all events are weighted on a unidirectional dimension. Every event is considered to increase disease risk with no consideration for the predictability or desirability of the event. Vinokur and Selzer (1975) have argued that the desirability of events is a critical factor in determination of the amount of stress resulting from LEs.[57] Consistently, negative valence life events are more consistently found to increase disease risk. Nevertheless, the role of more positive valence LEs in modifying or contributing to stress is still unclear.[58] Therefore, within the scope of Aim 1 of this doctoral work, I address whether valence of salient life events influences the association between life events and breast cancer risk.

Hoberman and Cohen (1983) also suggested that positive or desirable LEs may serve as a buffer, moderating the effects of undesirable/negative life event stress by giving the body time to restore its natural homeostasis.[59] This buffering effect has been observed in subjects with depression, psychological disorders and somatic symptomology such as headache.[59–61] Nevertheless these studies were limited in scope since they primarily focused on daily positive valence events while neglecting to include more salient positive valence LEs such as marriage and childbirth. Although the epidemiological evidence regarding the relationship of stress and breast cancer is mixed, there is a substantial amount of information from molecular, cellular and animal experiments supporting a causal mechanism behind psychological stress and breast cancer risk.

Potential mechanisms linking psychological stress and breast cancer etiology

Chronic cortisol exposure predisposes to various medical conditions including cancer.[62] Psychological stress induces sympathetic nervous system activation leading to acetylcholine release on the adrenal medulla culminating in epinephrine and norepinephrine release. Animal models of breast cancer have demonstrated that prolonged stress contributes to breast carcinogenesis.[63] The role of the GC/GR interaction in normal breast biology supports the epidemiologic investigation into the role of stress in breast cancer etiology.[37]

Consistently, the GR participates in anti-apoptotic pathways in breast cancer cell lines. Through the GR, NFKB is activated and stimulates inhibitors of apoptosis (IAPs).[40] Glucocorticoids interfere with P53 and thus cell cycle surveillance.[42,43] Stress signaling

impairs DNA repair capabilities and causes direct DNA damage. Rats exposed to stress in the form of inescapable foot shock and forced swims showed an increase in sister chromatin exchange, increasing the chance of mutation and cancer.[64] Murine 3T3 cells treated with cortisol, epinephrine or norepinephrine showed a 5-fold increase in DNA damage and compromised ability to repair DNA damage caused by ultra-violet radiation.[65]

The carcinogenic properties of the stress hormones cortisol, epinephrine and norepinephrine have been demonstrated in cell culture and animal models.[63,64,66,67] Cortisol shifts the immune response from Th1 to Th2 dominated. Decreased IFN-gamma production and a decline in natural killer cells have also been observed in response to increased HPA axis signaling. These alterations impair the ability of the immune system to identify and neutralize cancer cells.[68,69] With prolonged stress, tissues become insensitive to cortisol regulation contributing to an inflammatory state.[70] This state is characterized by increased cell proliferation and elevated reactive nitrogen and oxygen species that contribute to DNA damage, dysplasia and resultant neoplasia.[71] Consistently, the pro-inflammatory cytokine Interleukin-6 (IL-6) is elevated in various cancers.[72] Recently, blocking the effects of chronic stress in mice models by antagonizing the ß-adrenergic receptor was shown to protect against breast tumor dissemination through lymphatic spread.[73]

Alterations in the HPA axis have been observed years after stressful life event exposure. In a mouse model of stress, even a one-time exposure to inescapable foot

shock increased HPA axis sensitization and responsiveness to subsequent stressful situations.[74] Additionally research shows a direct relationship between a history of traumatic childhood experiences such as physical and emotional abuse and an increased production of ACTH.[75] Early stressors in conjunction with the experience of traumatic life events in adulthood were shown to most substantially increase HPA axis reactivity.[76] Therefore, within the scope of this project, I will investigate the cumulative effect of lifetime life events on future breast cancer risk.

Conclusion

The relationship between psychological stress and breast cancer etiology remains unclear. The biological plausibility of the association between severe, prolonged psychological stress and cancer risk warrants continued research. Evidence of elevated GR expression in breast malignancy implies the importance of stress signaling in breast cancer specifically. Improving our understanding of the interaction between psychosocial exposures and physiological processes in relation to breast cancer pathogenesis is expected to allow a more individualized view of breast cancer risk and women's health in general. Insight into the role of stress physiology in breast cancer etiology has could lead to improved screening, prevention and treatment approaches.

Project Overview:

Currently medical research and clinical practice is shifting toward a personalized approach. The precision medicine initiative takes into account a person's unique genetics, lifestyle and environment - components that are addressed in this project.[77] Establishing the impact of personalized risk factors such as exposure to stressful life events and its relationship to breast cancer etiology will improve our understanding of the interaction between psychological, neurological and endocrinological components in breast cancer pathogenesis. Such an understanding is expected to allow for better quantification of individualized breast cancer risk and more targeted preventive screening measures.

The first goal of this study is to examine the impact of life events that could be of positive or negative valence on breast cancer risk. Past studies investigating the relationship between life events and breast cancer risk showed conflicting evidence and for the most part did not specifically address life event valence and therefore it is important to continue to investigate. Furthermore, most of the studies have focused on negative life events. Yet, to my knowledge, no study has examined the differential impact of negative and positive stress as quantified by salient LEs in the etiology of breast cancer.

Secondly, the present research will analyze the data for all life events (whether perceived as stressful or not) and separately analyze those events that are perceived as

stressful. This is a novel approach to analysis of life event data in the context of breast cancer epidemiology, results of which will illuminate the importance of perceived stressfulness of life events as a marker of HPA axis dysfunction.

Further, I will investigate the influence of shared early environment and genetics on the association between stress and breast cancer by evaluating life events among cases compared to population-based controls and matched sister controls. Given that sister controls share similar environment and on average 50% of their genetic makeup, these analyses provide valuable information regarding the role of genetics and environment in the stress-breast cancer relationship.

The last and fourth goal of the proposed research is to determine whether breast cancer cases are more likely to have initiated an antidepressant medication prior to reference age. As mentioned above, one of the difficulties in measuring stress in human populations is that people respond differently to stressful events. Anti-depressant medication use will be used as a measurable indicator of elevated stress signaling which is increased in anxiety and depression. By analyzing whether breast cancer patients are more likely to have initiated anti-depressant medication use in light of stress in the form of life events, this study will examine the hypothesis that an overactive stress response predisposes to malignancy.

General Methods:

Data Source:

This study utilizes the Hereditary Breast and Ovarian Cancer (HBOC) dataset, which includes extensive information regarding epidemiological risk factors pertaining to environmental, behavioral and genetic characteristics. This rich dataset is expected to allow a comprehensive understanding of how life events influence breast cancer risk while controlling for known breast cancer risk factors.

Study Population:

Within the scope of the Hereditary Breast and Ovarian Cancer (HBOC) study (CA58860), a family registry of ovarian and breast cancer was established to investigate genetic and environmental contributions to breast cancer risk as well as gene-environment interactions.[78,79] The population included in this database is comprised of population-based incident, invasive primary breast cancer cases, population-based controls and sister controls that were part of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860).[78,79] The breast cancer cases were identified through the population-based cancer registry of the Cancer Surveillance Program of Orange County (CSPOC). Breast cancer cases included in the analyses were consecutive incident cases aged 24-75 years old that were diagnosed in Orange County, California, between March 1st, 1994 and February 28th, 1995. Physicians were notified about their patients being contacted to participate in the study. Each patient received an introduction letter and informed consent was obtained. Cases and controls were asked to answer an epidemiological risk factor questionnaire (RFQ)

that included extensive information regarding personal, social, medical and family history in addition to a section querying about life events.

One thousand and nineteen invasive breast cancer cases were eligible to participate in the study, out of which 809 completed the RFQ (79%). Seven hundred and nineteen breast cancer cases that completed the RFQ were in the age range of 24-75 years. Six hundred and sixty four of these women completed the Life Event section and were included in the present analyses (92%). Most cases (94%) completed the questionnaire within 3 years of breast cancer diagnosis. Six hundred twenty nine cases (95%) had information about antidepressant medication use prior to age at diagnosis.

Given the format of the RFQ changed midway through the study to no longer include a Life Event section, the number of controls for which we had information about LEs was smaller than for cases. Two hundred twenty six population-based controls in the appropriate age interval completed the RFQ, out of which 203 completed the LE section and were included here (90%). One hundred fifty six unaffected older sister controls completed the life events section of the RFQ and served as the sister control group. One hundred and ninety seven controls (97%) had information about AD medication use prior to reference age (Figure 1.1).

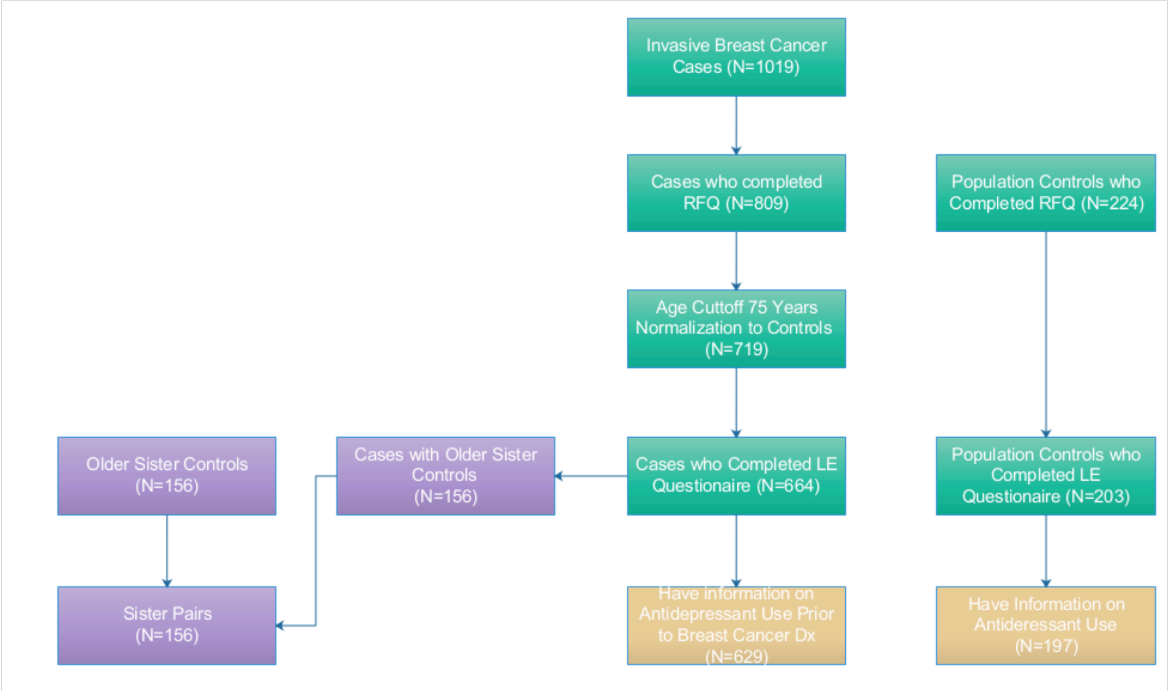


Figure 1.1: Study sample inclusion diagram: Green: Aims 1,2; Purple: Aim 3; Yellow: Aim 4

Study Parameters and Measures:

- *Age.* Continuous variable: age at diagnosis for cases and the age at RFQ completion for controls.
- *Age at life event.* Categorical variable: <20, 20-29, 30-39, 40-49, 50-59, and 60+ years old.
- *Smoking history.* Binary variable: 'ever smoked' and 'never smoked'. Ever smoked includes both current and past smokers.
- *Alcohol use.* Binary variable: 'none' and 'any'. Alcohol use will be determined by alcohol consumption in one year prior to year at diagnosis for cases and date of completion of RFQ for controls.

- *Physical activity.* Categorical variable. Physical activity in the previous year: not active, moderately active, and very active.
- *BMI.* Categorical variable based on NIH standardized BMI categories: underweight (BMI<18.5), normal weight (18.5≤BMI≤24.9), overweight (25≤BMI≤29.9) and obese (BMI≥30). Determined based on adult weight in kg and height in meters (BMI=kg/m^2) at RFQ completion.
- *Race/Ethnicity.* Categorical variable: White (European ancestry), Hispanic, Black (African), Asian and other.
- *Education.* Binary variable: less than college and some college or more.
- *Family history (FH).* Binary variable: yes or no family history of at least one 1^{st} degree female relative (sister, mother, daughter) with breast cancer
- *Hormone replacement therapy (HRT).* Binary variable: 'ever use' and 'never use'.
- *Age at menarche:* Categorical variable: less than 11, 12-13, and 14+.
- *Age at first full term pregnancy (FFTP):* Categorical variable: 0 (for nulliparous), <25, 25-29 and 30+.
- *Parity:* Binary variable: nulliparous, parous.
- *Menopausal Status:* Binary variable: Pre/perimenopausal, post-menopausal.
- *Anti-depressant medication use.* Binary Variable: 'never used', and 'ever used' (including both past and current use). Age at start of anti-depressant usage and number of years of antidepressant use is available.
- *Depression/Anxiety Comorbidity Index:* medical conditions that could influence the risk of depression/anxiety and thus anti-depressant medication use will be summed to create a depression/anxiety comorbidity index. Included medical conditions:

adrenal gland conditions, arthritis, chronic bronchitis, diabetes, emphysema, epilepsy, gout, heart attack or angina, hepatitis, kidney disease, lupus, scleroderma, mononucleosis, osteoporosis, thyroid disease, tuberculosis, ulcerative colitis, and non-breast/ovarian cancer.

Occurrence/summary LE parameters of interest:

Life Events: LE measures in the RFQ were based on a subset of the Holmes and Rahe scale.[49] LEs from the Holmes and Rahe scale (1967) that were inquired about here were: 1) marriage, 2) death of a spouse, 3) death of an offspring, 4) death of a close person: sister, brother, relative or friend, 5) death of a parent, 6) job loss, 7) relocation, 8) separation/divorce, 9) foreclosure of a mortgage loan or bankruptcy, 10) pregnancy, 11) pregnancy of a child, 12) illness and 13) illness in the family. We also asked participants about a history of abortion, marriage of an offspring, and buying a house. Participants were asked whether specific LEs occurred prior to their breast cancer diagnosis or corresponding reference age for controls, defined as age at questionnaire completion.

The Paykel (1971) Life Event Scale was used to operationally define which events were more likely to be positive valence or negative valence. This scale included 61 life events that were rated on a scale of 0-20 for degree of distressfulness. In general, events in the top half of the scale were more distressing and hence operationally defined as having negative valence. Events in the bottom half of the scale were less distressing and more likely to be desirable and hence defined as having positive valence.[80] Events that were

inquired about but did not match the categorization of events of Paykel (1971) were abortion and divorce/separation. Because abortion could be induced or spontaneous and therefore have equivocal valence, it was not included as a positive or negative valence life event. Similarly, because divorce is more likely to have negative valence and separation not due to argument more of a positive valence, separation/divorce was not included as a positive or negative valence event.

Negative valence LEs. A subset of LEs that were identified as being more likely to be undesirable and unpredictable and therefore have negative valence were identified and analyzed separately. LEs included in this sum were: 1) death of a spouse, 2) death of an offspring, 3) death of a sister, brother, close relative or friend, 4) death of a parent, 5) job loss, 6) foreclosure of a mortgage loan or bankruptcy, 7) illness and 8) illness in the family.

Positive valence LEs. A subset of LEs that were identified as being more likely to be desirable and predictable and therefore have positive valence were identified and analyzed separately. LEs included in this sum were: 1) marriage, 2) marriage of an offspring, 3) relocation, 4) buying a house, 5) pregnancy, 6) pregnancy of a child.

Stressful/non-stressful life events. Participants were asked if the above life events, whether positive or negative, brought about a “moderate to severe” amount of stress. If “yes”, the life event was considered a ‘stressful life event’ (SLE), if “no”, the life event was considered a ‘non-stressful life event’ (NSLE). In a similar fashion described for

LEs, age interval at SLE/NSLE was determined. SLEs/NSLEs were analyzed using similar parameters used for LE analyses above: 1. *SLE/NSLE occurrence*, 2. *negative valence SLE/NSLE sum* 3. *positive valence SLE/NSLE sum*, and 4. *SLE/NSLE sum*. Only SLEs/NSLEs occurring before reference age were included in the analyses.

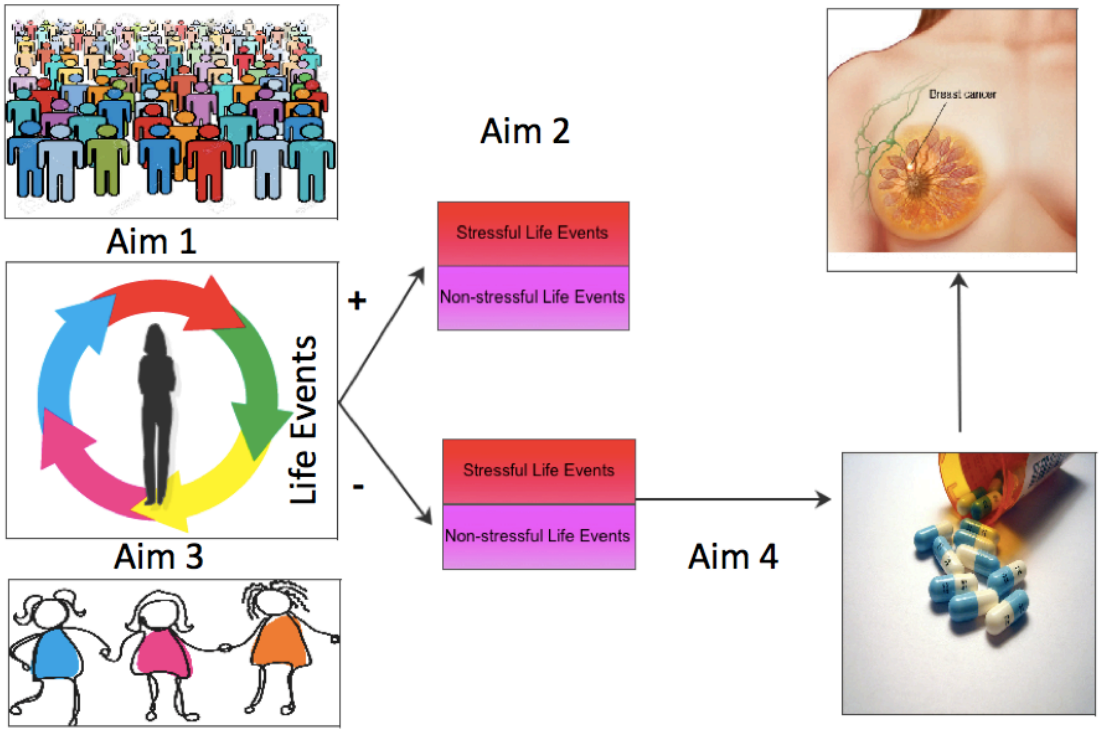
Anti-depressant ever use/duration of use. Participants who had a history of taking AD medication in the past or were taking AD medication at date of questionnaire completion were classified as *'ever users'* of antidepressant medication as compared to *'never users*' who had no history of AD use. The following intervals were used to evaluate *duration of AD use*: never use (baseline), $\leq$1 year and $\geq$2 years of use.

Data Analysis:

Will be described separately per each specific Aim (1-4) in the subsequent chapters (2-5).

Project Outline:

Figure 1.2: Graphical presentation of specific aims



Chapter 2:

Negative Valence Life Events Promote Breast Cancer Development

Abstract:

Background: The influence of stress on breast cancer risk remains unknown. The goal of this study was to determine the effect of stress in the form of salient positive and negative valence (+/-) life events (LEs) on primary invasive breast cancer risk. We hypothesized that salient (-) LEs increase breast cancer risk, while salient (+) LEs attenuate this increased risk.

Methods: A case-control design including 664 cases identified through the Cancer Surveillance Program of Orange County (CSPOC) and 203 population-based controls was used. Participants completed a risk factor questionnaire, which included a life event section. Fourteen salient LEs of positive or negative valence were used to quantify stress exposure. A baseline model was constructed and odds ratios (ORs) calculated using multivariate unconditional logistic regression.

Results: (-) LEs were associated with increased breast cancer risk. The OR for ≥4 (-) LEs showed a 2.81 fold increase in breast cancer risk (OR=2.81, 95% CI=1.47-5.36). A significant dose-response relationship between lifetime (-) valence LEs and breast cancer risk was found. Previous personal illness increased breast cancer risk by 3.6 fold (OR=3.60, 95% CI=2.50-5.20). Conversely, abortion was associated with a 45% decrease in breast cancer risk (OR=0.55, 95% CI=0.34-0.89). Salient (+) LEs did not have a significant effect on breast cancer risk. However, they seemed to buffer the adverse effect of salient (-) LEs on breast cancer risk.

Conclusion: This study supports the role of salient (-) LEs in promoting breast cancer development with a possible buffering effect of salient (+) life events.

