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Life Course Health Effects of Early Life Trauma

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LIFE COURSE HEALTH EFFECTS OF EARLY LIFE TRAUMA

by

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ABSTRACT

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The experience of trauma and psychosocial stress over the life course can have lasting impacts on health. Childhood and adolescence may serve as a particularly sensitive period during which trauma may become biologically embedded and affect adult health. Evaluating the pathways through which early life trauma can affect biological mechanisms of disease development provides insight into the early origins and etiology of adverse health outcomes in adulthood and provides potential targets to help mitigate their effects.

Using data from the Sister Study, a large prospective cohort study of women residing in the U.S. or Puerto Rico aged 35 to 74 with a sister previously diagnosed with breast cancer, we examined the relationship between early life trauma and three indicators of adult health—1) incident breast cancer risk, 2) DNA methylation of the glucocorticoid receptor gene (*NR3C1*), and 3) leukocyte telomere length. Much of the previous literature has depended upon summative trauma scores, traditional trauma domains (e.g., sexual trauma, physical trauma, household dysfunction), or single traumatic events to represent early life adversity. However, early life traumatic experiences rarely occur in isolation and the experience of trauma in early life is a risk factor for future revictimization. This dissertation utilizes a latent class approach to evaluate the effect of specific profiles of early profiles on the three measures of adult health and biological embedding of trauma, as well as uses a sensitive period life course model to examine whether

early life trauma impacts 1) *NR3C1* methylation and 2) leukocyte telomere length in adulthood independent of later life trauma or if adult trauma mediates these associations.

The findings from this dissertation suggest that the relationship between early life trauma and these three indicators of adult health are complex. There were no associations between traditional measures of early life trauma (e.g., summative scores or trauma domains) and incident breast cancer risk; however, compared to women who were classified in the latent class of early life trauma consisting of low early life trauma (i.e., the average probability of reporting any type of early life trauma was less than 2% across all possible traumatic events), the rate of incident breast cancer overall, as well as pre- and post- menopausal breast cancer, appeared higher among women belonging to the latent class of early life trauma consisting of both sexual trauma and family drug, alcohol, and/or mental health issues. This latent class of early life trauma was also associated with hypomethylation of cytosine-phosphate-guanine (CpG) sites located in the gene body of *NR3C1*. This association was also observed in a sensitive period life course model whereby the direct effect of early life trauma on decreased methylation was independent of trauma in adulthood. Finally, leukocyte telomere length was statistically significantly shorter in women classified in the latent class consisting of high early life trauma experience (i.e., the average probability of reporting each early life traumatic event was approximately 32%) compared to women classified in the latent class of low early life trauma experience independent of any indirect effect through trauma in adulthood.

The findings from this dissertation emphasize that the effect of early life trauma on incident breast cancer risk, NR3C1 methylation, and leukocyte telomere length in adulthood may be more nuanced than what is captured via a cumulative trauma score, assessment of a single traumatic event, or traditional trauma domains can capture. Traumatic experiences differ in

duration and intensity and various traumatic experiences tend to cluster and cooccur, likely contributing to unique patterns of biological embedding, behavioral responses, and socioenvironmental trajectories that may further differentiate how early life trauma affects adult health. Measures that capture the unique combinations of concomitant early life trauma profiles are needed to fully elucidate the effects of different experiences of early life trauma on adult health. Doing so can assist in designing programs and early life interventions to address those trauma profiles that are most detrimental to future health. Furthermore, the experience of trauma can occur across the life course. Utilizing life course models can help to better understand the relationship between the experience of early life trauma and adult manifestations of the biological embedding of stress and trauma. The findings from this dissertation contribute to a more robust understanding of the relationship between early life trauma and incident breast cancer risk, adult circulating NR3C1 methylation, and leukocyte telomere length in adulthood and support the need to identify opportunistic windows for interventions over the life course in order to minimize these negative health outcomes and their impacts. Future research will need to continue to utilize complex measures of early life trauma and a life course framework before a unified picture of the association between the nuanced roles of early life trauma in shaping these health outcomes can be fully elucidated.

To my family,

Dr. E. Yong-Woo, Dr. M. Woo, Dr. K. Woo, and Dr. B. Woo.

Finally completing the set.