Introduction:

Despite years of debate over the link between mind and body in breast cancer risk, this relationship remains unknown and is currently under active investigation.[37,81] Breast cancer patients continually express concern regarding psychological stress contributing to their breast cancer. A meta-analytical study (Dumalaon-Canaria *et. al*, 2014) reviewing the perceived causes of breast cancer in studies published from 1982-2012, showed that survivors reported stress as one of the main contributors to the development of their breast cancer[82]. Because breast cancer cases are more likely to remember past experiences and exposures that could explain their breast cancer, there is a strong possibility for recall bias. This study uses different time intervals when analyzing life events to best overcome this limitation.

The understanding of the intricate communication of the nervous system with the immune and endocrine systems is slowly unfolding. Through extensive molecular, cellular, animal and human research, the link between psychological and physiological stress and cancer initiation and progression is becoming apparent.[66] In vitro and in vivo experiments demonstrate a clear relationship between stress signaling and breast cancer pathogenesis (see Chapter 1 for further discussion). Animal models of breast cancer indicate that prolonged activation of the physiological stress response contributes to breast carcinogenesis.[63] Stress signaling impairs DNA repair capabilities and causes direct DNA damage.[64]

Epidemiological studies using life event (LE[1]) stress such as death of a spouse or serious illness as quantified measures of stress exposure have shown a stronger association with breast cancer risk in comparison to job or daily stress.[47] Nevertheless, the role of LEs in breast cancer pathogenesis remains ambiguous given that some studies indicated a direct relationship between a history of LEs and breast cancer risk,[48,50,51] while others found no such relationship.[52,53] A recent cohort study published in 2016 concluded that there is no relationship between adverse life events and breast cancer risk. However, this study had a relatively short period of follow-up (averaging 6 years), focused on events in the previous 5 years, did not inquire about cumulative lifetime events and included a relatively young cohort (average age of 46.6 years).[83] Another study that found a null association used hospital controls with a suspicious breast lump and therefore has limited generalizability.[53] An additional negative study examined the effect of individual LEs on breast cancer risk without examining the cumulative effect of these events.[52]

This case-control study utilizes a large sample of breast cancer cases and population-based controls and examines the effect of cumulative lifetime LE measures in addition to individual LEs on breast cancer risk, and includes information about LEs spanning 40+ years. Further, the present study aims at understanding how to better quantify stress resulting from LEs by addressing the importance of LE valence. Thus, the present study allows for a better understanding of the stress-breast cancer relationship by overcoming limitations of previous studies.

Our working hypotheses were that salient negative valence, undesirable LEs increase

breast cancer risk in a dose-response fashion, while positive valence, desirable LEs would moderate this increased risk. Positive valence life events are hypothesized to allow a break in the stress response and hence allow restoration of hormone levels and immune function back to baseline and therefore protect against the deleterious effects of negative, undesirable events on breast cancer risk.

Methods:

Study Population:

Six hundred and sixty three population-based incident primary invasive breast cancer cases and 203 population-based controls were part of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860) are included in the present analysis [78,79] (see Chapter 1 for more details).

Measures:

Four different LE parameters were used to evaluate the effect of LEs on breast cancer risk: (1) *LE occurrence:* (yes/no), (2) *negative valence LE sum:* 0 (baseline), 1, 2, 3 and 4+ events, (3) *positive valence LE sum:* 0 (baseline), 1, 2-3, 4-5 and 6+ events and (4) *total LE sum:* 0 (baseline), 1-3, 4-5, 6-8 and 9+ events. The grouping of the sum of positive/negative valence LEs and *total LE sum* was based on creating a zero event baseline and dividing the remaining distribution of events in the control group into quartiles (see Chapter 1 for more details).

In order to test the buffering hypothesis of positive valence salient LEs on the effect of negative valence LEs on breast cancer risk, they were analyzed together as *sum total LEs.* Abortion and separation/divorce were excluded from this sum because they were determined to be of equivocal valence and hence not easily categorized into positive/negative valence.

Data Analysis:

Descriptive statistics were calculated for cases and controls. For continuous variables, means and standard deviations were computed and for categorical variables, frequencies and percentages were computed. Covariates identified in the literature as breast cancer risk factors and candidates for baseline model inclusion were *age* (age at diagnosis for cases and the age at RFQ completion for controls), *smoking history (*ever smoked/never smoked), *alcohol use* (none/any), *BMI* (underweight [BMI<18.5], normal weight [18.5≤BMI≥24.9], overweight [25≤BMI≥29.9] and obese [BMI≥30]), *race/ethnicity* (non-Hispanic white [European ancestry], Hispanic, non-Hispanic black [African American], Asian and other), *education* (less than college/some college or more), *family history* (yes/no), determined based on family history of breast cancer in first degree relative, *hormone replacement therapy (HRT)* (ever use/never use), *age at menarche* (≤11, 12-13 and ≥14), *age at first full term pregnancy* (*FFTP):* (<25, 25-29 and ≥30)*, parity* (nulliparous/parous)*, menopausal status* (premenopausal/perimenopausal/post-menopausal), and *physical activity* (not active/moderately active/very active). A stepwise unconditional logistic regression selection process was used and goodness of

fit diagnostics were evaluated in selection of the baseline model used in the multivariate unconditional logistic regression.

Univariate analyses were performed on life event parameters with the dependent measure being breast cancer and the independent measures being individual *LE occurrence* parameters, *negative valence LE sum, positive valence LE sum* and *total LE sum.* Frequency tables and χ^2 statistics were used to compare *LE occurrence, negative valence LE sum, positive valance LE sum* and *total LE sum* among cases and controls. The influence of LE parameters on breast cancer odds ratios (ORs) was determined using simple unconditional logistic regression where ORs and 95% confidence intervals (CIs) were computed. Significant LE parameters were then included one at a time into the baseline multivariate regression model and adjusted ORs and CIs were determined based using multivariate unconditional logistic regression. The *negative valence LE sum* was also included in the logistic regression model as a continuous variable and Wald χ^2 statistics were used to test for trend.

To reduce the potential for recall bias and determine the influence of timing of LEs on breast cancer risk, two different analyses were performed. The first analysis included all lifetime events, including those in the decade of the reference age, to determine if LEs occurring in this decade influenced breast cancer risk. A probability weighting process was utilized when including LEs in the decade of reference age so that only events occurring prior to reference age would be included in the analysis since timing of LEs was based on ten-year time intervals (20-29, 30-39, etc.). To minimize the possibility of

information bias arising from cases over-reporting their recent breast cancer diagnosis as a severe life event, the second analysis included only LEs that occurred prior to the decade of reference age and therefore did not include the time frame at which the breast cancer diagnosis occurred. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results:

The distribution of demographics and characteristics among cases and controls are outlined in Table 2.1. Overall, cases were slightly older than controls with a mean age of 55.6 years for cases and 53.6 years for controls. Cases were also more likely to have a positive family history for breast cancer (24.7%) compared to controls (15.3%). Both cases (88.9%) and controls (87.7%) were predominantly of white ethnicity. Mean age at first full term pregnancy was significantly younger for controls (23.7) when compared to cases (25.2). Cases were more active than controls with 60.6% of cases compared to 49.3% of controls reporting being moderately or very active. There were no significant differences between cases and controls for the following parameters: mean BMI, age at menarche, parity, number of children, menopausal status, hormone replacement therapy, education level, smoking history and alcohol history.

Table 2.1: Distribution of demographics and other characteristics among 664 cases and 203 controls

Characteristic	Cases (n=664)		Controls (n=203)		*P* value
Reference age, years: mean, s.d.	55.6	10.9	53.6	12.3	0.039
BMI (kg/m2): mean, s.d.	25.8	5.3	26.5	5.7	0.134
Race/ethnicity: no. (%)					
White	590	88.9	178	87.7	0.124
Hispanic	38	5.7	19	9.4	
Black	2	0.3	1	0.5	
Asian/Pacific Islander	34	5.1	5	2.5	
Age at menarche: mean, s.d.	12.7	1.6	12.8	1.9	0.422
Age at first full-term pregnancy: mean,s.d.	25.2	5.0	23.7	4.7	0.001
Parity: no. (%)					
Nulliparous	107	16.1	32	15.8	0.865
Parous	556	83.7	171	84.2	
Number of children: mean., s.d	2.1	1.5	2.2	1.4	0.386
Menopausal status: no. (%)					
Pre/peri-menopausal	239	36.0	74	36.5	0.832
Post-menopausal	425	64.0	127	62.6	
Hormone replacement therapy: no. (%)					
Never	311	46.8	80	39.4	0.063
Ever	347	52.3	121	59.6	
Family history of breast cancer in first degree relative : no. (%)					
No	499	75.2	172	84.7	0.005
Yes	164	24.7	31	15.3	
Education: no. (%)					
<College	419	63.1	121	59.6	0.357
Some college or more	241	36.3	81	39.9	

Smoking: no. (%)					
Never	332	50.0	112	55.2	0.240
Ever	326	49.1	91	44.8	
Alcohol use in year prior					
None	243	36.6	69	34.0	0.533
Any	399	60.1	126	62.1	
Physical Activity In previous year					
Not active	262	39.5	103	50.7	0.011
Moderately active	209	31.5	60	29.6	
Very active	193	29.1	40	19.7	

* Due to unknown values, subcategories may not sum to total numbers of cases and controls

The univariate analysis of lifetime events showed that abortion, personal illness, death of a parent and *negative valence LE sum* were significantly associated with breast cancer risk (Table 2.2). Table 2.3 shows significant results from the univariate analysis along with adjusted estimates in the multivariate logistic regression model. After adjusting for covariates, abortion was found to be significantly associated with a 45% decrease in breast cancer risk (OR=0.55, 95% CI=0.34-0.89). Personal illness prior to reference age was associated with a 3.6 times increase in breast cancer risk (adjusted OR=3.60, 95% CI=2.50-5.20) and relocation was significantly associated with a 33% reduction in breast cancer risk (adjusted OR=0.67, 95% CI=0.47-0.97) (Table 2.3).

Table 2.2: Breast cancer univariate odds ratios for lifetime life event parameters

	Cases (N=664)		Controls (N=203)			95% CI		
	N	%	N	%	OR	Lower CI	Upper CI	*P* value
Negative valence events								
Death of child	55	8.28	16	7.88	1.06	0.59	1.89	0.855
Death of parent	440	66.27	116	57.14	1.47	1.07	2.03	0.018
Death of sibling	288	43.37	93	45.81	0.91	0.66	1.24	0.540
Death of spouse	102	15.36	31	15.27	1.01	0.65	1.56	0.975
Foreclosure	53	7.98	16	7.88	1.01	0.57	1.82	0.963
Illness	393	59.19	59	29.06	3.54	2.52	4.97	<.0001
Illness in family	383	57.68	113	55.67	1.09	0.79	1.49	0.612
Job loss	207	31.17	62	30.54	1.03	0.73	1.45	0.865
Positive valence events								
Buying home	340	51.20	103	50.74	1.02	0.74	1.40	0.908
Marriage	447	67.32	144	70.94	0.84	0.60	1.19	0.333
Marriage of child	188	28.31	53	26.11	1.12	0.78	1.60	0.539
Pregnancy	327	49.25	109	53.69	0.84	0.61	1.15	0.267
Pregnancy of child	119	17.92	39	19.21	0.92	0.61	1.37	0.677
Relocation	174	26.20	65	32.02	0.75	0.54	1.06	0.105
Equivocal valence events								
Abortion	76	11.45	34	16.75	0.64	0.41	1.00	0.047
Separation/divorce	253	38.10	81	39.90	0.93	0.67	1.28	0.645
Summary variables								
Sum negative valence events								
0 events	25	12.32	38	5.72	1.00			
1 events	30	14.78	108	16.27	2.37	1.24	4.52	0.009
2 events	44	21.67	103	15.51	1.54	0.83	2.85	0.169
3 events	39	19.21	125	18.83	2.11	1.14	3.92	0.018
4+ events	65	32.02	290	43.67	2.94	1.66	5.20	0.000
**P trend* = 0.0065								
Sum positive valence events								
0 events	111	16.72	29	14.29	1			
1 event	97	14.61	32	15.76	0.79	0.45	1.40	0.424
2-3 events	207	31.17	61	30.05	0.89	0.54	1.46	0.636
4-5 events	146	21.99	52	25.62	0.73	0.44	1.23	0.240
6 + events	103	15.51	29	14.29	0.93	0.52	1.66	0.801
Sum total events								
0 Events	21	3.16	8	3.94	1			
1-3 Events	134	20.18	48	23.65	1.06	0.44	2.56	0.891
4-5 Events	157	23.64	46	22.66	1.30	0.54	3.13	0.558

6-8 Events	176	26.51	54	26.60	1.24	0.52	2.96	0.626
9 + Events	176	26.51	47	23.15	1.43	0.59	3.42	0.426

Table 2.3: Univariate and multivariate odds ratios for significant life event occurrence parameters

Life event occurrence	Cases (N=664)		Controls (N=203)		OR	95% CI		$_{adj}$OR*	95% CI	
	N	%	N	%						
Lifetime events including decade of reference age										
Abortion	76	11.45	34	16.75	0.64	0.41	1.00	0.55	0.34	0.89
Illness	393	59.19	59	29.06	3.54	2.52	4.97	3.60	2.50	5.20
Death of Parent	440	66.27	116	57.14	1.47	1.07	2.03	1.43	0.98	2.08
Relocation	174	26.20	65	32.02	0.75	0.54	1.06	0.67	0.47	0.97
Lifetime events excluding decade of reference age										
Illness	279	42.02	51	25.12	2.16	1.52	3.07	2.15	1.46	3.17
Abortion	71	10.69	32	15.76	0.64	0.41	1.00	0.57	0.35	0.93
Relocation	159	23.95	61	30.05	0.73	0.52	1.04	0.65	0.45	0.95

* adjusted for reference age, age at first full term pregnancy, menopausal status, family history of breast cancer, HRT use, smoking history, education level, race/ethnicity and physical activity

After adjusting for covariates in the multivariate analysis, negative valence LEs remained significantly associated with increased breast cancer risk in a dose-response fashion (Table 2.4). Breast cancer risk approximately tripled in the highest category of *negative valence LE sum* (≥4) compared to baseline with (0) negative valence LEs (OR=2.81, 95% CI=1.47-5.36). The *P*-value for trend in the univariate (*P*=0.0065) and multivariate (*P*=0.0334) logistic regression models indicated a statistically significant monotonic rise in the association between increasing number of negative valence LEs and breast cancer risk. Positive valence LEs were not significantly associated with breast cancer risk (Table 2.2). However, the individual OR estimates for *positive*

valence LE sum were trending below 1, suggesting a mild protective effect of positive valence events on breast cancer risk. The increased risk of breast cancer resulting from negative valence LEs disappeared when positive and negative valence LEs were summed together (*P*-values for all values of *sum total events* were not statistically significant *P*>0.05), suggesting the moderating effect of positive valence LEs on the detrimental negative valence LEs in breast cancer risk.

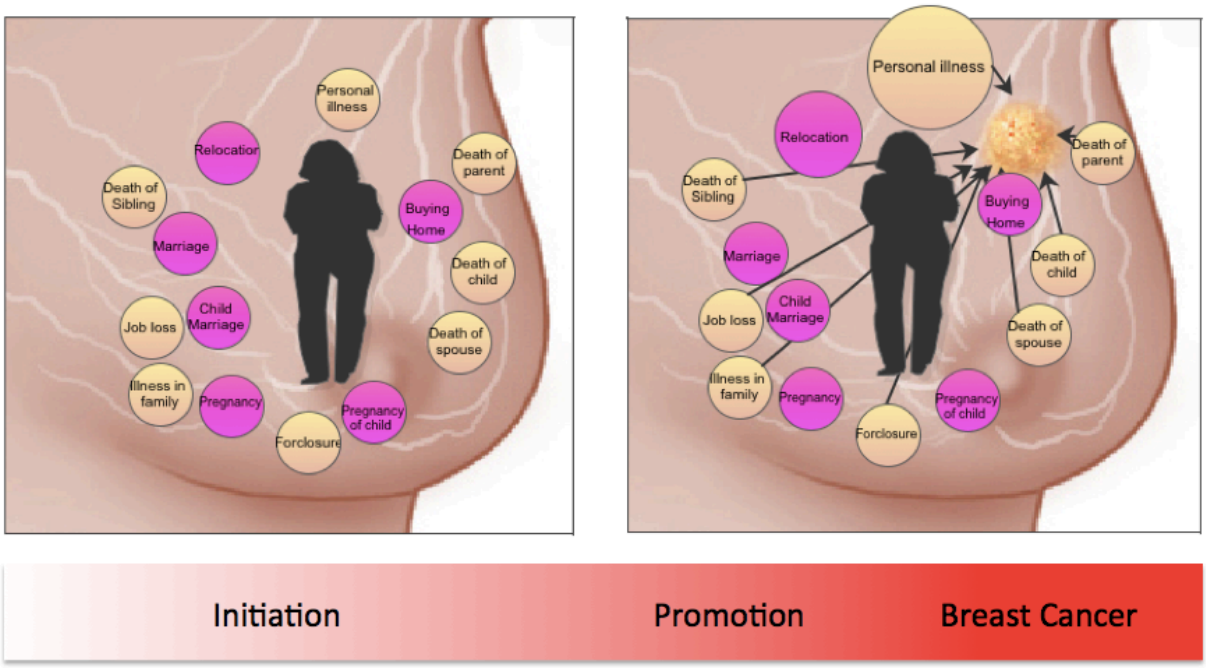
Table 2.4: Univariate and multivariate odds ratios for negative life event sum

	Cases (N=664)		Controls (N=203)		OR	95% CI		Adj OR*	95% CI	
	N	%	N	%						
Lifetime events including decade of reference age										
0 events	38	5.72	25	12.32	1			1		
1 event	108	16.27	30	14.78	2.37	1.24	4.52	2.26	1.11	4.59
2 events	103	15.51	44	21.67	1.54	0.83	2.85	1.74	0.88	3.44
3 events	125	18.83	39	19.21	2.11	1.14	3.92	2.20	1.11	4.35
4+ events	290	43.67	65	32.02	2.94	1.66	5.20	2.81	1.47	5.36
*P trend***					*P*=0.0013			*P*=0.0078		
Lifetime events excluding decade of reference age										
0 events	103	15.51	38	18.72	1			1		
1 events	117	17.62	35	17.24	1.23	0.73	2.10	1.25	0.70	2.22
2 events	94	14.16	42	20.69	0.83	0.49	1.39	0.92	0.52	1.63
3 events	103	15.51	33	16.26	1.15	0.67	1.98	1.12	0.62	2.03
4+ events	247	37.2	55	27.09	1.66	1.03	2.66	1.49	0.86	2.57
*P trend***					*P*=0.035			*P*=0.193		

* adjusted for reference age, age at first full term pregnancy, menopausal status, family history of breast cancer, HRT use, smoking history, education level, race/ethnicity and physical activity
** P-trend determined based on modeling negative events as a continuous variable in the logistic regression

Similar to the inclusive analysis with all lifetime events, when excluding the decade of breast cancer diagnosis, the same *LE occurrence* parameters significantly influenced breast cancer risk. History of personal illness was associated with approximately double breast cancer risk ($_{adj.}$OR= 2.15, 95% CI=1.46-3.17) and abortion ($_{adj.}$OR=0.57, 95% CI=0.35-0.93) and relocation ($_{adj.}$OR=0.65, 95% CI=0.45-0.95) had protective effects (Table 2.3). The effects of cumulative LEs on breast cancer risk were more modest when the only LEs occurring prior to the decade of reference age were included (Table 2.4). The highest category of negative valence LEs (≥4 events compared to 0 events) evaluated in the univariate analysis showed a significant increase in breast cancer risk (OR=1.66, 95% CI=1.03-2.66). However, this effect was no longer significant in the multivariate analysis ($_{adj.}$OR=1.49, 95% CI=0.86-2.57).