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ACE: Adverse childhood experience

BC: Breast cancer

BMI: Body mass index

C: Cytosine

CFI: Comparative fit index

CpG: Cytosine-phosphate-Guanine

DNA: Deoxyribose nucleic acid

ER: Estrogen receptor

G: Guanine

GR: Glucocorticoid receptor

H_0: Null hypothesis

H_a: Alternative hypothesis

HER2: Human epidermal growth factor receptor 2

HPA: Hypothalamic-pituitary-adrenal axis

kbp: Kilobase pairs

mDNA: DNA methylation

m*NR3C1*: *NR3C1* methylation

mRNA: messenger ribonucleic acid

NR3C1: Nuclear receptor subfamily 3, group C, member 1

PR: Progesterone receptor

RMSEA: Root mean square error of approximation

RNA: Ribonucleic acid

SE: Standard error

SEM: Structural equation modeling

SEP: Socioeconomic position

T/S ratio: ratio of the relative amount of telomeric DNA (T) to the relative amount of DNA from the single-copy control gene (S)

ACKNOWLEDGEMENTS

I find it apropos that the final curveball of my Epidemiology PhD journey has been thrown hard and fast by a global pandemic. It is intriguing to think that we are experiencing an event that will be studied by epidemiologists for years to come alongside the 1854 cholera outbreak in London that lead to Dr. John Snow's famous map and the 1918 influenza pandemic. Furthermore, as I have been writing this dissertation on the biological embedding of trauma during early life, I cannot help but ponder on how the COVID-19 pandemic is affecting the future health of young people around the world. The actions of parents/caregivers and society as a whole and the individual resilience of young people will all likely play a role in the long-term effects of COVID-19 on the health of these individuals.

I would first like to thank my advisor, Dr. Helen Meier, for continuing to foster my love of epidemiology and for her constant support and motivation throughout my PhD journey. I am so grateful that she took a chance on me when I needed an escape route and I am proud to not only be the first graduate of the Epidemiology PhD program at the University of Wisconsin-Milwaukee Joseph J. Zilber School of Public Health, but also her first PhD advisee. I would like to thank my entire committee, Dr. Paul Auer, Dr. Amanda Simanek, Dr. Rebecca Headley Konkel, and Dr. Dale Sandler, for their mentorship and guidance both on this dissertation, on other projects, and in life. Thank you to those Zilber faculty and staff who have supported me and have helped me grow throughout my PhD experience.

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CHAPTER 1

Introduction

1.1 Introduction

The experience of trauma and psychosocial stress over the life course has the potential to impact current as well as future health outcomes[1, 2]. Childhood trauma and adversity, experienced before age 18, have been associated with health risk behaviors[3-6], mental health conditions[7-10], chronic disease[11-16], and overall poor health[17, 18]. Life course theory posits that early life environments may affect later health outcomes by "getting under the skin" and acting during developmentally sensitive periods or by contributing damage to biological systems that accrue over time. In a sensitive period model, early life trauma and adversity have lasting effects on biological structures and functions and long-term health outcomes, independent of subsequent adverse exposures[19]. Alternatively, in accumulation models, early life trauma and adversity may take part in a web of risk factors that span the life course and contribute to the accrual of biological damage or they may serve as early links in a chain of risk factors that lead to a final risk factor (i.e., adult trauma) that ultimately triggers the health outcome of interest[19, 20]. Evaluation of the pathways through which early life trauma acts on biological mechanisms of disease development and cellular aging provides insight into the early life origins and etiology of adverse health outcomes.

Three indicators of adult health that may be accelerated by the biological embedding of psychosocial stress over the life course are 1) breast cancer tumorigenesis, 2) epigenetic modification of stress management pathway genes, and 3) cellular aging. These three mechanisms are ongoing biological processes and may, therefore, be more susceptible to the influence of chronic stress. First, psychosocial stress experienced over the life course has been

associated with the development of breast cancer[21-23], which is one of the most commonly diagnosed cancers among women in the U.S.[24, 25], affecting approximately 12% of women during their lifetime[26], and stress experienced at potentially vulnerable periods of breast development[27] (i.e., childhood and adolescence) may differentially affect breast cancer risk via various behavioral and biological pathways. Second, both DNA methylation and telomere length have been linked to disease development via biological embedding of trauma[28, 29]. DNA methylation is a form of epigenetic modification that can alter gene expression by interrupting gene transcription without modifying the underlying genetic code[30]. Methylation of genes on the hypothalamus-pituitary-adrenal (HPA) axis, the primary stress management pathway, contributes to modified expression of genes related to immune response, inflammatory pathways, DNA repair, and cell apoptosis, contributing to disease risk and negative health impacts[31]. Finally, telomere length is a marker of cellular division and aging[32]. Telomeres are repeating DNA protein complexes found at the end of chromosomes that shorten with subsequent cell divisions until a critical length is reached and cellular senescence occurs[33]. Telomere length is sensitive to perceived and chronic stress via increased exposure to stress response-related oxidative stress and lowered telomerase activity[34].