Figure 2.1: Aim 1 graphical presentation



Discussion:

We confirmed our first hypothesis concerning the cumulative effect of salient negative valence LEs in increasing breast cancer risk. The time it takes from stressful LEs to breast cancer manifestation is unclear.[84] In this study the association between negative valence LEs and increased breast cancer risk was observed only when including LEs that had occurred in the 1-10 years prior to age at diagnosis in addition to previous LEs. Therefore, LEs seem to have a cumulative and promoting effect in the pathway to breast cancer pathogenesis. This observation is consistent with previous studies

showing a positive relationship between breast cancer and severe LEs occurring in the 10 years prior to diagnosis.[48,50,51,84,85]

As the number of lifetime salient negative valence LEs increased so did the likelihood of breast cancer. This finding is supported by previous studies indicating the impact of distressing LEs in increasing breast cancer risk.[48,50,51] In a recent meta-analysis (Lin *et. al,* 2013) that analyzed seven studies published from 1995-2012, the pooled OR was 1.51 (95% CI=1.15-1.97, P=0.003) for LEs and primary breast cancer.[54] This estimate is understandably discrepant with ours since the valence/desirability of LEs summed in these studies was not considered.[48,50,52,84] Previous research that took into account desirability of LEs and risk of psychological and physical illness supports our findings by showing a consistent positive relationship between negative valence events and disease and no consistent effect of positive valence LEs on disease.[58]

LEs may increase breast cancer risk by suppressing immune function and tumor surveillance thus causing direct and indirect mutagenesis.[64,68,86,87] Cortisol, the main glucocorticoid in humans, impairs the ability of the immune system to identify and neutralize cancer cells.[68] Cells treated with the stress hormones cortisol, epinephrine or norepinephrine, showed a 5-fold increase in DNA damage and impaired DNA damage repair.[65] Additionally, a rat model of life event stress showed that exposure to tail shock increased serum estradiol levels,[87] which directly and indirectly contribute to breast carcinogenesis.[11]

This study supports the importance of history of personal illness, defined as “serious illness or injury of oneself” as a major stress factor increasing breast cancer risk. This durable finding, even when excluding events in the decade of breast cancer diagnosis for cases, makes it unlikely that this result is a mere artifact of over-reporting by cases. This result is consistent with previous results indicating that a history of personal illness increases breast cancer risk by 2.6 fold (OR=2.6, 95% CI=1.63-4.62).[50] In a validation analysis, cases more commonly reported a positive history of fibrocystic breast disease and gallstones compared to controls (P<0.05) (Appendix 1). Personal illness contributes to both physical and psychological stress. Therefore, it is likely that these effects have an additive influence on the stress system thus cumulatively contributing to breast cancer development.

The LEs abortion and relocation were shown to significantly reduce breast cancer risk. Abortion reduced breast cancer risk by 45% after adjusting for reproductive and other covariates. A plausible explanation for this finding is that abortion takes place during the reproductive years, and that psychological stress resulting from abortion during this time period reduces circulating estrogen levels and hence moderates breast cancer risk. Nevertheless, more studies are needed to verify this stipulation.

The second hypothesis concerning the negative relationship between salient positive valence LEs and breast cancer risk was not supported. However, these events seemed to have a buffering effect that moderated the adverse impact of negative valence LEs. The significant dose-response relationship between negative valence LEs and breast

cancer risk disappeared when positive and negative valence LEs were summed in the *total LE sum*. It is possible that negative valence LEs are severely distressing and therefore perturb reproductive and immune functions [80] while positive valence LEs promote the recovery of these systems. This finding emphasizes the importance of including the valence or desirability of events in the study of how LEs influence breast cancer risk.[80,88] These results provide comfort and reassurance to women who have faced hardships since it is likely that desirable, salient positive lifetime events moderate the effect of stressful and distressing undesirable events. Women are advised to continue to engage in meaningful, prolific lives despite negative valence LEs given that their physiology and tumor microenvironment are likely influenced by both the positive and negative events occurring in their lives.

One of the main limitations of this study, as with other case-control studies, is the reliance on memory and hence subjectivity to recall bias. However, highly salient events such as death in the family and marriage have been shown to be reported with high reproducibility and accuracy.[89] We have also shown that the same *LE occurrence* parameters were significant whether including events in the decade of reference age or not. Therefore, it is unlikely that our results are an artifact of recall bias. Another limitation of this study relates to the classification of desirability of events that did not perfectly match with that reported in the Paykel (1971) Life Event Scale. Paykel's scale classifies divorce as a highly distressing event and therefore attributes to it negative valence. Separation, although stressful, is potentially a desirable event and therefore may be assigned positive valence.[80] Since the Life Event questionnaire we used

grouped these events into one category “separation/divorce”, the data derived from this survey did not allow for the differentiation of these two salient LEs. Similarly, the LE “abortion” could be perceived as desirable if terminating an undesirable pregnancy (induced abortion) or undesirable if occurring spontaneously. Hence the exclusion of these salient LEs from the positive and negative valence sums due to difficulty matching the events to those in the Paykel scale. Lastly, we acknowledge the limitation of having fewer controls than cases.

When assessing the effect of LEs on breast cancer risk, studies have generally focused on severe LEs measured by the Holmes and Rahe Social Readjustment Rating Scale.[48,50,52] A major limitation of this scale is that events are weighted on a unidirectional dimension where all events are considered to increase disease risk with no consideration for the predictability or desirability of events.[49] Vinokur and Selzer (1975) have argued that the desirability of events is a critical factor in determining the amount of stress resulting from LEs.[57] Hoberman and Cohen (1983) also suggested that positive or desirable LEs may serve as a buffer, moderating the effects of undesirable/negative life event stress by giving the body the time to restore its natural homeostasis.[59] This buffering effect has been observed in subjects with depression, psychological disorder and somatic symptomology such as headache.[59–61] Nevertheless, these studies were limited in scope since they primarily focused on daily positive valence events while neglecting to include more salient positive valence LEs such as marriage and childbirth.

To the authors' knowledge, this is the first epidemiological study to examine the effect of LEs on breast cancer risk while taking into consideration valence of salient life events. Considered LEs were generally easily categorized as desirable or not based on the Paykel (1971) scale. This study included assessment of additional, less-studied LEs such as abortion.[48,51,84,85] The significant reduction in breast cancer risk resulting from abortion indicates that the scope of LEs investigated in the context of LEs and breast cancer risk should be expanded. The use of population-based controls increases generalizability and external validity of our findings. Demonstration of a dose-response relationship for *negative valence LE sum* and breast cancer risk increases the likelihood that this association is not merely due to chance. Further, this study included an extensive breast cancer risk factor data pool allowing the adjustment for known breast cancer risk factors. To our knowledge this is the only study of its kind focusing on the relationship of LEs and breast cancer risk in the population of Southern California.

In conclusion, this study emphasizes the importance of assessing valence of LEs over the life course when evaluating the relationship between stress in the form of life events and breast cancer risk. We demonstrated that salient negative valence LEs are cumulatively associated with increased breast cancer risk. The additional finding that positive valence LEs may act as a buffer, thereby moderating the adverse effects of negative valence LEs, points to the need to increase the understanding of how LE valence directly or indirectly moderates breast cancer risk. Assessments of life events in the clinic in conjunction to other breast cancer risk factors such as alcohol, BMI and diet, could allow a more targeted approach to understanding individualized breast

cancer risk and guide screening recommendations. We recommend expanding the categorization of major LEs and the modification of the LE scale into one with a -5 to +5 continuum. The evaluation of LEs on a gradient from positive-negative valence will allow a more personalized approach to evaluating a women's breast cancer risk in context of her own personal experiences.

Chapter 3:

Perception Matters: Stressful Life Events Increase Breast Cancer Risk

Abstract:

Objective: The relationship between psychological stress and breast cancer risk is unclear. The present study sought to understand how stressfulness appraisal of salient Life Events (LEs) influences breast cancer risk.

Methods: A case-control design was used and included 664 female cases identified through the Cancer Surveillance Program of Orange County, CA and 203 female population-based controls. A LE questionnaire determined if events occurred prior to breast cancer diagnosis and if these events were considered to be stressful or not. Multivariate unconditional logistic regression was used to calculate ORs while adjusting for known breast cancer covariates.

Results: Cumulative adverse LEs perceived as stressful were associated with increased breast cancer risk in a dose response fashion (OR=1.63, 95% CI=1.00-2.66, P_{trend}=0.045*)*. Conversely, events perceived as non-stressful did not have a significant impact on breast cancer risk. Previous personal illness was directly related to increased breast cancer risk, whether perceived as stressful (OR=2.84, 95% CI=1.96-4.11) or non-stressful (OR=3.47, 95% CI=1.34-8.94). Abortion and relocation were observed to have a protective effect on breast cancer risk only when reported as stressful (OR=0.54, 95% CI=0.32-0.92; OR=0.63, 95% CI=0.43-0.93, respectively). Pre/Peri-menopausal women who were nulliparous or who had their first child at ≥30 years of age were especially prone to the effects of appraised stress on increased breast cancer risk.

Conclusions: This study underscores the importance of stressfulness appraisal when determining the effect of major LEs on breast cancer risk. Our results support incorporating assessments of perceived stressfulness in future epidemiological investigation of this topic.

Background:

One of the main challenges in stress research is measuring and quantifying stress levels. When analyzing the effect of stress due to LEs on breast cancer risk, researchers have attempted to standardize measurement of stress exposure. The main measures of stressful LEs utilized in epidemiological studies thus far have been the Holmes and Rahe Social Readjustment Rating Scale [48,50,52,84] and the Brown and Harris LEs and Difficulties Schedule (LEDS).[51,53] However, in both of these measures individual stress appraisal is not directly addressed.[49,56]

Contributors to psychological stress

Psychological stress arises when a person appraises the environmental demands to overwhelm his/her ability to cope with the situation.[90] The stress researcher Selye, M.D. was the first to recognize that the individual is the one to determine based on their perception of the situation whether it is eustressful or distressful. When a stressor is appraised to be positive, beneficial or desired, it leads to eustress. In contrast, when a stressor is appraised to be negative, unwanted or exceeding coping abilities, it will cause a state of distress.[91,92] Personal perception of stress has been shown to be important in determining how stressors impact physiology.[25]

Daily stress and breast cancer risk

In a large prospective cohort study investigating the influence of perceived daily stress on subsequent breast cancer risk during 24 years of follow-up, a 2-fold increase in

breast cancer risk was observed for women who reported high levels of stress compared to women who reported no or minimal mental stress.[45] Conversely, another prospective study focusing on perceived daily stress found a protective relationship between higher stress levels and breast cancer risk.[46] However, these studies quantified stress based on reports of nervousness and anxiety in everyday life and did not address stressfulness of more salient LEs such as death of a close relative or serious illness.

Stress appraisal

The subjective reporting of daily stress and breast cancer incidence has been investigated previously.[45] However, to our knowledge, the relationship between perceived stressfulness of salient LEs and breast cancer risk has not been directly addressed. Higher perceived stress has been linked to increased cortisol levels[93] and therefore can provide easily ascertained information regarding HPA perturbations resulting from LEs. Recent work has revealed that higher perceived stress is linked to higher resting amygdalar activity [94], a key component of the physiological stress system. The synergistic influence of a stressor and the perception that 'stress influences health outcomes' on increasing mortality, further supports the importance of stress perception [95].

Modulators of stress appraisal

Antoni *et. al* (2006), developed a bio-behavioral model for the relationship between psycho-social factors and cancer incidence and progression. Based on this model, the cancer 'macroenvironment' is comprised as a result of the interaction between

environmental stress and an individual's attitudes toward the stressor, perception of treat, and coping abilities.[67] When the state of distress persists chronically, it is more likely to influence neuroendocrine stress hormones contributing to cancer growth.[96] Consistent with this model, blocking the effects of chronic stress in mice models by antagonizing the ß-adrenergic receptor was shown to protect against breast tumor dissemination through lymphatic spread.[73] The individual's internal working model and attachment style are important factors in determining perceived stressfulness and subsequent activation of the physiological stress response.[97] A recent study found that an 'optimal' relationship with at least one parent leading to a secure attachment style reduced the likelihood of LN involvement of breast malignancies by 62% [98].

Importance of life event stress appraisal in breast cancer risk

To better study the effects of stress in the form of LEs, we sought to understand if a woman's personal stress appraisal influences the association between LEs and breast cancer risk. As discussed, perceived stress is associated with elevated cortisol levels [93], and higher resting amygdalar activity.[94] Considering our understating of the plausible role of cortisol in cancer initiation and progression [63,64,66,67], we hypothesize that the perception of stress resulting from major life events will increase cortisol signaling and hence impair immune surveillance [68,69] and contribute to breast cancer risk. The aim of the present analysis is to investigate whether LEs increase breast cancer risk depending on the individually reported experience of stress. Understanding how to better epidemiologically measure and quantify stress is expected to allow a more accurate assessment of whether stress influences breast cancer development.

Methods:

Study Population:

Six hundred and sixty three population-based incident primary invasive breast cancer cases and 203 population-based controls were part of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860) are included in the present analysis [78,79] (see Chapter 1 for more details).

Measures:

We further assessed if events that occurred were individually perceived as 'stressful' or 'non-stressful'. The questionnaire prompted participants to identify their age at which each event occurred using the following age intervals: 10-19, 20-29, 30-39, 40-49, 50-59 and ≥60 (see Chapter 1 for more details).

In order to analyze if perceived event stressfulness was impacted by event valence, we used the Paykel (1971) LE Scale to dichotomize events based on whether they were more likely to be desirable or undesirable, i.e. of positive or negative valence (Chapter 1). Events that were at the top half of the scale were determined to be of negative valence, while events at the bottom half of the scale determined to be of positive valence.[80]

Stressful/non-stressful life events: Participants were asked if the above life events, whether positive or negative, brought about a “moderate to severe” amount of stress. If “yes”, the life event was considered a ‘stressful life event’ (SLE), if “no”, the life event was considered a ‘non-stressful life event’ (NSLE) (Figure 3.1). In a similar fashion described for LEs, age interval at SLE/NSLE was determined. Three different SLE/NSLE parameters were used to evaluate the effect of SLEs/NSLEs on breast cancer risk: (1) S*LE/NSLE occurrence:* (yes/no)*,* (2) *negative valence SLE/NSLE sum:* 0, 1, 2, 3 and ≥4 events, and (3) *positive valence SLE/NSLE sum:* 0, 1, 2-3, 4-5 and ≥6 events,

For the S*LE/NSLE occurrence* variable, the LE category received a value of ‘0’ if the event did not occur and ‘1’ if any number of events happened in the same category (if someone was pregnant twice, the ‘pregnancy’ *occurrence* variable obtained a value of ‘1’). The grouping of the sum of positive/negative valence *SLEs/NSLE*s was based on creating a zero event baseline and dividing the remaining distribution of events in the control group into quartiles. Here each event in a LE category contributed an unweightd value of ‘1’ to the sum (if a women was pregnancy twice, ‘2’ LEs would be added to the *SLE/NSLE sum*. For the *SLE sum*, LEs that were appraised as non-stressful (NSLEs) were included in the zero event baseline and for the *NSLE sum*, LEs that were appraised as stressful (SLEs) were included in the zero event baseline.

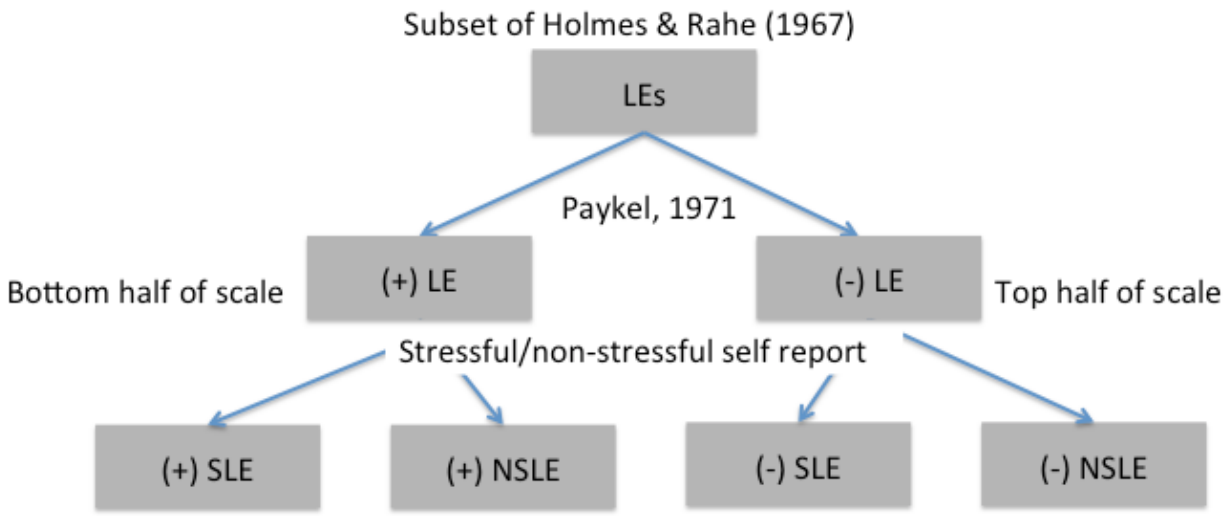


Figure 3.1: Categorization of life events according to valence and perceived stress: LE: Life Event, SLE: Stressful Life Event, NSLE: Non-Stressful Life Event

(+): Positive valence, (-): Negative valence

Data Analysis:

Descriptive statistics were calculated for cases and controls. Means and standard deviations were computed for continuous variables, whereas frequencies and percentages were computed for categorical variables (see Chapter 2). Additional descriptive statistics were generated in a stratified analysis according to occurrence of events appraised as stressful or non-stressful.

Candidate variables for model building were selected based on a literature review of breast cancer covariates. A standard stepwise model building process with statistical threshold for inclusion into the model of P_{entry}=0.25 and P_{stay}=0.3 was performed in selection of a baseline model for the multivariate analyses. The final multivariate logistic

regression model adjusted for *age* (age at diagnosis for cases and age at RFQ completion for controls), *smoking history (*ever smoked/never smoked), *race/ethnicity* (non-Hispanic white/all other groups), *education* (less than college/some college or more), *family history* (yes/no) (determined based on family history of breast cancer in first degree relative), *hormone replacement therapy (HRT)* (ever use/never use), *age at menarche* (≤11, 12-13 and ≥14), *age at first full term pregnancy* (*FFTP):* (<25, 25-29 and ≥30)*, menopausal status* (pre-menopausal/peri-menopausal/post-menopausal) and *physical activity* (not active/moderately active/very active). To examine potential confounding, we examined the estimates for the covariates in the model when including negative life events and when not including negative life events. The estimates of the ORs associated with covariate coefficients did not differ by more than 5% and therefore we conclude that there is no significant association between stress in the form of life events and the breast cancer covariates examined in the model.

SLE and NSLE parameters were evaluated in separate univariate and multivariate analyses. Univariate associations between breast cancer and S*LE/NSLE occurrence* parameters, *negative valence SLE/NSLE sum, positive valence SLE/NSLE sum* and *total SLE/NSLE sum* were determined. Frequency tables and χ^2 statistics were used to compare *SLE/NSLE* variables among cases and controls. Likelihood of an event being 'stressful' was determined based on the fraction of (SLEs)/(total LEs) reported for cases and controls.

The influence of *SLE/NSLE* parameters on breast cancer odds ratios (ORs) was determined using simple unconditional logistic regression where ORs and 95% confidence intervals (CIs) were computed. Significant *SLE/NSLE* parameters were then included one at a time into the baseline multivariate regression model and adjusted ORs and CIs were determined using multivariate unconditional logistic regression. The *negative valence SLE/NSLE sums* were also included in the logistic regression model as continuous variables and Wald χ^2 statistics were used to test for trend.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results:

Demographics and Characteristics Among Breast Cancer Cases and Controls

Descriptive characteristics of cases and controls are presented in Chapter 2 (Table 2.1). Cases were slightly older than controls with a mean age of 55.6 years for cases and 53.6 years for controls. Cases were more likely to have a positive family history of breast cancer (24.7%) compared to controls (15.3%). Both cases (88.9%) and controls (87.7%) were primarily of white ethnicity. Mean age at first full term pregnancy was significantly younger for controls (23.7) compared to cases (25.2). The majority of controls had children at the age of ≤24 years (60.8%) in comparison to 49.8% in the case group. No significant differences between cases and controls were observed for the following parameters: BMI, age at menarche, parity, number of children,

menopausal status, hormone replacement therapy, education level, smoking history and alcohol history.

Descriptives of Cases and Controls who Perceived Events as 'Stressful'

Pre-menopausal or peri-menopausal women who appraised at least one event as stressful were more likely to be cases (*P*=0.002). Similarly, nulliparous women (*P*=0.043) and women who had their first child at age ≥30 and appraised at least one event as stressful were more likely to have had breast cancer (*P*=0.024). There were no significant differences observed among cases and controls on the following parameters: age, BMI, race/ethnicity, age at menarche, number of children, HRT, family history of breast cancer, education level, smoking history and alcohol history (Table 3.1).

Table 3.1: Demographics and characteristics of women who appraised events as stressful*

Characteristic	Cases		Controls		***P* value
Reference age, years: mean, s.d.	54.5	11.9	55.6	10.6	0.296
BMI: no. (%)					
Underweight	10	71.4	3	75.0	0.467
Normal Weight	262	80.6	69	72.6	0.094
Overweight	163	84.9	42	77.8	0.215
Obese	95	83.3	42	89.4	0.466
Race/ethnicity: no. (%)					
Non-Hispanic White	479	83.5	139	78.1	0.103
Other	51	76.0	19	71.8	0.687

Age at menarche: no. (%)					
≤10 years	28	77.8	9	75.0	1
11-13 years	367	83.4	107	77.5	0.117
≥14 years	121	81.8	38	79.2	0.690
Age at first full-term pregnancy: no. (%)					
≤24 years	226	84.0	85	81.7	0.595
25-29 years	137	76.1	36	81.8	0.418
≥30 years	78	85.7	15	65.2	0.024
Parity: no. (%)					
Nulliparous	89	84.8	22	68.8	0.043
Parous	441	81.7	139	79.5	0.501
Number of children: mean., s.d	2.2	1.4	2.1	1.5	0.386
Menopausal status: no. (%)					
Pre/peri-menopausal	194	84.4	50	67.6	0.002
Post-menopausal	336	81.0	106	83.5	0.525
Hormone replacement therapy: no. (%)					
Never	244	80.8	61	76.3	0.368
Ever	282	83.4	97	80.2	0.416
Family history of breast cancer in first degree relative: no. (%)					
No	397	82.2	133	77.3	0.163
Yes	133	82.6	25	80.7	0.793
Education: no. (%)					
<College	331	80.7	95	78.5	0.590
Some college or more	197	84.9	62	76.5	0.086
Smoking: no. (%)					
Never	261	81.1	85	75.9	0.242
Ever	264	83.3	73	80.2	0.497
Alcohol use in year prior: no. (%)					
None	192	81.4	56	81.2	0.971
Any	324	83.7	97	77.0	0.087

Physical Activity In previous year: no. (%)					
Not active	207	81.5	78	75.7	0.219
Moderately active	172	84.7	46	76.7	0.145
Very active	151	80.3	34	85.0	0.492

* appraised stressful events: had at least one event that was appraised as stressful

** table total values could be discrepant from the total cases and controls since some participants reported only NSLEs

*** *P* values were calculated based on χ^2 statistics unless cell counts were below 5 where fisher's exact test was used

(-) test statistic could not be computed because of insufficient data

Likelihood of Events being 'Stressful':

The pattern of stressfulness of individual *LE occurrence* variables did not differ among cases and controls (Figure 3.2). As expected, negative valence LEs were more likely to be perceived as stressful compared to positive valence LEs. On average, 85% of the time negative LEs were categorized as 'stressful' among cases, as compared to 89% of the time among controls. On the other hand, positive LEs were categorized as 'stressful' only 61% of the time among cases and 64% of the time among controls. Job loss and foreclosure were the negative valence LEs most likely to be reported as 'stressful' among cases (89%) and controls (100%).

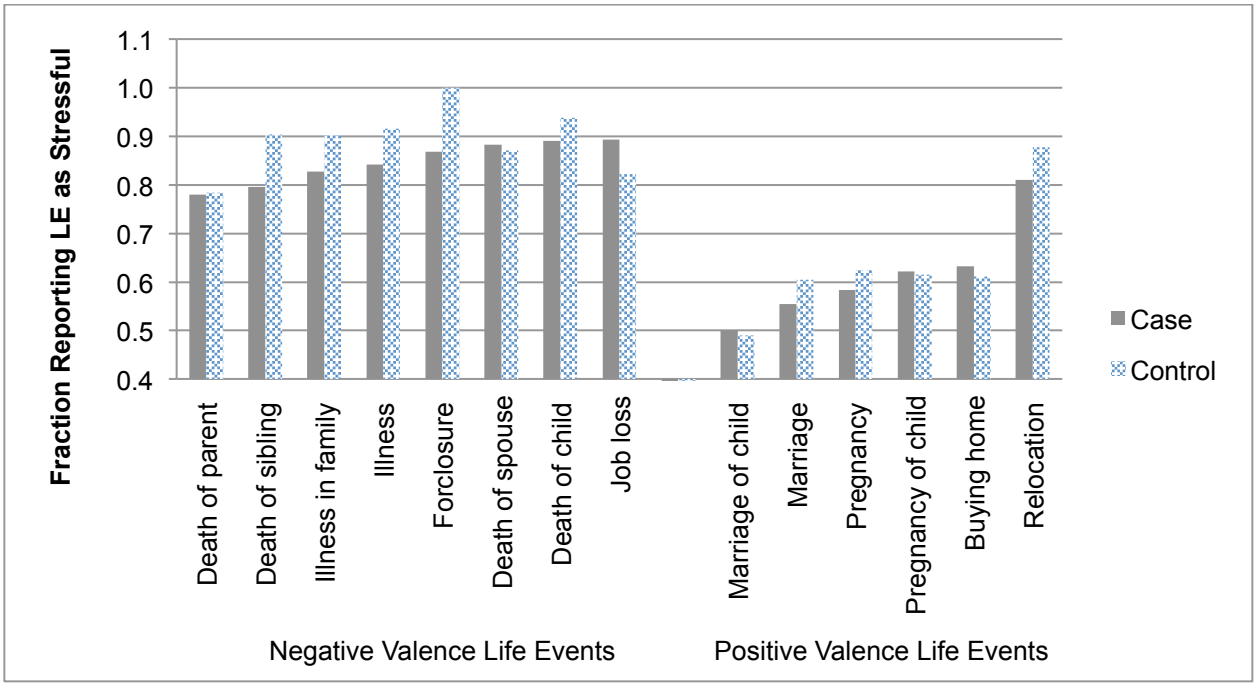


Figure 3.2: Likelihood of LEs being perceived as 'stressful' among breast cancer cases and controls LEs (LEs) were categorized into 'positive' or 'negative' valence based on the Paykel (1971) LE scale (see text for details). LEs are organized based on events least to most likely to be stressful among cases in both valence categories. The likelihood an event category to be classified as 'stressful' was determined by the fraction: (# stressful LEs)/(total # of LEs).

Stressful/Non-Stressful LEs and Breast Cancer Risk

In the univariate and multivariate analyses, relocation (OR=0.65, 95% CI=0.44-0.95) and abortion (OR=0.54, 95% CI=0.32-0.91) were associated with decreased breast cancer risk only when perceived as stressful. History of personal illness was associated with increased breast cancer risk both when appraised as stressful (OR=2.80, 95% CI=1.94-4.04) and non-stressful (OR=3.40, 95% CI=1.32-8.75) (Table 3.2).

Table 3.2: Univariate and multivariate odds ratios for significant stressful and non-stressful life event occurrence parameters

	Cases (N=664)		Controls (N=203)		OR	95% CI		$_{adj}$.OR*	95% CI	
	N	%	N	%						
Life events perceived as stressful										
Abortion	60	9.0	28	13.8	0.62	0.38	1.00	0.54	0.32	0.91
Illness	331	49.9	54	26.6	2.74	1.94	3.88	2.80	1.94	4.04
Relocation	141	21.2	57	28.1	0.69	0.48	0.99	0.65	0.44	0.95
Life events perceived as non-stressful										
Death of sibling	59	8.89	9	4.43	2.10	1.02	4.32	1.75	0.83	3.68
Illness	62	9.34	5	2.46	4.08	1.62	10.29	3.40	1.32	8.75
Illness in family	66	9.94	11	5.42	1.93	1.00	3.72	1.53	0.77	3.03

* Adjusted for reference age, age at first full term pregnancy, menopausal status, family history of breast cancer, HRT use, smoking history, education level, race/ethnicity and physical activity

N: Number of exposed; %: Percent exposed

Negative valence LEs were associated with a significant dose-response increase in breast cancer risk only when perceived as stressful (P_{trend}=0.049). The highest category of negative valence SLEs was associated with a 62% increase in breast cancer risk after adjusting for breast cancer covariates (OR=1.62, 95% CI=0.99-2.63) (Table 3.3). Conversely, positive valence LEs were cumulatively associated with a non-significant trend toward decreased breast cancer risk only when events were perceived as stressful (data not shown).

Table 3.3: Univariate and multivariate odds ratios for sum negative life events

	Cases (N=664)		Controls (N=203)		OR	95% CI		$_{adj.}$OR*	95% CI	
	N	%	N	%						
Stressful negative valence LEs										
0 events	123	18.5	45	22.2						
1 event	111	16.7	29	14.3	1.40	0.82	2.39	1.43	0.81	2.53
2 events	84	12.7	46	22.7	0.67	0.41	1.10	0.81	0.47	1.38
3 events	109	16.4	29	14.3	1.38	0.81	2.34	1.57	0.89	2.77
≥4 events	237	35.7	54	26.6	1.61	1.02	2.52	1.62	0.99	2.63
*P trend***					0.042			0.049		
Non-stressful negative valence LEs										
0 events	526	79.2	161	79.3						
1 event	46	6.9	18	8.9	0.78	0.44	1.39	0.77	0.42	1.42
2 events	30	4.5	13	6.4	0.71	0.36	1.39	0.58	0.28	1.19
3 events	21	3.2	6	3.0	1.07	0.43	2.70	0.80	0.31	2.10
≥4 events	41	6.2	5	2.5	2.51	0.98	6.46	2.04	0.77	5.41
*P trend***					0.227			0.760		

* Adjusted for reference age, age at first full term pregnancy, menopausal status, family history of breast cancer, HRT use, smoking history, education level, race/ethnicity and physical activity

** *P trend* computed by incorporating *negative valence LE (NSLE) sum* in the logistic regression model as a continuous variable
N: Number of exposed; %: Percent exposed

Discussion:

The results presented here suggest that LEs differentially influence breast cancer risk according to stress perception. This result is consistent with previous research indicating the importance of stress appraisal in altering the physiological stress system [93]. Prior research supports this finding given that LEs were overall perceived as more impactful among breast cancer patients as compared to controls [99].

Our results are in line with those reported by Helgesson *et. al* (2003) where perceived stress was associated with an approximately 2-fold increase in breast cancer risk. However, in this study stress was determined based on experiencing daily stress "occasionally or more" during the past 5-years and did not address LE stress [45]. Likewise, a recent study that focused on perceived stress in daily life and increased breast cancer risk in a cohort of 29,098 Japanese women showed that women who reported "high perceived stress", had a 1.71 (95% CI=1.02-2.85) increased risk of developing breast cancer prospectively [100]. It is possible that women who reported higher perceived stress also had more negative valence LEs that they perceived as stressful since these measures are significantly correlated [59].

Our findings support the need to examine salient LEs in combination with other factors influencing perception of stress such as coping style and social support [101]. We found that repeated exposure to negative valence LEs perceived as 'stressful' increased the risk of breast cancer. Therefore, it is likely that negative LEs cumulatively contribute to

appraised overload of environmental demands compared to available coping mechanisms leading to a state of distress [91,92,96]. Consistently, a significant interaction was observed between LEs perceived as highly stressful and social support in predicting breast cancer risk, with the highest risk observed for those with highly stressful events and low social support [101].

As expected, we found that negative valence LEs were those to have the greatest likelihood of being perceived as 'stressful' (Figure 3.2). Therefore, negative valence, cumulative LEs perceived as stressful seem to be most influential in increasing breast cancer risk. Interestingly, 'relocation' was the positive valence event to be reported as 'stressful' most often and was the only positive LE to be significantly associated with decreased breast cancer risk. Therefore, it seems that to experience the benefits of positive stress, an event needs to be salient enough to induce significant eustress. Consistent with this hypothesis, the cumulative benefit of positive valence LEs on decreasing breast cancer risk was only observed when events were perceived as stressful.

History of personal illness, defined as 'serious illness or injury of oneself' was significantly associated with increased breast cancer risk both when perceived as stressful and when perceived as non-stressful. In line with our findings, a higher incidence of major personal illness has been reported in the past among breast cancer patients than among controls [50,99]. In a separate validation analysis, fibrocystic breast disease and gallstones were more commonly reported among breast cancer cases than

among controls (P<0.05). This may indicate the possibility of physical illness contributing to immune system breakdown and facilitation of a pro-cancer microenvironment, regardless of individual psychological stress perception. Consistent with this finding, previous research has shown that physical and psychological stressors activate the HPA stress axis in a similar fashion and independently contribute to immune system breakdown [102]. Pre-existing immune system function and HPA reactivity from transgenerational [103] or early-childhood [98] experiences may predispose women to both physical illness and breast cancer.

Younger women seem to be more impacted by the effects of perceived stress. Our results suggest that stressful LEs have a stronger influence on pre/peri-menopausal breast cancer risk than on post-menopausal breast cancer risk. In the only study identified focusing on breast cancer in young women, LEs were cumulatively associated with a 62% increase in breast cancer risk [104]. It is possible that LE stress has a stronger impact on basal estrogen levels during reproductive years.

Estrogen causes proliferation of breast tissue and may promote the progression from normal cellular proliferation to hyperplasia and neoplasia [8]. Further, estrogen metabolites are directly genotoxic [10,11]. Premenopausal women have higher average levels of estradiol [105,106]. In a mouse model of LE stress, tail shock and forced swim tests resulted in increased estrogen levels and persistently thereafter [87]. Consequently, decreased estrogen metabolism resulting from LE stress [107] likely more substantially contributes to increased premenopausal estrogen exposure.

Further, LE stress seems to influence breast cancer risk specifically among nulliparous women and women who delayed childbirth. Many studies have shown that the younger a woman is at her first full term pregnancy, the lower her risk of breast cancer [15]. It is hypothesized that the mammary gland undergoes complete differentiation during pregnancy, leading breast tissue to increased resistance against carcinogenic initiation [108]. Therefore, young women who are nulliparous or had an older age at first full-term pregnancy, not only have the highest endogenous estrogen levels, but their breast tissue is also the most susceptible to mutagenic changes. Hence this population is hypothesized to be the most vulnerable to the effects of LE stress on breast cancer risk. Future studies examining the interaction between parity/age at first full term pregnancy and negative valence LEs would deepen our understanding of this observation and the interplay between reproductive and psychological factors in breast tumorigenesis.

Premenopausal breast cancer tends to be more aggressive with estrogen-independent tumors being more common [109]. Further, approximately 30% of female breast malignancies are diagnosed among women younger than 50 years of age [110]. We are limited in our understanding of etiologic and mechanistic contributors to this disease. A study from 2016 achieved significance for the population attributable fraction when considering known risk factors for post-menopausal breast cancer but not premenopausal breast cancer [111]. Therefore, this research has important implications toward understanding contributing factors to development of breast malignancy in young women.

Breast cancer cases and controls did not exhibit a different pattern of LE stress perception. Stressfulness perception followed a predictable trend among cases and controls. The likelihood of an event being reported as 'stressful' followed our valence categorization based on the Paykel scale (1971) [80]. Negative valence LEs were more often perceived as stressful as compared to positive valence LEs. These findings implicate that cases and controls do not differ in what events they perceive as stressful. However, given the cumulative effect of stressful events on breast cancer risk, it seems that cases are experiencing more stress through negative valence LEs and possibly are not as well equipped to cope with stress compared to the control group.

Future Directions

Additional research focusing on the effect of perceived LE stress on breast cancer risk among premenopausal women is needed. Incorporating assessments of cortisol, catecholamines and estradiol would allow researchers to better understand the mechanism behind the proposed alterations in neuroendocrinological processes accelerating breast carcinogenesis. Further investigating the contributing factors that distinguish cases and controls in their response to stressful situations should be considered. Controls may be more likely to find meaning in their stressful situations and as a result gain resiliency, while cases are perhaps more debilitated in the face of stress.

Epidemiological studies examining the relationship between LEs and breast cancer risk thus far do not directly incorporate individualized stress appraisal measures. Questionnaires and structured interviews are currently widely used in an attempt to standardize stress exposure [48,50,51,53,84]. However, this approach ignores the importance of an individual's interaction with their environment along with their coping mechanisms and resulting appraised stress [112,113]. Higher scores on the most commonly used measure of stress appraisal, the Perceived Stress Scale (PSS), have been associated with prior occurrence of LEs and the presence of somatic and depressive symptomology [59]. However, this measure addresses perceived stress in the preceding 30 days. Our results support the utilization of an adapted PSS in future research focusing on LEs and breast cancer risk. Consistent with this recommendation, studies incorporating self-ratings of event stressfulness were superior predictors of health outcomes compared to studies that did not incorporate this information [57,88].

Limitations and Strengths:

When interpreting our findings, it is important to consider study limitations. Firstly, this is a case-control study, where information was ascertained retrospectively and hence the possibility of recall bias. However, since LEs are reported with high reproducibility[114], it is unlikely that cases were over-reporting the occurrence of LEs. Reflective of the population of Orange County, the majority of participants were of White ethnicity. Therefore, generalizability to other racial/ethnic groups is questionable. Additional studies examining a more diverse population are warranted. Because we asked about an additional 3 events not included in the validated Holmes and Rahe (1967) scale[115],

we did not sum events according to assigned weights. A further limitation to our study pertains to the use of a dichotomized assessment of stressfulness and assignment of positive/negative valence to LE categories.

To gain a better understanding of the effect of perceived stressfulness on breast cancer risk, it would be more accurate to quantify stress appraisal on a gradient from non-stressful to extremely stressful. Further, it would be advised to include a positive-negative valence gradient for each event to better quantify the interplay of valence and stressfulness in breast cancer risk. Another limitation pertains to valence categorization of some events that did not perfectly match with the Paykel (1971) LE Scale categories [80]. For example, we could not differentiate the valence of our grouped category 'separation/divorce' since based on our dichotomization 'divorce' would be assigned negative valence and 'separation not due to argument' positive valence. Similarly, abortion could be desirable (terminating an undesirable pregnancy) or undesirable (spontaneous abortion) and therefore was not assigned valence.

This study provides novel insight into the relationship between LE stress and breast cancer risk. To our knowledge, this is the first study of its kind to investigate the influence of LE appraised stress on breast cancer risk. This assessment was facilitated by the information we had available to us on the personal categorization of events as 'stressful'/'not-stressful' by participants in the study. Further, our results shed light onto the effect of stress in the form of LEs on breast cancer risk in young women specifically. The use of population-based controls strengthens the external validity of our findings.

Our rich dataset allowed for the adjustment of breast cancer covariates in our multivariate models, enabling a more accurate understanding of the influence of LEs on breast cancer risk while limiting the possibility of confounding.

Conclusion:

This study demonstrates that stress appraisal should be addressed in future epidemiologic investigation of LEs and breast cancer risk. Negative valence LEs seem to increase breast cancer risk in a dose-response fashion only when perceived as stressful. Younger women, women who are nulliparous and women who delayed age at childbirth seem to be particularly sensitive to the effects of LE stress. Additional research should be performed specifically examining the effect of LE stress appraisal on premenopausal breast cancer risk according to breast cancer molecular subtypes.

Chapter 4:

Life Events and Breast Cancer Risk Among Sister Pairs

Abstract:

Background: Early life stress and later life events (LLEs) in combination with genetic vulnerability contribute to neuroendocrine stress reactivity and subsequent stress-related pathology. Here we investigate the association between LEs and breast cancer risk among sister pairs.

Methods: 156 cases of primary invasive breast cancer and 156 matched older sister controls with no history of cancer were identified through the family registry of ovarian and breast cancer of Orange County, CA. Participants completed an epidemiological risk factor questionnaire querying about breast cancer risk factors and life events. Conditional logistic regression was used to estimate ORs and 95% CIs.

Results: There were 36 sister pairs where the control sister had an earlier age at first full term pregnancy (≤24 years/>24 years) compared to 9 pairs where the opposite was true (P<0.001). Control sisters had a higher level of education (49 pairs where the control sister had 'some college or more' and the case sister '<college education') compared to 11 where the opposite was true (P<0.001). Previous personal illness was associated with an approximately 3-fold increase in breast cancer risk ($_{adj.}$OR=3.36; 95% CI=1.86-6.10). Reporting ≥3 and ≥4 negative LEs events was associated with a non-significant increase in breast cancer risk ($_{adj.}$OR=1.46; 95% CI=0.81-2.62, $_{adj.}$OR=1.37; 95% CI=0.69-2.71 respectively). Among familial breast cancers, there was a stronger non-significant association between LEs and breast cancer than among non-familial breast cancers for every level of LEs.

Conclusions: These results support the importance of a life course gene-environment approach to understanding breast cancer risk associated with LEs. Previous personal illness stands out as a breast cancer risk factor above and beyond familial factors shared among sisters. Further study of developmental stress vulnerability periods in combination with genetic predisposition and LEs in relation to breast carcinogenesis is recommended.

Background:

Predisposition to the effects of life event stress:

Individuals respond differently to stressful situations. Some individuals will become resilient in the face of stress as compared to others who will develop pathology.[116] It is well established that the two main contributors to psychopathology are genetics and stress exposure.[28] The most commonly acknowledged stress-related psychiatric disorders are posttraumatic stress disorder (PTSD), major depressive disorder (MDD) and anxiety disorders. However, stress exposure also contributes to somatic conditions such as coronary heart disease and hypertension.[27]

Stress reactivity is believed to be the result of complex gene-environment interactions. Specifically, early life stress and later stressful life events in combination with genetic vulnerability have been identified as important contributing factors to stress-related pathology.[28,116] The magnitude of the glucocorticoid stress response has been associated with breast tumor incidence.[117] Here we investigate the impact of adulthood stress in the form of life events (LEs) on breast cancer risk among sister pairs.

Epidemiology: genetics and life event stress

The only study to our knowledge that has examined the effect of LEs on subsequent breast cancer risk among family members was performed by Lillberg *et. al* (2003). In this cohort and nested case-control study the OR estimate for major life events among twin pairs discordant for breast cancer (OR=1.88; 95% CI=1.12-3.13) was similar to the HR estimate from the population cohort design (HR=1.27; 95% CI=1.04-1.56).[48] As a

result, the authors concluded that shared familial factors do not influence the relationship between LEs and breast cancer risk.[48] However, because this was the only study to our knowledge to examine this relationship, we sought to further investigate the interplay of genetics, early life experiences and later life event (LLE) stress on breast cancer risk.