Research in the impact of childhood adversity and early life trauma on adult health gained traction in the 1990's with the seminal Adverse Childhood Experience (ACE) Study conducted by Felitti et al., which identified a core set of seven domains of trauma that were linked to the leading causes of adult mortality[35]. Since then, researchers have not only expanded the field of study to assess this relationship in other adult health outcomes, but have also begun to study the mechanisms contributing to the biological embedding of these early life exposures. These studies have primarily utilized summative trauma scores[36-40], distinct traumatic events

(e.g., death of a parent)[41-45], or singular domains of trauma (e.g., sexual trauma)[46-49] to study the effects of early life trauma and adversity. Due to the heterogeneity of these methods, findings reported on the association between early life trauma and the measures of adult health that are investigated in this dissertation have been mixed[23, 36, 37, 40, 47, 50-59]. While traumatic experiences rarely occur in isolation, with about 80 percent of individuals who report one adverse experience also reporting at least one additional adverse experience[35], few studies have investigated how specific combinations of early life traumatic events (not just the total number of events or individual domains) may interact and differentially impact mechanisms of stress embodiment and adult health outcomes.

Using data from the Sister Study, a large prospective cohort study of women residing in the U.S. and Puerto Rico free of breast cancer at baseline but with a biological sister diagnosed with breast cancer, the objectives of this dissertation are: to investigate the association between early life trauma and 1) incident breast cancer risk, 2) DNA methylation of the HPA axis gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*), and 3) leukocyte telomere length.

1.2 Specific Aims and Hypotheses

Aim 1: Investigate the association between early life trauma and incident breast cancer.

Hypothesis 1a**:** Traditional measures of early life trauma (e.g., summative trauma scores or singular events) will be positively associated with breast cancer incidence among women enrolled in the Sister Study.

Hypothesis 1b: The association between early life trauma and incident breast cancer will vary based on latent models of concomitant early life traumas (i.e. the substantive nature of a cluster of experienced traumatic experiences may be more nuanced than the actual number of experienced traumas).

Aim 2: Examine the relationship between early life trauma and DNA methylation of the HPA axis gene, *NR3C1*.

Hypothesis 2a: The effect of early life trauma on levels of adult DNA methylation of *NR3C1* in peripheral blood will differ based on latent class of early life trauma.

Hypothesis 2b: Early life trauma will directly affect adult *NR3C1* levels independent of potential mediating pathways via adult trauma and other adult health and lifestyle factors.

Aim 3: Assess the relationship between early life trauma and leukocyte telomere length.

Hypothesis 3a: The association between early life trauma and adult leukocyte telomere length will differ based on latent classes of early life trauma.

Hypothesis 3b: Early life trauma will directly affect adult leukocyte telomere length independent of potential mediating pathways via adult trauma and other adult health and lifestyle factors.

1.3 Background

Approximately 50% of women in the U.S. report experiencing at least one trauma in their lifetime[47, 60], including physical, sexual, or emotional abuse, household dysfunction, and experience of natural disasters or major accidents. The first formal study of early life trauma, the Adverse Childhood Experiences Study, identified a core set of seven domains or categories of trauma (e.g., emotional, physical, and sexual abuse, physical and emotional neglect, and household challenges associated with substance abuse, mental illness, violent treatment of a female care giver, parental separation or divorce, or imprisonment of a member of the household) that were linked to the leading causes of adult mortality[35].

Since the seminal ACE Study, numerous studies have reported associations between ACEs and health risk behaviors[3-6] (e.g., alcohol use, drug abuse, smoking, physical inactivity,

and poor diet), mental health conditions[7-10], chronic disease[11, 12] (e.g. heart disease[12], autoimmune disease[13], and different cancers[14-16]), and overall poor health[17, 18]. Research has also begun to assess the biological mechanisms through which psychosocial stress may become embodied and link the exposure of early life stress to these health outcomes. Early life trauma has the potential to impact adult health through the process of biological embedding[61] during which initial regulatory homeostatic functions related to neural, immune, inflammatory, and metabolic processes negatively alter physiology with prolonged activation[62]. These processes can be driven by epigenetic modification, which include DNA methylation, histone modification, and telomere regulation.

1.4 Life Course Perspective

The life course perspective posits that physical and social exposures during gestation and early life through adulthood can have long term effects on the development of chronic disease later in life, such as breast cancer[19]. This framework highlights the fallacy of assessing social and biological pathways independently[63] and instead promotes the integration of multiple psychosocial and biological pathways over the life course to identify causal pathways of disease development[19].