Genetics of stress reactivity:

To study the heritability of HPA axis reactivity, cortisol and ACTH levels are commonly measured and compared among monozygotic (MZ) and dizygotic (DZ) twin pairs in response to stressful stimuli.[27] For example, in response to repeated stress in the Trier Social Stress Test, MZ twins showed greater plasma cortisol levels and higher inter-pair correlations compared with DZ twins with a heritability estimate of up to 0.98.[118] Holsboer *et. al* (1995) investigated HPA reactivity to the dexamethasone-CRH (DEX-CRH) test in family members at risk for affective disorders and found that high risk individuals had higher cortisol levels and thus impaired HPA regulation in response to the DEX-CRH test compared to controls.[119]

Early life environment and stress reactivity:

Programming of neurobiological systems early in life impacts emotional regulation capabilities and susceptibility to stress and stress-related disorders later in life. Early life stress (ELS) such as low SES, maltreatment and social isolation[120] has been observed to modify brain structures and connectivity.[116] The lasting effects of ELS on the brain and its outflow systems including: autonomic, endocrine and immune lead to increased

sensitivity to stress and subsequent somatic disorders.[116] Perinatal and peripubertal stress are thought to be particularly important in programming long lasting modifications to the HPA (hypothalamic-pituitary-adrenal) and HPG (hypothalamic-pituitary-gonadal) axes.[121] Twin studies confirm the role of ELS beyond genetic predisposition in psychopathology such as major depression.[122] ELS influences stress regulatory systems including the autonomic nervous system and endocrine system.[116] Through aberrant regulation of stress systems, individuals may be at increased risk for both mental and physical illness.[123]

The perinatal period is thought to be a critical period during which the developing brain and endocrine system are particularly sensitive to the effect of endogenous steroid hormones.[124] Steroid hormones, particularly estrogens, are associated with breast cancer risk.[125] Therefore the neonatal period is of particular interest when studying early programming of breast cancer risk. Mouse models of ELS have shown that social isolation and prolonged maternal separation contribute to altered mammary gland ductal development which is associated with mammary pathology in adult mice.[124] Additionally, exposure to higher steroid hormone levels have been observed to increase the mouse mammary stem cell pool which are theorized to be an origin on breast cancer cells.[126]

Gene-environment interactions: stress reactivity

Childhood experiences seem to modulate physiological stress reactivity patterns in response to stressful events in adulthood.[127] Hence, it is important to examine how LEs influence breast cancer risk when controlling for the effects of ELS and shared genetics.

Subjects carrying genetic polymorphisms in transferases important for estrogen metabolism have greater breast cancer risk when also reporting a history of separation/divorce compared to subjects without these polymorphisms.[107] Given the evidence for susceptibility polymorphisms to the effects of LEs and the scarcity of studies on this topic, the goal of this study is to further investigate the association between LEs and breast cancer risk when partially controlling for shared genetics and ELS among sister pairs.

We hypothesized that LEs increase breast cancer risk in sisters beyond shared genetics and ELS. Sisters share on average 50% of their genetic material and overall have more similar early life environments compared to women not growing up in the same household. We therefore hypothesized that breast cancer risk associated with LEs among sisters in the present analysis will be different compared to the population analysis in Chapter 1. The present analysis of sisters discordant for breast cancer will facilitate the elucidation of a clearer picture regarding the impact of LEs on breast cancer risk.

Methods:

Study Population:

One hundred and fifty six sister pairs discordant for breast cancer were included in the present analysis. Population-based incident primary invasive breast cancer cases were identified within the database of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860) and 156 unaffected older sister controls with no history of cancer were included in the present analysis[78,79] (see Chapter 1 for more details).

Measures:

The parameters to significantly influence breast cancer risk in the population-based analysis performed in Chapter 2 were the specific adulthood *LE occurrence* parameters: previous personal illness (OR=2.15; 95% CI= 1.46-3.17), abortion (OR=0.57; 95% CI=0.35-0.93) and relocation (OR=0.65; 95% CI= 0.45-0.95) and *sum of negative LEs* (P_{trend}=0.008). Hence, the parameters of interest included in the present analysis were: (1) *LE occurrence:* (yes/no) and (2) *negative valence LE sum.* In the present investigation, the sum of negative LEs was analyzed as a continuous variable and as a binarized variable according to different thresholds to facilitate the study of dissimilar pairs for the *negative LE sum*. Cut points of *2 negative valence LEs* (≤2, >2), 3 *negative valence LEs* (≤3 , >3), and 4 *negative valence LEs* events (≤4 , >4) were used. The rational for these cut points was based on the distribution of negative valence LEs among control sisters.

Data Analysis:

Demographics and characteristics were compared among sister pairs discordant for breast cancer. Variables were binarized to allow adequate sample sizes for comparisons between sister pairs on particular covariates. McNemer's test was used to determine significant differences (P<0.05) between covariates among sister paris discordant for breast cancer. Univariate conditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) and compare *LE occurrence* (yes/no) and *sum negative LE* parameters among sister pairs. *Sum negative LEs* was analyzed as a continuous and dichotomized variable based on the following cut points: 2, 3, and ≥4 events. For example for the 2 event threshold dichotomization, the distribution of sisters with a congruent or incongruent number of events where one sister had >2 events and the other sister ≤2 events was determined. To assess genetic contributions to the LE and breast cancer relationship, a stratified conditional logistic regression was performed based on previous family history of breast cancer (+FH/-FH).

Variables identified in the literature[16,128] as influencing breast cancer risk and hence eligible for multivariate model inclusion were: *age* (age at diagnosis for cases and age at RFQ completion for controls), *smoking history* (ever/never), *alcohol use in previous year* (none/any), *BMI* (underweight [BMI<18.5], normal weight [18.5≤BMI≥24.9], overweight [25≤BMI≥29.9] and obese [BMI≥30]), *education* (less than college/some college or more), *physical activity level*, (not active/moderately active/very active) and reproductive variables: *age at menarche* (≤10, 11-13 and ≥14), *age at first full term pregnancy* (FFTP) (<25, 25-29 and ≥30), *parity* (nulliparous/parous), *menopausal status*

(pre/peri-menopausal/post-menopausal) and *hormone replacement therapy (HRT)* use (ever/never).

A manual forward selection model-building process was used to identify covariates significantly ($P<0.05$) influencing breast cancer risk, which were then added sequentially into the model. This addition continued until all variables entered into the conditional logistic regression model maintained a significance level of $P<0.30$ based on Wald-Chi square tests. We Used PROC LOGISTIC with a STRATA statement for family ID in matched pair conditional logistic regression analysis. Thereafter, we compared model AIC, BIC and R-squared statistics to select the best-fit model.

We adjusted the final multivariate model for education level and the composite variable parity/age at FFTP (nulliparous, age FFTP $\leq$24 age and age FFTP >24). We then used this model for the multivariate conditional logistic regression to calculate adjusted ORs and 95% CIs to determine significant differences in LE parameters. The significant *LE occurrence* parameters ($P<0.05$) indicated in the univariate analysis were then entered one at a time into the multivariate conditional logistic regression model. Additionally we used the multivariate model to delineate significant differences in the distributions of *negative LEs* among matched sister pairs after adjusting for covariates.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA)

Results:

Table 4.1 shows the distribution of demographics and characteristics among sister pairs discordant for breast cancer. In 36 sister pairs, the case sister had her FFTP at an older age (>24 years) than the control sister (≤24 years). Whereas, there were 9 sister pairs where the reverse was true (*P*<.0001). In 27 sister pairs the control sister was post-menopausal and the case sister pre/peri-menopausal whereas the reverse was true for only 2 sister pairs (*P*<.0001). The same pattern was observed for education level with the control sister having a higher education level in 49 sister pairs as compared to 11 pairs where the opposite was true (*P*<.0001). No significant (*P*>0.05) differences were observed for the variables: BMI, age at menarche, parity, HRT use, smoking, alcohol use and physical activity.

Table 4.1: Distribution of demographics and characteristics among 156* sister pairs discordant for breast cancer

Characteristic	Control Sister	Case Sister		**P-value
BMI: (N=153)		Normal Weight	Overweight/Obese	
	Normal Weight	46	31	1.000
	Overweight/Obese	31	45	
Age at menarche: (N=148)		≤13 years	>13 years	
	≤13 years	91	25	0.456
	≥14 years	20	12	
Age at first full-term pregnancy: (**N=107)		≤24 years	>24 years	
	≤24 years	31	36	<.0001
	>24 years	9	31	
Parity: (N=156)		Nulliparous	Parous	
	Nulliparous	7	20	0.758
	Parous	22	107	

Menopausal status: (N=149)		Pre/peri-menopausal	Post-menopausal	
	Pre/peri-menopausal	32	2	<.0001
	Post-menopausal	27	88	
Hormone replacement therapy: (N=140)		Never	Ever	
	Never	37	21	0.208
	Ever	30	52	
Education: (N=154)		<College	Some college or more	
	<College	49	11	<.0001
	Some college or more	49	45	
Smoking: (N=152)		Never	Ever	
	Never	61	26	0.777
	Ever	24	41	
Alcohol use in year prior: (N=155)		None	Any	
	None	25	31	0.796
	Any	29	64	
Physical activity In previous year: (N=94)		Not active	Active	
	Not Active	12	13	0.289
	Active	19	50	

*Number of pairs used for characteristics varied depending on different number of missing values
***P*-values: calculated based on McNeimer's test statistic for paired data

Univariate analyses of *LE occurrence* parameters indicated significant differences among sister pairs discordant for breast cancer for the parameters ‘history of personal illness’ (OR=2.91; 95% CI: 1.77, 4.77), ‘buying a home’ (OR=1.76; 95% CI: 1.08, 2.88) and ‘marriage of a child’ (OR=0.47; 95% CI: 0.26, 0.85) (Table 4.2). However, once controlling for education level and parity/age at FFTP, only ‘history of personal illness’ remained statistically significant. After adjustment of covariates, previous personal

illness was associated with an approximately 3-fold increase in breast cancer risk ($_{adj.}$OR=3.36; 95% CI: 1.86, 6.10) (Table 4.3).

Table 4.2: Odds ratios for breast cancer according to specific life event occurrence parameters among sister pairs discordant for breast cancer

		Case		OR	95% CI		**P* value
	Control	Yes	No				
Negative Valence Life Event Occurrence*							
Death of child	Yes	2	13	0.85	0.38	1.89	0.683
	No	11	130				
Death of parent	yes	82	12	1.67	0.82	3.41	0.162
	no	20	42				
Death of sibling	yes	31	38	0.71	0.43	1.16	0.175
	no	27	60				
Death of spouse	yes	6	20	1.05	0.57	1.94	0.876
	no	21	109				
Foreclosure	yes	3	12	0.50	0.19	1.33	0.166
	no	6	135				
Illness	yes	33	21	2.91	1.77	4.77	<.0001
	no	61	41				
Illness in family	yes	69	36	0.67	0.40	1.12	0.124
	no	24	27				
Job loss	yes	18	30	1.07	0.65	1.76	0.799
	no	32	76				
Positive Valence Life Event Occurrence							
Buying home	yes	42	25	1.76	1.08	2.88	0.024
	no	44	45				
Marriage	yes	58	31	1.36	0.85	2.16	0.200
	no	42	25				

Marriage of child	yes	17	34	0.47	0.26	0.85	0.013
	no	16	89				
Pregnancy	yes	39	30	1.33	0.83	2.14	0.234
	no	40	47				
Pregnancy of child	yes	4	29	0.76	0.44	1.32	0.329
	no	22	101				
Relocation	yes	13	32	0.81	0.48	1.36	0.432
	no	26	85				
Equivocal valence Life Event Occurrence							
Abortion	yes	3	21	0.67	0.34	1.31	0.240
	no	14	118				
Separation/divorce	yes	14	37	0.81	0.50	1.31	0.391
	no	30	75				

* Event valence was determined based on the Paykel Scale (1971) (see text in Chapters 1&2 for details)
** *P*-values calculated based on the McNeimer's test statistic for paired data

Table 4.3: Conditional multivariate logistic regression for significant univariate life event occurrence parameters

		Case		OR	95% CI		$_{adj.}$OR*	95% CI	
Life Event	Control	Yes	No						
Illness	yes	33	21	2.91	1.77	4.77	3.36	1.86	6.10
	no	61	41						
Buying home	yes	42	25	1.76	1.08	2.88	1.71	0.98	3.00
	no	44	45						
Marriage of child	yes	17	34	0.47	0.26	0.85	0.55	0.28	1.12
	no	16	89						

*$_{adj.}$OR: adjusted for parity/age at first full term pregnancy (nulliparous, age FFTP ≤24 age and age FFTP >24) and education level (less than college/some college or more)

As shown in Table 4.4, with our study sample we were unable to identify significant differences in the distribution of negative valence LEs among sister pairs. In 61 pairs the control sister had a greater total *negative valence LE sum* than the case sister. The opposite was true for 63 pairs in which the case sister had more negative LEs than the control sister ($_{adj.}$OR=1.10; 95% CI: 0.94, 1.28). However, when examining the distributions of events according to different binarization thresholds, we observed that as the number of events increased, LEs had a stronger impact on breast cancer risk. For occurrence of ≥3 and ≥4 events, there was a non-significant 46% increase ($_{adj.}$OR=1.46; 95% CI=0.81-2.62) and 37% increase ($_{adj.}$OR=1.37; 95% CI=0.69-2.71) in breast cancer risk respectively.

Table 4.4: Conditional multivariate logistic regression of negative life event summary parameters among sister pairs discordant for breast cancer

		Number of Pairs		OR	95% CI		$_{adj}$OR*	95% CI	
Variable	Congruent # of events	(# events case) > (# events control)	(# events control) > (# events case)						
Total Events[Ţ]	32	63	61	1.06	0.93	1.20	1.10	0.94	1.28
2 Event Cutoff[¥]	114	19	23	1.11	0.71	1.76	1.11	0.65	1.90
3 Event Cutoff[Δ]	91	37	28	1.32	0.81	2.16	1.46	0.81	2.62
4 Event Cutoff[†]	106	29	21	1.38	0.79	2.42	1.37	0.69	2.71

*P trend = 0.373

Ţ Negative Life Events included: Death of spouse, death of child, death of parent, death of sibling, foreclosure, illness in family, personal illness and job loss. Included as a continuous variable.

*$_{adj.}$OR: adjusted for parity/age at first full term pregnancy (nulliparous, age FFTP ≤24 age and age FFTP >24) and education level (less than college/some college or more)

¥ Incongruence for 2 event threshold: one sister had >2 events and the other sister ≤2 events

Δ Incongruence for 3 event threshold: one sister had >3 events and the other sister ≤3 events

† Incongruence for 4 event threshold: one sister had >4 events and the other sister ≤4 events

* P_{trend} calculated by incorporating negative sum as a continuous variable

Table 4.5: Multivariate logistic regression of life events among sister pairs discordant for breast cancer stratified by family history of breast cancer

Variable	Pairs concordant for negative life events	Pairs in which case sister had more events	Pairs in which control sister had more events	OR	95% CI		$_{adj.}$OR*	95% CI	
Total events									
Positive FH	10	20	11	1.26	0.94	1.68	1.22	0.89	1.67
Negative FH	22	43	50	1.01	0.88	1.17	1.08	0.89	1.30
Event Binarization Thresholds¥ :									
2 events									
Positive FH	23	12	6	2.00	0.75	5.33	1.71	0.54	5.40
Negative FH	59	27	29	0.93	0.55	1.57	1.00	0.53	1.90
3 events									
Positive FH	27	10	4	2.50	0.78	7.97	2.27	0.64	8.05
Negative FH	64	27	24	1.13	0.65	1.95	1.40	0.70	2.80
4 events									
Positive FH	26	10	5	2.00	0.68	5.85	2.04	0.62	6.75
Negative FH	80	19	16	1.19	0.61	2.31	1.20	0.50	2.87

¥ Example of binarization based on 2 event threshold: one sister had >2 events and the other sister ≤2 events

*$_{adj.}$OR: adjusted for parity/age at first full term pregnancy (nulliparous, age FFTP ≤24 age and age FFTP >24) and education level (less than college/some college or more)

FH: family history for breast cancer in 1st degree relative

When examining the association between LEs and breast cancer risk stratified according to +FH/-FH as a measure of shared genetics and epigenetics (Table 4.5), there was a non-statistically significant increase in breast cancer risk associated with *negative valence LE sum* specifically among familial breast cancers. For example, for the 3 and 4 event thresholds, the effect of LEs on breast cancer risk was more pronounced among familial breast cancers, (OR=2.27; 95% CI=0.64-8.05 & OR=2.04 95% CI=0.62-6.75 respectively) than among non-familial breast cancers (OR=1.4; 95% CI=0.7-2.8 & OR=1.2; 95% CI=0.5-2.87 respectively). The greater association between LEs and breast cancer risk was observed for all LE binarization thresholds.

Discussion

Despite the distribution of LEs being statistically similar between sister pairs discordant for breast cancer, a trend toward having higher numbers of LEs was observed among case sisters. Hence, LEs seem to contribute to breast cancer risk in adulthood regardless of shared familial factors as described previously.[48] However, when comparing breast cancer risk among sister pairs discordant for breast cancer, we are partially controlling for shared familial factors including early life environment and genetic makeup. Therefore, we conclude that LEs seem to be particularly impactful on future breast cancer risk when occurring to individuals who are susceptible to the effects of LEs in adulthood. Consistently, the OR for breast cancer associated with LEs was attenuated in the present sister analysis as compared to the population-based analysis in Chapter 2. Further supporting a gene x environment interaction, are the results from

the stratified analysis based on family history for breast cancer where increased breast cancer risk associated with LEs was observed specifically among sisters with a positive family history for breast cancer. Additional research is recommended to elucidate the precise genetic, epigenetic and early environmental factors contributing to susceptibility to later life stress on breast cancer pathogenesis.

Consistently, reactivity of the stress response is influenced by complex interactions between genetic and environmental factors. Specially, ELS and LEs along with genetic makeup influence glucocorticoid levels and vulnerability to stress-related disorders such as depression.[28,116] Furthermore, stressors in early life have been shown to alter mammary gland development.[124] and be associated with higher levels of the inflammatory marker C-reactive protein (hs-CRP).[120] Our recent work demonstrated that negative valence LEs promote breast cancer risk.[129] Therefore, it seems that LE stress are likely associated with increased breast cancer risk especially in individuals predisposed to elevated stress signaling and reactivity. Our findings are consistent with developmental theories describing stress during neonatal life, infancy and childhood as being particularly crucial for development of the neuroendocrine system and determining the baseline stress response and stress reactivity later in life.[121] The aforementioned alterations in stress reactivity and mammary susceptibility may contribute to carcinogenetic changes in the breast potentiated by LE stress.[130]

It is possible that despite statistically similar distributions of LEs among sister pairs, case sisters were more likely to appraise events as ‘stressful’ than controls. We have

recently demonstrated that the deleterious effects of adverse life events on breast cancer risk differ according to individual stress perception (Fischer, *et al*., in press). When an individual identifies a stressor to exceed coping abilities, distress arises.[91,92] Biopsychosocial models of cancer recognize the importance of individual perception of threat in addition to attitude toward the stressor and available coping resources in determining the contributions of psychosocial stressors to the tumor microenvironment.[67] Future research is recommended to determine if there are differences in stress perception among sisters discordant for breast cancer that experienced similar LEs.

Discordant sister pairs differed significantly in their age at FFTP, with control sisters having their FFTP at an earlier age. Breast tissue is not completely differentiated until completion of the FFTP, at which point it is hypothesized to become less susceptible to carcinogenic changes.[108] Therefore, the influence of LEs on breast cancer risk in the control group could have been counteracted by the protection conferred by earlier pregnancy. This finding is consistent with our previous observations (Fischer, et al, in press), where women who delayed age at first full term pregnancy were at increased breast cancer risk resulting from stressful life events.

This observation emphasizes the need to investigate interactive effects between LEs and other known breast cancer risk factors and especially reproductive risk factors.[66] Despite no observed statistically significant differences in the distribution of the *negative LE sum*, there were more sister pairs in which the case sister had a greater number of

>3 and >4 LEs events than the control sister (37 vs. 28 LEs and 29 vs. 21 respectively). This finding suggests that high exposures to LEs could increase breast cancer risk despite other protective characteristics such as earlier age at first full term pregnancy.

Other than having an earlier age at FFTP, the control sister usually had a higher level of education. It is possible that given similar SES at baseline, higher education acts as a protective factor diminishing breast cancer risk later in life by prompting more healthy life habits such as reduced alcohol consumption, healthy eating and increased physical activity. This finding nevertheless is discrepant with those reported in previous studies indicating a direct relationship between a higher education and increased breast cancer risk.[131] However, previous investigation has recognized that higher education is associated with later age at first full term pregnancy which researchers hypothesize to at least partially mediate the association between higher education and increased breast cancer risk.[132] In the present analysis, the controls who were more educated had an earlier age at FFTP which could be the factor driving the observed relationship between higher levels of education and decreased breast cancer risk. Consequently, the effect of education level on breast cancer risk among sisters is to be further investigated.

The only other epidemiological study to examine the effect of LE stress on breast cancer risk among siblings was conducted by Lillberg *et. al* (2003).[48] In their analysis of twin pairs discordant for breast cancer, a significant association between LEs and breast cancer risk was observed. The authors subsequently concluded that familial

factors do not play a substantial role in the relationship between LEs and breast cancer risk. In contrast, our results suggest that LEs contribute to breast cancer risk especially when occurring in combination with predisposing factors such as early life experiences and shared genetics. Consequently, we recommend integrating assessments about early life adversity such as SES, parental divorce, childhood abuse and neglect into life event questionnaires to better understand how these early life experiences in conjunction with LEs alter future risk of breast malignancy.

Stress in the form of previous personal illness was the only life event associated with a significant increase in breast cancer risk among matched sister pairs after adjusting for breast cancer covariates (OR=3.36; 95% CI: 1.86, 6.10). This finding is similar to that observed when comparing the same case group to a population-based control sample (OR=3.54; 95% CI: 2.52, 4.97).[129] This robust finding emphasizes previous comorbidities as an important risk factor for breast cancer risk. It is likely that physical and psychological distress resulting from previous personal illness additively contribute to neuroendocrine alterations and immune system breakdown thus facilitating breast cancer development regardless of genetic or early life susceptibility.[25]

There are important limitations that must be considered when interpreting our findings. First and foremost, we had a limited sample size of 156 sister pairs. This sample became significantly reduced when examining the distributions of LEs among discordant pairs. Therefore, it is possible that our sample lacked sufficient power to identify significant differences in LE parameters among sister pairs. Additional studies

with larger samples are therefore recommended. Our study, as in other case-control studies, relied on subjects' recall of LEs. Hence recall bias may reduce the validity of our findings. However, research shows that subjects recall major LEs with great accuracy and reproducibility.[89] Additionally, the robustness of the relationship between 'previous personal illness' in previous studies[48,50,129] and increased breast cancer risk reduces the likelihood that our results are an artifact of recall bias. Finally, our study was based on a sample of paired sisters who identify themselves as White. Consequently to increase the generalizability of the findings, future studies should include more ethnically diverse populations.

Despite some limitations, the strengths of the present study should be mentioned. To our best knowledge, this is the first study analyzing the effects of LEs on breast cancer risk among sister pairs. The case-control design including sister pairs, with older sister controls, has an advantage over the twin study performed by Lillberg *et. al* (2003).[48] By selecting older-sister controls we increased the degree of certainty with which the researchers could ascertain that these sisters were actually controls for the disease of interest. Older sister controls who have passed the age of breast cancer onset of their paired younger sister are less likely to develop breast cancer since the age at breast cancer onset is genetically influenced. Cases were identified from the population-based cancer registry of Orange County, which increases the generalizability of our findings. This study is the first epidemiological study to examine the effect of LEs on breast cancer risk while controlling for familial factors in this population and in the U.S. in general.

Future studies are recommended to validate the hypotheses of the present study regarding the importance of ELS in the development of breast cancer. It is advised that following studies assess the unique and interactive effects of exposure to ELS (violence, abuse, neglect, severe family dysfunction and low SES) and LEs within a larger sample of sister pairs discordant for breast cancer. Such exposures have been associated with adulthood morbidity specifically in relation to cardiovascular disease, autoimmune disorders and premature mortality.[133] Thus the possible impact of stress exposure in childhood on the programming of the neuroendocrine mechanisms responsible for stress signaling and epigenetic alterations contributing to immunological and hormonal dysfunction and increasing breast cancer risk in later life.

The present research sheds light on the importance of familial factors in establishing susceptibility to the effects of LEs on increased breast risk. The identification of critical time periods of stress exposure in relation to breast cancer risk will allow for more targeted approaches to breast cancer prevention. The present analysis suggests that targeted interventions to reduce breast cancer risk should begin as early as pre-natal life and continue throughout childhood and adulthood to mitigate the harmful effects of negative stress. It is expected that such interventions will decrease vulnerability to the deleterious effects of later life stress on breast cancer development.

Chapter 5

The Integrative Relationship Between Life Events, Antidepressant Medication and Breast Cancer Risk

Abstract:

Background: Breast cancer is the most prevalent female malignancy and antidepressant (AD) medication use is increasingly common among women. However, the link between stress, depression and breast cancer risk is still speculative. Within the scope of this study, we aim to better understand the relationship between stress and breast cancer by studying the integrative relationship between life events, antidepressant medication use and breast cancer risk. The goal of the present research is to examine patterns of AD use in light of LEs among breast cancer cases and controls and study the relationship between AD medication use and duration of use and breast cancer risk.

Methods: A case-control design including 609 cases and 194 population-based controls was used. Multivariate logistic regression was used to calculate ORs and 95% CIs.

Results: Breast cancer cases began use of ADs on average 5 years later (44.3 years) than controls (39.3 years) (P<0.05). 'Ever' use of ADs was associated with a 43% reduction in breast cancer risk (OR=0.57; 95% CI=0.34-0.97) when controlling for previous personal illness or negative life event sum. Use of '1 year or less' of ADs was associated with a 51% reduction in breast cancer risk ($_{adj.}$OR =0.49; 95% CI= 0.25 0.96). This protective effect was no longer observed for '2 or more years' of use ($_{adj.}$OR= 1.07; 95% CI=0.58-1.98).

Conclusions: AD use is correlated with the occurrence of negative valence LEs. Breast cancer cases and controls are similar in their AD use patterns in response to LEs. Further analysis controlling for depression when investigating the AD and BC relationship is needed.

Introduction:

Stress, life events and depression

Depression and anxiety result from the collective and interactive effects of biology, environment and adverse life events.[134] The majority of evidence supporting the relationship between exposure to stress and onset of depression is based on occurrence of episodic negative or undesirable stressors that have clear start and end points, in other words, the occurrence of adverse life events.[135] A linear relationship between the number and distressfulness of adverse life events and onset of depression has been repeatedly observed, with the likelihood of depression increasing with higher numbers of adverse life events.[136,137] The nature of specific life events leading to depression onset has also been explored with depression most commonly arising after interpersonal loss such as death or separation.[138]

Depression, antidepressants and breast cancer epidemiology

Approximately 9-11 percent of middle-aged women in the United States are depressed and 23% of women between the ages of 40-59 are currently taking anti-depressant medication.[139] Breast cancer is the most prevalent female malignancy and comprised an estimated 30 percent of incident cancers among women in the United States in 2017.[140] The high prevalence of depression and anti-depressant medication use among women along with breast cancer being the most common female malignancy, leads to speculation regarding the relationship between the two.

Epidemiology of depression and breast cancer risk

The potential association between depression and subsequent breast cancer risk has been topic of debate for many years. The epidemiological evidence regarding this association has been mixed and therefore a firm conclusion has been difficult to reach.[141] A recent meta-analysis of cohort studies showed a statistically insignificant 13% increase in breast cancer risk resulting from depression (RR=1.13; 95% CI=0.94-1.36).[141] However, there was substantial variability in the results of epidemiological investigation into this association. The studies included in the meta-analysis that measured depression with the Diagnostic Interview Schedule (DIS) based on the DSM diagnosis of depression, showed a significant increase in breast cancer risk for depressed individuals. Therefore, some of the variability in the findings could be due to differences in the way depression was operationally defined. The magnitude of effect varied from 4.4 fold (HR=4.4, 95% CI=1.08-17.6)[142] to 17.2 fold (OR=17.2, 95% CI=3.76-78.08)[143] increase in breast cancer risk. Nevertheless, other studies have reached null findings and do not support an association between depression and subsequent breast cancer risk.[144–146]

Depression physiology and cancer initiation

Depression may etiologically contribute to breast cancer initiation and/or promotion. Depressed individuals have dysregulated HPA signaling resulting in chronically elevated cortisol levels and an impaired ability to normalize cortisol levels after stressful stimuli have subsided.[147] In vitro experiments have shown that glucocorticoids could increase mammary tumor growth and at dysfunctionally elevated levels interfere with normal

immune surveillance.[68,86,148] Depression also results in a chronic inflammatory state characterized by high levels of inflammatory cytokines and abnormally low levels of anti-tumor cytotoxic T cell and natural killer cell activity.[149] Individuals with anxiety and major depressive disorder (MDD) have elevated levels of the pro-inflammatory cytokine IL-6 that have been associated with multiple myeloma, non-Hodgkin's lymphoma and chronic lymphocytic leukemia.[150]

Epidemiology of anti-depressant medication use and breast cancer risk

In a similar fashion to the study of depression and subsequent breast cancer risk, the investigation into the relationship between antidepressant medication use and subsequent breast cancer risk has led to inconclusive results. Anti-depressant medications may serve as a marker of depression severe enough to require medical attention or may themselves alter breast cancer risk. In the New York University Women's health study, following 15,270 women prospectively, there was a 39 percent increase in breast cancer risk for women taking any psychotropic medication (RR=1.39; 95% CI=1.11-1.74).[151] Similarly, another study found that the use of selective serotonin reuptake inhibitor (SSRI) antidepressant medication was associated with a 53 percent increase in breast cancer risk (RR=1.53; 95% CI=1.14-2.03).[152] Conversely, many studies have reached null findings. The direction of association also varied among null studies with some studies showing a protective effect[153–155] and others a deleterious effect of antidepressant medication on breast cancer risk.[156,157]

Antidepressant medication and breast cancer risk

Anti-depressant (AD) medications are commonly used to treat depression and anxiety disorders.[158] The use of ADs has been steadily increasing, with the rate of AD use doubling from 1996 to 2005.[159] Currently, ADs are the most frequently used medication among 18-44 year olds in the United States.[139] An estimated 78% of AD prescriptions are intended to treat depression and depressive disorders.[158,160] Selective serotonin reuptake inhibitors (SSRIs) are currently the most commonly prescribed class of antidepressants.[159] Tricyclic antidepressants (TCAs) were the most commonly prescribed antidepressant medication to treat depression until the introduction of SSRIs in the 1980s.[161] The long-term therapeutic use of available ADs has been shown to normalize glucocorticoid levels, despite the precise mechanism behind this phenomenon being unclear.[147,162] Further, ADs have anti-inflammatory qualities and thus may neutralize the deleterious inflammatogenic state characterizing depression.[160] These properties support a potential protective effect of AD medication on subsequent breast cancer risk.

However, there is also evidence suggesting that antidepressant medications may actually increase cancer risk.[163] Both TCAs and SSRIs have been associated with breast cancer risk and cancer promotion in animal models via stimulation of the anti-estrogen receptor.[163] SSRIs have also been associated with increased circulating prolactin levels, a peptide hormone that has been observed to increase cellular proliferation and angiogenesis.[164] Epidemiological investigation has related increased prolactin levels with both premenopausal and postmenopausal breast cancer risk,

despite there being a stronger association with postmenopausal breast cancer risk.[165,166]

Thus far, we have shown that stressful adverse life events are cumulatively associated with increased breast cancer risk (Chapters 2 & 3). We also know that life events are causally associated with depression.[136] Patients with depression have elevated cortisol levels, and therefore depression is thought to be a mediating factor in the relationship between negative valence LEs and breast cancer risk. Consequently, we will use the initiation of anti-depressant medication as a method of quantification of dysfunctional regulation of the physiological stress response. To our knowledge, this is the first study of its kind to attempt to integrate the relationship between adverse life events, depression and breast cancer risk. We hypothesize that women who experienced more adverse life events are more likely to be depressed, initiate AD medication and resultantly be at increased breast cancer risk.

It is possible that women who go on to develop breast cancer after negative valence LEs are fundamentally more susceptible to the deleterious effects of stress on breast cancer risk. The interaction between adverse life events and breast cancer case/control status in predicting AD medication use will be used to evaluate the hypothesis that breast cancer cases are more susceptible to stress in the form of life events. Antidepressant medication will be used as a proxy to the presence of a hyperactive stress response, theorized to predispose women to the harmful effects of life events on breast cancer risk.

Methods:

Study Population:

Six hundred and twenty nine population-based incident primary invasive breast cancer cases and 197 population-based controls were part of the Hereditary Breast and Ovarian Cancer (HBOC) study of the University of California, Irvine (CA58860) [78,79] are included in the present analysis (see Chapter 1 for more details).

Measures:

Participates were asked whether they had ever taken antidepressant medication. Those who were taking antidepressant medication at time of questionnaire completion or who had taken antidepressants in the past were considered *'ever'* antidepressant medication users as compared to *'never'* antidepressant users who had no history of ever taking antidepressant medication. Further, participants were asked to report age at initial use of AD medication and duration of use. Because of small numbers, we were not able to achieve high resolution of duration of antidepressant medication use and subsequently the following intervals were used to evaluate duration of AD use: never use (baseline), $\leq$1 year and $\geq$2 years of use. Individuals who reported age at initial antidepressant medication use to be later than age at breast cancer diagnosis were excluded from subsequent analyses. Life event measures used were described in the context of Chapters 2 & 3 and included: (1) *LE occurrence:* (yes/no), (2) *negative valence LE sum:* 0 (baseline), 1, 2+ events. Only life events occurring prior to age at initial use of AD medication were considered here.

Data Analysis:

Demographics and characteristics were evaluated based on 'ever'/'never' use of antidepressant medication prior to breast cancer diagnosis. Means and standard deviations were calculated for continuous variables and frequencies and percentages were computed for categorical variables. Student t-test and Chi-square statistics were evaluated with a $P<0.05$ significance threshold to determine statistically significant differences between descriptive variables among AD users and non-users.

The following covariates were recognized in the literature[167,168] as risk factors for depression and hence were candidate variables for model building: *age* (age at first use of antidepressants or age at RFQ completion), *BMI* (underweight [BMI<18.5], normal weight [18.5≤BMI≥24.9], overweight [25≤BMI≥29.9] and obese [BMI≥30]), *race/ethnicity* (non-Hispanic white [European ancestry], Hispanic, non-Hispanic black [African American], Asian and other), *marital status* (not married/cohabitating; married/cohabitating) , *smoking in past year* (ever/never) , *education* (less than college/ some college or more), *employment* (employed, homemaker, unemployed/disabled, retired, other), *physical activity in past year* (not active, moderately active, very active), *number of chronic medical conditions* $(0,1,\geq 2)$. Medical conditions included in the chronic somatic illness index were: arthritis, asthma, bronchitis, diabetes, emphysema, epilepsy, gout, kidney disease, lupus, neuropathy, thyroid disease, ulcerative colitis, cancer and endometriosis. Medical condition selection was based on information in Herva *et. al* and medical knowledge of the researchers.[168] A standard stepwise unconditional logistic regression model building process took place and a baseline

model was established based on goodness of fit statistics. The baseline model was used for subsequent multivariate unconditional logistic regression analyses.

Univariate and multivariate logistic regression was used to determine the association between the *sum of negative LEs* and anti-depressant use. Categories of *sum negative LEs* (0, 1 and ≥2) were based on creating a '0 event' baseline and dividing the remaining distribution of events in the control group. 'Ever' use of antidepressant medication was compared to 'never' use in the unconditional logistic regression models. A stratified analysis based on breast cancer case/control status was performed to evaluate if the association between *sum negative LEs* and *AD use* was different among cases and controls. A (case/control status)x (*sum negative LEs*) interaction term was used to test whether breast cancer status was a modifying variable in the association between *sum negative LEs* and AD medication use. These analyses were performed for LEs and separately for LEs that were perceived by the participants as 'stressful' i.e. stressful life events (SLEs).

A similar model building processes took place to identify covariates associated with breast cancer risk. Details of this model building process are outlined as part of Chapters 2 & 3. Univariate unconditional logistic regression was used to compute odds ratios (ORs) and 95% confidence intervals (CIs) for breast cancer risk depending on 'ever'/'never' use and 'duration of use' of antidepressant medication prior to breast cancer onset. Breast cancer case/control status was the outcome variable of interest. 'Ever use' of AD and 'duration of AD use' were the independent variables investigated. When analyzing the effect of 'duration of AD' use on breast cancer risk, '≤1 year' and '≥ 2 years' use of AD medication was compared to a 'never use' baseline in the logistic

regression models. The multivariate breast cancer model from the stepwise model building process was used to adjust for known breast cancer risk factors and covariates and compute adjusted ORs. Two additional multivariate models were used to assess the relationship between AD 'use' and 'duration of use' and breast cancer risk while adjusting for 'previous personal illness' or 'LE negative sum' in addition to adjusting for the other breast cancer covariates identified in the stepwise model building process. This allowed the investigation into the association between antidepressants and breast cancer among individuals with similar stress exposure known to influence depression risk and previously demonstrated to influence breast cancer risk.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results:

The distributions of demographics and characteristics according to 'ever'/'never' use of ADs are presented in Tables 5.1. AD 'ever' users were more likely to be of white ethnicity (92.1% vs. 87.8%), have a history of smoking (60.4% vs. 45.6%), less than a college education (72.3% vs. 60.7%), be unemployed/disabled (13.8% vs. 3.9%), and have a history of '2 or more' chronic medical conditions (37.4% vs. 5.1%). The overall mean age of antidepressant medication initiation of use was 42.8 years. Age at start of AD use was significantly younger (P=0.048*)* among breast cancer controls (39.3 years) compared to breast cancer cases (44.3 years). AD users were also more likely to have

a positive history of HRT use (66.7% vs. 52.1%) and to have had their first child either before the age of 25 (51.1% vs. 42.5%) or after 30 years of age (18% vs. 12.5%).

Table 5.1: Demographics and characteristics among antidepressant users vs. non-users

	Anti-Depressant Medication Use				
Characteristic	Never		Ever		*P* value
Age, years: mean, s.d.	57.03	11.60	42.76	11.30	<.0001
	N	%	N	%	
Breast cancer status					
Case	509	76.7	100	71.9	0.238
Control	155	23.3	39	28.1	
BMI					
Underweight	16	2.4	1	0.7	0.556
Normal Weight	335	50.6	70	50.7	
Overweight	195	29.5	39	28.3	
Obese	116	17.5	28	20.3	
Race					
White	583	87.8	128	92.1	0.031
Hispanic	44	6.6	6	4.3	
Black	1	0.2	7	1.4	
Asian/Pacific Islander	36	5.4	3	2.2	
Marriage status					
Not married/cohabitating	222	33.5	56	40.9	0.098
Married/cohabitating	441	66.5	81	59.1	
Smoking (past year)					
Never	359	54.4	55	39.6	0.002
Ever	301	45.6	84	60.4	
Education					
<College	402	60.7	99	72.3	0.011

Some college or more	260	39.3	38	27.7	
Employment					
Employed	346	52.4	75	54.4	<.0001
Homemaker	150	22.7	20	14.5	
Unemployed/Disabled	26	3.9	19	13.8	
Retired	131	19.9	20	14.5	
Student/Other	7	1.1	4	2.9	
Physical activity in past year					
Not active	282	42.5	57	41.0	0.937
Moderately active	201	30.3	44	31.7	
Very active	181	27.3	38	27.3	
Chronic medical conditions					
0	450	67.8	53	38.1	<.0001
1	180	27.1	34	24.5	
≥2	34	5.1	52	37.4	
Alcohol frequency					
Never	232	36.0	56	42.1	0.186
Ever	412	64.0	77	57.9	

* sums may not add up to totals because of missing data

Negative life events were individually and cumulatively associated with increased AD use (Table 5.2). Experiencing even one adverse life event substantially increased the odds of taking an antidepressant medication ($_{adj.}$OR=10.22; 95% CI=2.99-34.92). Experiencing '2 or more' adverse life events increased AD use 32-fold ($_{adj.}$OR=32.03; 95% CI=12.31-83.35). When modeling adverse life events as a continuous variable, there was a significant dose response relationship between negative valence life events and the odds of AD medication use ($p<0.0001$). For each additional adverse life event

reported there was close to a 3-fold increase in odds of antidepressant medication use ($_{adj.}$OR=2.76; 95% CI=2.14-3.57). 'Previous personal illness' as a life event was identified as a breast cancer risk factor (Chapter 2,3). Here we demonstrate that history of 'stressful personal illness' was associated with an approximately 5 fold increase in odds of taking an antidepressant medication ($_{adj.}$OR=4.93; 95% CI=2.51-9.68).

Table 5.2: Life events and subsequent use of antidepressant medication

Life event parameter		Antidepressant Use									
		Never		Ever							
		N	%	N	%	OR	95% CI		*$_{adj.}$OR	95% CI	
Illness	No	394	88.0	54	12.05						
	Yes	270	76.1	85	23.94	2.30	1.58	3.34	2.08	1.09	3.96
Stressful Illness											
	No	577	88.0	79	12.04						
	Yes	87	59.2	60	40.82	5.04	3.36	7.55	4.93	2.51	9.68
Negative LE Sum											
	0 Events	500	92.6	40	7.4						
	1 Event	66	83.5	13	16.46	2.46	1.25	4.84	10.22	2.99	34.92
	≥2 Events	98	53.3	86	46.74	10.97	7.11	16.92	32.03	12.31	83.35
	Continuous LE Scale					2.15	1.88	2.47	2.76	2.14	3.57
						P_{trend}<.0001			P_{trend}<.0001		

*$_{adj.}$OR: Odds Ratios adjusted for age, race/ethnicity, smoking, education, physical activity level, illness index, employment, marital status

CI: Confidence Interval

Breast cancer case/control status does not appear to moderate the impact of negative life events on AD medication use ($P_{(interaction\ status\ x\ negsum)}$=0.892). However, the impact of adverse life events on AD medication use seemed to be stronger among breast cancer

cases then among controls (Table 5.3). Both cases and controls had substantially higher odds of taking AD medication given a history of ≥2 adverse life events ($_{adj.}$OR=27.14; 95% CI=4.93-149.29 for controls and $_{adj.}$OR=43.18; 95% CI=12.16-153.38 for cases). However, experiencing a single adverse life event was significantly associated with AD medication use only among cases ($_{adj.}$OR=4.92; 95% CI=0.72-33.53 for controls, $_{adj.}$OR=15.25; 95% CI=3.00-77.59 for cases). However, because of a larger sample size available for cases, this result may be the result of higher power in the case group. The results were similar when restricting the analyses to only stressful adverse life events.

Table 5.3: Negative life events and antidepressant use by breast cancer case/control status

Controls (N=194)										
Sum Neg. LEs	Anti-Depressant Use									
	Never		Ever							
	N	%	N	%	OR	95% CI		**$_{adj.}$OR	95% CI	
0 Events	119	90.8	12	9.2	Reference					
1 Event	24	82.8	5	17.2	2.07	0.67	6.41	4.92	0.72	33.53
2+ Events	12	35.3	22	64.7	18.18	7.24	45.64	27.14	4.93	149.29

Cases (N=609)										
Sum Neg. LEs	Anti-Depressant Use									
	Never		Ever							
	N	%	N	%	OR	95% CI		**$_{adj.}$OR	95% CI	
0 Events	381	93.2	28	6.9	Reference					
1 Event	42	84.0	8	16.0	2.59	1.11	6.05	15.25	3.00	77.59
2+ Events	86	57.3	64	42.7	10.13	6.13	16.73	43.18	12.16	153.38

*$P_{interaction}$ (status*negsum)=0.8917

**$_{adj.}$ORs: Adjusted Odds Ratios for age, race/ethnicity, smoking, education, physical activity level, illness index, employment, marital status

The results of the univariate and multivariate logistic regression analyses examining the relationship between AD medication 'use' and 'duration' and their impact on subsequent breast cancer risk are presented in Table 5.4. 'Ever use' of antidepressant medication did not alter breast cancer risk when adjusting for breast cancer covariates ($_{adj.}$OR=0.87; 95% CI=0.55-1.38). However, when further adjusting for the stressful life event 'previous personal illness', AD use had a significant 55% reduction in breast cancer risk ($_{adj.}$OR=0.45; 95% CI=0.27-0.77). Similarly, when further adjusting for 'negative LE sum', AD 'ever' use was associated with a 43% reduction in breast cancer risk

(OR=0.57; 95% CI=0.34-0.97). When analyzing the effect of duration of antidepressant medication use on the risk of breast cancer, '1 year or less' use of antidepressant medication was associated with 51% reduction in breast cancer risk compared to 'never use' of antidepressant medication ($_{adj.}$OR=0.49; 95% CI= 0.25-0.96). This protective effect was no longer observed for '2 or more years' of antidepressant medication use ($_{adj.}$OR= 1.07; 95% CI=0.58-1.98). This effect persisted for all models.

Table 5.4: Anti-depressant use and duration and breast cancer risk

	Controls		Cases							
AD Use	N (194)	%	N (609)	%	OR	95% CI		*adj.OR	95% CI	
Never	155	80.7	509	85.8	Reference					
Ever	39	20.1	100	16.4	0.78	0.52	1.18	0.87	0.55	1.38
					**adj.OR	95% CI		***adj.OR	95% CI	
					0.45	0.27	0.77	0.57	0.34	0.97

AD Duration of Use	Controls		Cases							
	N (194)	%	N (609)	%	OR	95% CI		*adj.OR	95% CI	
Never	155	80.7	509	85.8	Reference					
≤1 year	19	9.9	31	5.2	0.50	0.27	0.90	0.49	0.25	0.96
≥2 years	18	9.4	53	8.9	0.90	0.51	1.58	1.07	0.58	1.98
					**adj.OR	95% CI		***adj.OR	95% CI	
					0.24	0.11	0.52	0.34	0.16	0.70
					0.68	0.35	1.31	0.80	0.42	1.54

AD: Antidepressant

*adj: adjusted for age, age at FFTP, menopausal status, family history of breast cancer, HRT use, smoking, education, race/ethnicity and physical activity level

**adj: further adjusted for occurrence of stressful previous illness other than breast cancer

***adj: further adjusted for LE negative sum

≤1 year and ≥2 years duration of use are compared to 'never' use when computing Odds Ratios

Totals for "duration of us' and 'ever use' numbers may be discrepant because some individuals that reported AD use did not report duration of use

Discussion:

This analysis does not support our original speculation that depression, as we attempted to quantify by the use of anti-depressant medication, is a mediating variable in the pathway from adverse LEs to increased breast cancer risk. We were able to demonstrate that LEs are significantly associated with the use of antidepressant medication in a dose response fashion, consistent with the causal relationship between stressful life events and depression demonstrated in the literature.[136] Nevertheless, we think that we were not able to distinguish the effect of depression from that of ADs given the data available to us. Therefore, it could still be possible that depression is a mediating variable but with the data available to use we were unable to disentangle the effect of depression from the effect of AD medication use on breast cancer risk.

'Ever' use of antidepressant medication had a significant protective effect on breast cancer risk only when controlling for basal stress among women, whether in the form of previous personal illness or as the sum of negative life events. Controlling for the effect of 'previous illness' had the most prominent effect and revealed a 64% reduction in breast cancer risk. This result underscores the importance of studying the relationship between antidepressants and breast cancer among individuals that have similar baseline physiological stress levels, which are influenced by both physical illness and psychological stress.[102]

Antidepressant medication use for the duration of 'one year or less' was demonstrated here to have a protective effect on subsequent breast cancer risk in all models tested.

There is evidence pointing toward AD medication having a protective effect on subsequent breast cancer risk through normalization of the stress response, immune and hormonal systems.[147,160,162] On the flip side, studies have shown AD medication use to be associated with increased breast cancer risk through their direct stimulation of the anti-estrogen receptor[163] and through their indirect effects of increasing prolactin levels.[164–166]

Therefore, it is conceivable that ADs have a differential influence on breast cancer risk depending on duration of use. It seems that at shorter durations of use the benefits of AD medication could outweigh their potential harm. Conversely, at longer durations of use, the adverse effects of ADs seem to counteract their potential benefits. However, this is just speculation and would need to be studied further with larger samples and a more fine-tuned assessment of AD duration of use, serum measures of cortisol levels and immune function assays. In a meta-analysis from 2012, the investigators found a statistically significant increase in breast cancer risk for shorter durations of SSRI use (less than 1-2 years, OR=1.10; 95% CI=1.02-1.19) but were not able to show statistical significance with longer durations of use.[169] Despite the direction of effect being discrepant from ours, this analysis supports the notion that antidepressants may impact breast cancer risk differently depending on duration of use.

Given our observation that controls initiated antidepressant medication on average 5 years earlier than cases, it is likely that they had more contact with their medical provider for follow-up visits. Therefore, they may have been treated earlier for

suspected breast cancer and hence prevented the development of invasive breast cancer studied here. However, since we are observing a different effect of antidepressants on breast cancer risk, depending on duration of use, we think that the antidepressants themselves are likely responsible for the protective effect we are observing.

It is possible that the protective effect we observed for shorter durations of AD use is specific to TCAs since our study population was ascertained in the 90s, a time when the use of TCAs was still common for the treatment of depression. Current use of TCAs was observed to have a marginally protective effect on breast cancer risk (OR=0.86; 95% CI=0.73-1.00).[155] Although not statistically significant, a meta-analytic study demonstrated that 'less than 1-2 years' use of TCA had a protective effect on breast cancer risk (OR=0.93; 95% CI=0.84-1.04). This finding contrasted the trend toward a harmful effect of longer use of TCAs and any duration of SSRI use.[169]

Breast cancer cases and controls seem to be somewhat different in their response to stressful life events. Women who did not have a history of breast cancer were more likely to begin an antidepressant medication 5 years earlier in life as compared to breast cancer cases. This could be the result of personality differences among cases and controls. Controls may be more willing to face their problems and seek help in the face of depression/anxiety. Breast cancer patients have been observed to score higher on scales of anti-emotionality (lack of emotional behavior and trust in personal feelings) prior to development of their cancer relative to controls.[170] Therefore individuals who go

on to develop breast cancer may be more reluctant to acknowledge the need for medical attention and hence less likely to seek out help in the face of depression. These results do not support our original hypothesis that breast cancer cases have a more vulnerable stress response leading to higher likelihood of antidepressant use in the face of adverse life events. However, we postulate that that cases are actually more depressed than controls but because of personality differences are not seeking the medical attention they require.

Limitations:

There are important limitations to this study that should be considered when interpreting our findings. Because we did not have a direct measure of depression or depression severity in this study, we were not able to disentangle the effect of depression itself from the effect of antidepressant medications on breast cancer risk. Brown et al, 2015, adjusted for depression in models of AD medication and for AD medication use in models of depression in an attempt to analyze their specific effects. Analogously to our study, they did not observe a significant effect of antidepressants on breast cancer risk when adjusting for known breast cancer risk factors.[160] However, when we further adjusted for measures of life events, a significant protective effect of antidepressant medication on breast cancer risk was observed. Hence, it is possible that the true relationship between ADs and breast cancer may have been masked in previous studies that did not control for stress exposure parameters such as life events. Additionally, we were not able to determine the precise timing from life events to anti-

depressant initiation since were only had information on timing of life events in 10 year intervals.

A further limitation of our study is that we did not have information on individual AD medications used since they were all grouped under the category of 'anti-depressant medication' in our questionnaire. Therefore, we were unable to examine the effect of specific AD medication on breast cancer risk. Although we had a large sample size, when performing stratified analyses based on duration of antidepressant medication use our subcategories did not allow the examination of a more fine-tuned relationship between AD duration and subsequent breast cancer risk.

Strengths:

Our study is unique in that it attempts to synthesize the information we know thus far about the relationship between LEs, antidepressant use and breast cancer risk. This study highlights the importance of considering other covariates in models of breast cancer. We adjusted for the stress exposure variables: 'previous personal illness' or 'sum negative LEs' found to be significantly associated with increased breast cancer risk in Chapters 2 & 3.

These variables were not considered in previous multivariate models looking at the relationship between AD use and breast cancer risk.[169] Therefore, we believe that our results provide important information that may have been masked in previous studies regarding the association between AD medication and breast cancer risk. Our rich

dataset allowed adjustment for both depression risk factors and breast cancer risk factors in two separate multivariate models reducing the risk of confounding.

Future directions:

Future analyses should be performed on datasets including information on adverse life events, depression, antidepressant medication and breast cancer risk to disentangle the impact of depression from that of AD medication on breast cancer risk. The impact of duration of AD use on breast cancer risk should be evaluated in a more fine-tuned fashion with assessments on a monthly scale, as compared to yearly scales employed thus far. Additionally, we recommend further investigation into specific antidepressant medications, since they may have different effects on breast cancer risk. Further, to better understand the influence of AD medication on breast cancer, we propose to perform a case-only analysis where the effect of 'AD use' and 'duration of use' on tumor characteristics are studied. Specifically, we hypothesize that breast cancer cases who had taken antidepressant medication prior to their breast cancer onset, will have a later age at diagnosis, more favorable stage at diagnosis and improved survival.

Conclusions:

This study underscores the importance of controlling for baseline psychological and physiological stress when addressing the impact of AD medication on breast cancer risk. Breast cancer cases and controls are very similar in their AD use patterns. However, there is a possibility that a beneficial balance between pro and anti tumorigenic properties exists at shorter durations of AD use. However at longer

durations, the pro-tumor properties seem to counterbalance the anti-tumor properties of AD drugs. The influence of depression and AD medication on breast cancer risk and the role of LEs in this relationship should be further examined in larger sample sizes.

Chapter 6:

Discussion and Future Directions:

What We Know and Where We are Going

State of the art: breast cancer

Despite our progress toward understanding risk factors and pathophysiology of breast cancer, the cause of breast cancer initiation is largely unknown.[171] Consequently, clinicians and researchers are still unable to predict breast cancer onset in many individuals.[3,172] Overall, breast cancer mortality rates have been improving. Nevertheless, incidence rates are trending upward in the U.S. and around the world.[173,174] Strategies focusing on prevention and early detection are expected to be the most effective toward reducing breast cancer incidence.[173] In order to improve prevention efforts, we need to improve our understanding of normal breast physiology, identify additional breast cancer risk factors and use the knowledge we have gained thus far to better target women at heightened breast cancer risk.

Breast cancer prediction models

The Gail model[175] (1989) was developed to aid the prediction of 5-year and lifetime invasive breast cancer risk. The Breast Cancer Risk Assessment Tool (BCRAT) is available online for public use through the National Cancer Institute as a resource to help approximate breast cancer risk (https://www.cancer.gov/bcrisktool/). This assessment tool is based on risk factors described in the Gail model and includes: history of carcinoma in situ, BRCA1/2 mutation status, age (>35 years), age at menstruation, age at first full term pregnancy, number of first-degree relatives with breast cancer, previous breast biopsy results, and race/ethnicity.[176] However, the

predictive ability of this model in quantifying individualized breast cancer risk is limited and therefore additional models are being explored.[172]

Limitations of current predication models

Given ER+ breast cancer is both the most common subtype of invasive breast carcinoma (~75%)[173] and the subtype that we have greatest understanding of risk factors, prediction models are most useful for predicting ER+ breast cancers. A women's reproductive characteristics, such as age at menstruation and age at first full term pregnancy included in the Gail model are most pertinent to HR+ breast cancers.[16] Epidemiological risk factors for TNBC seem to be substantially different than risk factors for non-TNBCs (HR+/HER2+).[23] The most well established protective factor for TNBC is longer duration of breast-feeding.[16,23] Nevertheless, additional research is needed to better understand risk factors specific to breast cancer molecular subtypes to facilitate individualized breast cancer risk prediction.

Psychosocial stress as an additional breast cancer risk factor

The role of stressful negative valence life events in promoting breast cancer risk, as discussed in Chapters 2 and 3, supports the investigation of inclusion of psychosocial risk factors in future predictive models of invasive breast cancer. Our analyses point to the role of stress in breast cancer pathogenesis particularly among younger women (Chapter 3). Although breast cancer is relatively rare among women <40 years of age (5-7% of cases), the incidence of breast cancer in this age group is increasing.[177]

Further, breast cancer in young women is generally more aggressive, carries a poorer prognosis and has a higher proportion of tumors that are TN for endocrine markers.[16,178] Therefore, breast cancer prediction models that include psychosocial risk factors may be most applicable to younger women for which available models are limited.

Life event stress and breast cancer risk according to molecular subtype

The only study identified which analyzed the effect of life events on subsequent breast cancer risk when stratifying according to ER+/ER- subtypes was performed by Schoemaker *et. al* (2016).[83] The authors concluded that overall life events in the preceding 5 years are not associated with breast cancer risk. However, when assessing risk depending on ER status, a history of separation/divorce was associated with a 54% (OR=1.54; 95% CI=1.01-2.34) increase in ER- breast cancer risk. This study supports our hypothesis that stress signaling may differentially influence breast cancer according to breast cancer molecular subtypes and particularly influence hormone receptor negative breast cancers. We suggest performing additional analyses with a more comprehensive stratification according to ER/PR/HER-2 status and particularly TNBC, which we anticipate to be most influenced by the effects of stressful life events.

GC/GR signaling in the breast

Identification of additional aberrant signaling pathways on the way to breast cancer will hopefully allow identification of signaling receptors and cascades to target in breast cancer prevention. Elucidating additional targets for treatment will be especially important in preventing and treating TNBCs that do not express markers we have

targeted treatments for. The role of the glucocorticoid receptor (GR) in the normal mammary gland[33,34] and mammary tumorigesis[35] has attracted research interest. Glucocorticoids are important in normal mammary development and physiology.[33,34] The GR is a nuclear transcription factor that influences gene expression in response to glucocorticoids.[35] Changes in expression of the GR have been identified to potentially influence tumorigenesis in the mammary gland.[39,40] Consistently, a recent study demonstrated GR expression in the majority of breast cancer specimens.[36] Glucocorticoid mRNA levels were observed to be higher in ER- breast cancers compared to ER+ breast cancers, highlighting the importance of glucocorticoid signaling specifically in ER- breast cancers.[179]

Glucocorticoids and chemotherapy outcomes

Prognosis of TNBCs has been observed to depend on levels of GR expression. Highest levels of GR expression are correlated with poorer long-term prognosis.[180] In a TNBC model, the synthetic glucocorticoid dexamethasone reduced the potency of Paclitaxel chemotherapy by impairing apoptosis.[181] The pro-survival effect of GC administration is at least in part mediated by activation of the anti-apoptotic genes *SGK1* (serum and glucocorticoid-inducible protein kinase-1) and *MKP1/DUSP1* (mitogen-activated protein kinase phosphatase-1).[182,183] Consistently, administration of the GR antagonist mifepristone significantly reduced expression of *SGK1* and *MKP1/DUSP1* in GC-treated ER- breast cancer cell lines[183] and potentiated the effects of chemotherapy on ER- and TN breast cancers.[184] Mutations in the GR-encoding gene (*NR3C1)* are among the most

commonly observed in TNBCs.[185] Taken together, this data supports the role of the GR as an important driver in TNBC and ER- breast cancer biology.[184]

The importance of understanding the influence of glucocorticoids in breast cancer outcomes is twofold. Firstly, glucocorticoids are widely used in clinical oncology as antiemetic and anti-inflammatory agents to stimulate appetite and to control adverse reactions to radiation therapy.[22,36] Secondly, the GR may be an important target to augment the efficacy of chemotherapeutics. The response to chemotherapy is influenced by GR+/GR- status of breast tumors. Specifically, tumors that express both the ER and GR are associated with better outcomes than those that do not express the GR.[36,180] The higher the GR expression in ER- tumors, the less responsive the tumors are to chemotherapy and the higher the rates of recurrence.[180]

Estrogens and glucocorticoids in the breast

Estrogens and glucocorticoids have interactive effects on cell survival and proliferation, which warrant further investigation to better understand the role of physiological stress signaling in breast growth and cancer pathogenesis.[186] The influence of increased GR signaling on inferior outcomes in ER- and TN breast cancers specifically emphasizes the need for further research.[22,180] Given the emerging understanding of the role of glucocorticoids in breast cancer prognosis and facilitation of apoptotic escape, the function of the GR in breast cancer initiation and promotion should be further examined. It would be particularly informative to examine the gene-environment interactions

between genes in the GR signaling pathway and life event stress in breast cancer tumorigenesis according to molecular subtypes.

Interaction between life events and other breast cancer risk factors

It is possible that the reason there has been so much ambiguity in the field investigating the relationship between life event stress and breast cancer risk is partially because of the limited assessment of interactions between known breast cancer risk factors and stressors such as adverse life events.[66] In support of this notion, our Chapter 4 analysis demonstrated that among sister pairs discordant for breast cancer, control sisters were more likely to have had children at an earlier age (<24 years). Given that the distribution of life events did not significantly differ among sister pairs discordant for breast cancer, it is possible that an earlier age at first pregnancy reduced the adverse effects resulting from negative valence life events.

Comorbidities and breast cancer risk

The robust finding that previous personal illness contributes to breast cancer risk even when accounting for appraised stress (Chapter 3) and familial factors (Chapter 4), supports the importance of previous comorbidities in increasing breast cancer risk. Personal illness defined as 'serious illness or injury of oneself', contributes to both psychological and physiological stress. Consistently, our analysis in Chapter 5 indicated that having history of 'previous personal illness' was associated with a 5-fold increase in odds of taking an antidepressant medication. This finding underscores the apparent relationship between physical illness and psychological stress. Further, the way an

individual responds to a stressful situation is impacted by their physical wellbeing.[25] Consequently, previous personal illness seems to be a significant stressor contributing to breast cancer risk. The magnitude of effect remained very similar across analyses and ranged from a 2.8 to 3.6-fold increase in breast cancer risk.

Future work:

Timing of life events

The presented research suggests that stress in the form of life events contributes to breast cancer risk when occurring repeatedly over time (Chapter 2). This is consistent with the known differences between the effect of chronic and acute stress. Acute stress could actually enhance immune function, while chronic stress impairs immune function thus contributing to adverse health effects and disease.[66] In addition to the effects of chronicity, stress may have a different impact depending on timing of stressors. It is therefore recommended to examine vulnerability periods to the effect of life event stress. Early life stressors are known to have an important influence on future HPA baseline activity and reactivity to stressors[121] in addition to influencing immunological function.[187] Consequently, stressors in early life are hypothesized to be particularly detrimental to breast cancer susceptibility later in life.

It is recommended that future research include assessments of early life events in addition to later events in adulthood. In our analysis in Chapter 4 we observed that when controlling for shared familial factors among sister pairs (both environmental and genetic), the effects of later life events were reduced. In the stratified analysis based on

FH of breast cancer, LEs were observed to be particularly impactful among sister pairs with a genetic predisposition to breast cancer. We therefore hypothesize that early life exposures, genetics and later life events interactively contribute to breast cancer risk. It is conceivable that specific adulthood life events are more likely to be particularly stressful after experiencing certain childhood events.[187] Future investigation is therefore warranted to decipher how and when stressors influence breast cancer risk.

Physiological stress quantification

In order to better quantify the effects of stress on breast cancer risk, it is recommended to incorporate stress biomarkers into the epidemiological study of life event stress and breast cancer pathogenesis. The main human glucocorticoid, cortisol, is thought to be a main mechanistic contributor to breast cancer development in response to stressors.[40,42,43,68,69] Perceived stress, shown to be important in influencing the effects of stress on breast cancer risk (Chapter 3), has been associated with higher hair cortisol concentrations in middle-aged women.[93] Cortisol is a lipophilic compound and diffuses into the hair from nearby capillaries. Hair grows at an average rate of 1cm per month and therefore can be used to quantify chronic physiological stress resulting from exposure to stressful life events.[188,189]

In addition to determining baseline cortisol levels, evaluation of individual variations in HPA reactivity in response to acute stressors will allow a more fine-tuned understanding of long-term cortisol exposure among individuals. Higher laboratory salivary cortisol

levels have been measured as a metric of stress reactivity.[190] We propose to measure experimental cortisol reactivity in addition to longer-term chronic cortisol levels from hair extracts to better understand how life event stress influences stress physiology and reactivity to future stressors. Analysis of the relationship between physiological stress markers and breast cancer risk will allow a better understanding of whether and how psychosocial stressors influence breast carcinogenesis.

Immunological assays

Stress hormones including glucocorticoids, norepinephrine and epinephrine selectively interfere with the Th1 cellular based immunity, which is the most important for tumor cell neutralization. Specifically, neuroendocrine stress hormones interfere with the production of IL-12, the main interleukin to stimulate Th1 activation, and do not interfere with IL-10, an inhibitor of cellular immunity.[66] These alterations contribute to a Th1 to Th2 shift in response to cortisol signaling. To elucidate the precise mechanism behind the relationship between life event stress and breast cancer risk, it is recommended to measure IL-12, IL-10 and TNF-alpha to establish the perturbations in immune function potentially contributing to breast cancer pathogenesis. Additionally, natural killer (NK) cells are activated by Th1 cells and are crucial players in tumor cell neutralization.[191] Compromised NK cell function has been observed after death of a spouse.[192] Similarly, women who had recently separated/divorced from their spouse demonstrated lower NK and Th1 cells[193]. Therefore, future work could utilize measurements of NK cell function toward a mechanistic understanding of the presented epidemiological findings.

Measures of chronic inflammation

Further, a state of chronic inflammation is thought to contribute to a tumor microenvironment. Chronic inflammation results in elevated reactive nitrogen and oxygen species, which could damage DNA and contribute to neoplastic change.[71] Therefore, future studies should also quantify inflammatory states using the pro-inflammatory cytokines considered to be the most established biomarkers of chronic inflammation including interleukins IL-6, IL-1, TNF-alpha along with C-reactive protein (CRP).[188] Higher levels of TNF-alpha impair expression of MHC-I complexes, which are important in presentation of tumor antigens to the immune system.[66] Therefore, not only does a state of chronic inflammation contribute to an environment conducive to cancer but also interferes with immune surveillance of existing tumor cells.

Genetic markers

Our analysis in Chapter 4 points to the importance of familial factors in determining the impact of later life events on breast cancer risk. Therefore, it is recommended to investigative genetic and early life contributors to stress signaling and vulnerability. Future examination of the significance of GC/GR signaling according to breast cancer molecular subtype and particularly in TNBC is recommended. Investigation into interactions between SNPs in genes involved in stress signaling such as *SGK1* and *MKP1/DUSP1* genes, which facilitate the pro-survival effects of glucocorticoids[182,183], and *NR3C1*, encoding the GR[185], with life event stress should be considered when evaluating gene-environment interactions contributing to breast cancer risk. Epigenetic

alterations in response to troubled childhood relationships have been observed in the exon 1F *NR3C1* promoter, which is associated with higher HPA activity at baseline and in response to stressful stimuli.[194] Such epigenetic modifications are the most likely link between early environment and future susceptibility to disease[187] and warrant further investigation in relation to breast cancer risk.

Improvements to epidemiological stress assessment

In addition to obtaining biomarkers and genetic data in order to improve the quantification of physiological stress and potential contributors to the tumor microenvironment, it is recommended to improve current epidemiological scales investigating the effect of life event stress on breast cancer risk. Specifically, it is advised to include a measure of event valence on a -5 to +5 continuum to future scales utilized for the study of life event stress and breast cancer risk. Additionally, the assessment of the subjective experience of life events will aid in understanding their impact. Thus, our investigation supports the development and use of an adapted life event scale, which incorporates levels of perceived stress on a gradient from non-stressful to highly stressful. The most commonly used life event scale used in the epidemiological investigation of life events and breast cancer risk only incorporates adulthood life events.[49] We therefore suggest integrating questions about early life adversity such as SES, parental divorce, childhood abuse and neglect to better understand how these early life experiences in conjunction with later life events alter future risk of disease and breast cancer in particular.

Psycho-behavioral interventions

Psycho-behavioral interventions aimed at improving stress management skills have showed improved survival and recurrence profiles among breast cancer patients. Specifically, a cognitive behavioral stress-management intervention[195] reduced all cause mortality and breast cancer specific mortality among breast cancer patients.[196] Such interventions are thought to allow restoration of psychobiological signaling to their healthy baseline.[188] Psychosocial interventions aimed at improving stress-management and coping skills through cognitive behavioral therapy (CBT) and mindfulness based stress reduction[197] (MBSR) in response to major life events should be investigated as a means to mitigating their effects on future breast cancer risk. Stress markers such as cortisol and immune system functional assays would be helpful in quantifying the effectiveness of such proposed interventions.[188]

Pharmacological intervention

Pharmacological interventions are another avenue to be explored in attempt to mitigate the deleterious effects of stress.[67] Specifically, antidepressant medication could be a promising avenue of future research. Antidepressants normalize cortisol levels[147,162] and suppress the inflammatory state[160] thought to contribute to the cancer microenvironment. The analysis as part of Chapter 5 suggested that shorter durations of antidepressant medication use (≤1 year) could be beneficial toward reducing breast cancer risk. When controlling for the experience of adverse life events, we observed that 'ever use' of antidepressant medication could be helpful in combating the effects of stress. However, a larger sample size is needed in addition to the direct assessments of

depression and specific antidepressant medication type in order to draw conclusions from these observations.

Summary:

The present research provides important insight into the influence of psychological stress on breast cancer risk. It has been demonstrated that not every major life change contributes adversely to breast pathology. Event desirability and predictability and hence event valence seems to dictate how events influence breast cancer risk. Negative valence life events appear to have a promoting effect on breast cancer risk. Conversely, positive valence life events potentially serve as a buffer to the harmful effects of distress arising from negative valence events. The cumulative nature of adverse events seems to be important in determining their influence on breast cancer tumorigenesis.

Personal appraisal of stress further impacts how stressors influence breast cancer risk. Among events that were negative in nature, only those that were also perceived as 'stressful' contributed to increased breast cancer risk. Therefore, it is not only the nature of the stressor that is important, but also the individualized perception of these events as 'stressful' which is influenced by attachment style, internal working models, coping resources and attitude toward stressors. Younger women, women who did not have children or who delayed age at first full-term pregnancy were the most influenced by appraised stress on future breast cancer risk.

The differing associations between LEs and breast cancer risk in the population analysis in Chapter 2 and sister analysis in Chapter 4, highlight the likelihood of genetics and early life experiences contributing to susceptibility to the effect of stress in the form of adulthood LEs on breast cancer risk. In support of the gene x environment paradigm proposed, the influence of LEs on breast cancer risk seems to be particularly prominent among sisters with a previous family history of breast cancer. Control sisters were more likely to have had their FFTP at an earlier age than their matched case sister. Therefore, it seems that the interactions between LE stress and other breast cancer risk factors, particularly those pertaining to reproductive history, are important when determining the effect of psychological stressors on breast cancer risk. The life event 'pervious personal illness' was associated with increased breast cancer risk in all analyses, emphasizing the importance of pervious comorbidities in increasing breast cancer risk.

Our integrative analysis of life events, antidepressant use, and breast cancer risk provided important insight into the possible mechanistic relationship between psychological stressors and elevated breast cancer risk. Despite overall no major differences in the pattern of antidepressant medication use among breast cancer cases and controls, shorter durations of antidepressant medication use seemed to be somewhat protective over future breast cancer risk. This observation underscores the importance of continued research into the potential stress and inflammation balancing properties of antidepressant medication as a potential intervention aimed at reducing the impact of adverse events on breast cancer promotion. Our findings underscore the

importance of controlling for baseline psychological and physiological stress when addressing the impact of AD medication on breast cancer risk.

Conclusions:

This work has led to the synthesis of a bio-psychosocial model of breast cancer (Figure 6.1). Psychosocial risk factors add to the tremendous complexity of breast cancer tumorigenesis. Stressful, negative valence life events appear to be important in breast cancer promotion, particularly when occurring repeatedly over time. Genetic predisposition and early life experiences likely modify the impact of later life events on future breast cancer risk. Pharmacological and psychosocial interventions should be further investigated in an attempt to normalize neuroendocrine and inflammatory biomarkers resulting from stressful exposures, which contribute to the breast cancer microenvironment. Psychosocial stress seems to be more influential on breast cancer risk in young women, who are not protected by other reproductive variables known to alter breast cancer risk such as parity and earlier age at first full term pregnancy. We hypothesize that life events are particularly important in increasing ER- and TN breast cancer risk. This research sheds a ray of light on the need to expand our personalized medicine approach to women's health while integrating mind and body to combat breast cancer development.

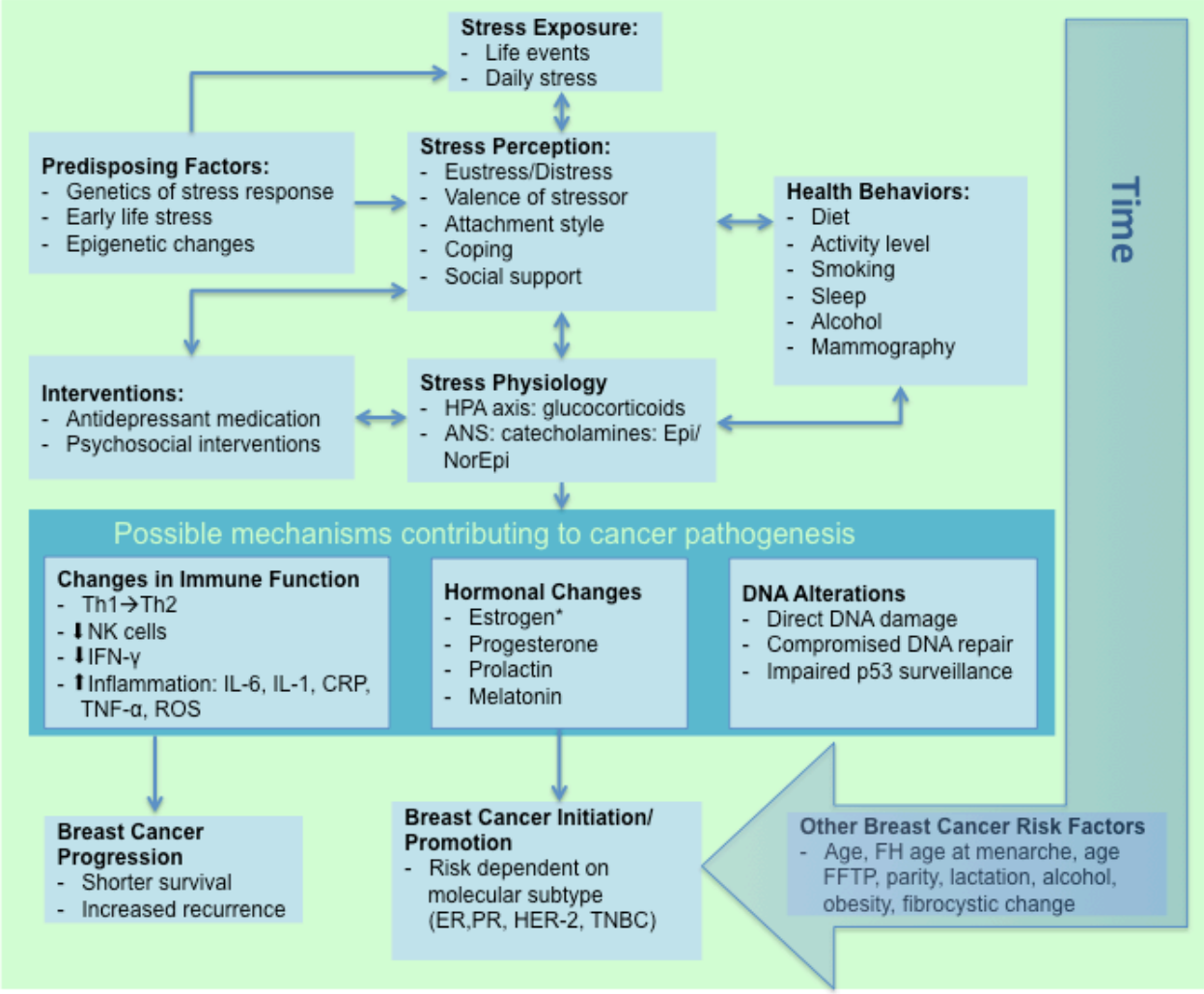


Figure 6.1: Bio-Psychosocial Model of Breast Cancer: The proposed model shows the integrative relationship between psychological and physiological stress and recognized breast cancer risk factors in breast cancer initiation and promotion. Psychological stress arises as a result of daily stress and life event exposure. Stressor valence, perception of stressors and available buffering resources alter the physiological stress response contributing to proposed carcinogenic changes in the breast. Genetic makeup and early life experiences modify mammary gland and physiological stress signaling susceptibility to the effects of later life stressors. Health behaviors are potential modifiers of stress carcinogenesis and can alter the way stressors influence physiology. Psychosocial and pharmacological interventions could attenuate stress physiology resulting from exposure to stressful stimuli. Together, the aforementioned factors interact with other breast cancer risk factors over time to determine individualized breast cancer risk and survival.

*Estrogen: main hormone known to be involved in breast cancer pathogenesis.
HPA axis: hypothalamic-pituitary-adrenal axis, ANS: autonomic nervous system, Epi: epinephrine, NorEpi: norepinephrine, CRP: C-reactive protein, ROS: reactive oxygen species, NK cells: natural killer cells, Th: T helper lymphocyte, IFN-γ: interferon gamma, TNF-α: tumor necrosis factor alpha, IL: interleukin.

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Appendix 1: Previous medical illnesses reported according to breast cancer case/control Status

Illness	Controls		Cases		OR	95% CI		P value
	N (203)	%	N (664)	%				
Anemia	62	30.5	173	26.1	0.80	0.57	1.13	0.208
Asthma	75	37.0	235	35.4	0.93	0.67	1.30	0.686
Bronchitis	22	10.8	71	10.7	0.99	0.59	1.63	0.954
Diabetes	6	3.0	19	2.9	0.97	0.38	2.46	0.944
Emphysema	1	0.5	7	1.1	2.15	0.26	17.60	0.464
Epilepsy	3	1.5	3	0.5	0.30	0.06	1.51	0.123
Fibrocystic Breast Disease	37	18.2	212	31.9	2.10	1.42	3.11	0.000
Gallstones	8	3.9	55	8.3	2.20	1.03	4.70	0.037
Goiter	23	11.3	113	17.0	1.61	0.99	2.59	0.051
Gout	6	3.0	8	1.2	0.40	0.14	1.17	0.083
Heart Attack	1	0.5	15	2.3	4.67	0.61	35.56	0.102
Hepatitis	6	3.0	19	2.9	0.94	0.97	0.38	2.455
High Cholesterol	47	23.2	102	15.4	0.60	0.41	0.89	0.010
Hypertension	24	11.8	104	15.7	1.39	0.86	2.23	0.177
Immune Disorder	4	2.0	10	1.5	0.76	0.24	2.45	0.646
Kidney Stones	5	2.5	29	4.4	1.81	0.69	4.73	0.221
Lupus	3	0.5	1	0.5	0.92	0.09	8.86	0.940
Osteoporosis	9	4.4	22	3.3	0.45	0.74	0.33	1.631
Neuropathy	3	1.5	9	1.4	0.92	0.25	3.42	0.896
Pneumonia	40	19.7	137	20.6	0.77	1.06	0.71	1.570
Psoriasis	8	3.9	25	3.8	0.91	0.95	0.42	2.148
Ulcers	11	5.4	39	5.9	0.81	1.09	0.55	2.168
Stroke	2	1.0	4	0.6	0.56	0.61	0.11	3.350
Thyroid Disease	13	6.4	67	10.1	0.11	1.64	0.89	3.037
Ulcerative Colitis	2	1.0	7	1.1	1.07	0.22	5.20	0.932
Endometriosis	22	10.8	54	8.1	0.73	0.43	1.23	0.233
Ovarian Cysts	39	19.2	111	16.7	0.84	0.56	1.26	0.411
Uterine Cysts	35	17.2	85	12.8	0.70	0.46	1.08	0.109
Pelvic Inflammatory Disease	10	4.9	17	2.6	0.51	0.23	1.13	0.090
Polycystic Ovaries	4	2.0	9	1.4	0.68	0.21	2.24	0.528

Appendix 2: Breast cancer univariate odds ratios for life events perceived as non-stressful

	Cases (N=664)		Controls (N=203)		95% CI			
	N	%	N	%	OR	Lower CI	Upper CI	P value
Negative valence events								
Death of child	6	0.9	1	0.5	1.84	0.22	15.39	0.567
Death of parent	97	14.6	25	12.3	1.22	0.76	1.95	0.411
Death of sibling	59	8.9	9	4.4	2.10	1.02	4.32	0.039
Death of spouse	12	1.8	4	2.0	0.92	0.29	2.87	0.880
Foreclosure	7	1.1	0	0.0				0.142
Illness	62	9.3	5	2.5	4.08	1.62	10.29	0.001
Illness in family	66	9.9	11	5.4	1.93	1.00	3.72	0.048
Job loss	22	3.3	11	5.4	0.60	0.28	1.26	0.170
Positive valence events								
Buying home	125	18.8	40	19.7	0.95	0.64	1.41	0.780
Marriage	199	30.0	57	28.1	1.10	0.77	1.55	0.605
Marriage of child	94	14.2	27	13.3	1.08	0.68	1.70	0.758
Pregnancy	136	20.5	41	20.2	1.02	0.69	1.50	0.930
Pregnancy of child	45	6.8	15	7.4	0.91	0.50	1.67	0.764
Relocation	33	5.0	8	3.9	1.27	0.58	2.81	0.546
Equivocal valence events								
Abortion	16	2.4	6	3.0	0.81	0.31	2.10	0.665
Separation/divorce	33	5.0	9	4.4	1.13	0.53	2.40	0.755
Summary variables *Sum negative valence events*								
0 events	526	79.2	161	79.3				0.227
1 events	46	6.9	18	8.9	0.78	0.44	1.39	
2 events	30	4.5	13	6.4	0.71	0.36	1.39	
3 events	21	3.2	6	3.0	1.07	0.43	2.70	
4+ events	41	6.2	5	2.5	2.51	0.98	6.46	
**P trend*	0.1706							

Sum positive valence events								
0 events	408	61.5	128	63.1				0.826
1 event	67	10.1	18	8.9	1.17	0.67	2.04	
2 events	53	8.0	17	8.4	0.98	0.55	1.75	
3-4 events	81	12.2	20	9.9	1.27	0.75	2.16	
5+ events	55	8.3	20	9.9	0.86	0.50	1.49	
Sum total events								
0 Events	385	58.0	123	60.6				0.4312
1-2 Events	118	17.8	30	14.8	1.26	0.80	1.97	
3-4 Events	58	8.7	21	10.3	0.88	0.52	1.51	
5-6 Events	37	5.6	15	7.4	0.79	0.42	1.48	
7+ Events	66	9.9	14	6.9	1.51	0.82	2.77	