The critical or sensitive period model is one of the primary theoretical frameworks used in the life course perspective. Critical or sensitive period models (known as latency models) posit that there are windows of vulnerability throughout the life course that correspond to periods of growth or disease susceptibility during which pertinent exposures have greater effect on adult health[19, 64]. Critical periods represent timeframes during which a given exposure must occur in order trigger a specific health outcome; if the exposure occurs outside this window, then the health outcome does not manifest in relation to the exposure. Conversely, sensitive periods

represent timeframes during which a given exposure will have a greater impact on disease risk; if the exposure occurs outside the sensitive period then the effect of the exposure may persist, but the impact is lessened. Figure 1.1 outlines a sensitive period model for the potential life course health effects of early life trauma. It proposes a sensitive period in early life, such that exposure to early life trauma has a direct effect on adult measures of health, independent of other periods (dashed line), but also allows for early life trauma to have downstream effects on health outcomes through mediating pathways via adult SEP, trauma, and behavioral and lifestyle factors outside of the sensitive period (solid black lines).

1.5 Early Life Trauma and Breast Cancer Incidence

Breast cancer pathogenesis and tumorigenesis

Invasive breast cancer is a heterogeneous disease and different molecular subtype milieus have been associated with different populations, disease pathogenesis, and response to treatment[65]. Breast cancer tumors are often classified by the presence or absence of cellular receptors, the most common of these being receptors for estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2). Tumors that lack receptors for one or more of these ligands (i.e., triple negative tumors) respond poorly to current breast cancer therapies and are associated with the worst outcomes[66]. In addition to the subtype classification, breast cancer severity is classified using additional clinical manifestations to determine tumor stage. Tumor stage is primarily determined using the system developed by the American Joint Committee on Cancer[67], which can range from stage 0 (*in situ* tumors) to stage IV with stage 0 representing non-invasive tumors, stage I representing the earliest invasive breast cancer where tumor cells have just started to invade normal surrounding tissue, and stage IV representing tumors that have metastasized to distant tissues in the body. Further classification within each

tumor stage is determined by the size of the primary tumor, involvement of the lymphatic system (a common conduit that allows for tumor cells to travel to distal tissues), metastatic status, and cellular grade (similarity in appearance of cancer cells compared to normal cells).

The development of breast cancer is thought to stem from the interaction of genetic and environmental factors; however, breast cancer etiology and the mechanisms through which risk factors contribute to differential pathology are still largely unknown. Less than 25% of risk for breast cancer is thought to be attributable to genetic risk associated with highly penetrant genetic mutations or intermediate risk-low frequency genetic variants[68]. A substantial portion of the unknown non-genetic risk for breast cancer is likely attributable to the exposure to and the embodiment of the spectrum of social determinants of health, including neighborhood context, SEP, and psychosocial stress experienced over the life course[69-72].

Childhood/adolescence as a sensitive period for breast cancer risk

Childhood and adolescence represent potential sensitive periods associated with later breast cancer risk due to ongoing breast development, characterized by rapid proliferation of undifferentiated mammary cells and heightened susceptibility to tumorigenic changes[73, 74]. Premature thelarche[75] (onset of breast development) and younger age at menarche (age at first menses)[76], both indicators of accelerated breast development, have both been associated with increased breast cancer risk. The period of breast duct development may serve as a sensitive period for carcinogenesis due to a prolonged period of cellular differentiation in the breast ducts. National trends indicate that although age at menarche has remained fairly constant over the past two decades, age at thelarche has experienced a downward trend, suggesting a lengthening of this inherent period of vulnerability at a population level[77]. Age at menarche has also been associated with the timing of a woman's first pregnancy; late onset of menarche is associated

with slightly increased risk for subfecundity and/or infertility[78]. Delayed age at first birth, parity, and time since last pregnancy are all known risk factors for breast cancer in young women[79, 80]. Early age at first birth and increased parity are associated with decreased long-term breast cancer risk[79, 81, 82], whereas pregnancy itself is associated with a transient increase in breast cancer risk within 5 years of delivery[83-85]. Although earlier age at menarche may serve as a predictor for additional breast cancer risk factors later in life, early life exposure to stress-related factors may also contribute to premature menarche and alter baseline breast cancer risk independent of future pregnancy-related breast cancer risk factors[75].

Early life trauma may affect breast cancer risk by contributing to high risk phenotypes of early life breast cancer risk factors, including early onset thelarche and menarche. Premature thelarche and menarche are patterned in the U.S. by adverse childhood experiences, such as food and resource insecurity[86], childhood sexual abuse[87], the presence of family conflict, and perception of environmental instability[88], which have all been associated with early age at menarche and fecundity[89]. Taken together with their potential impact on the timing of breast development, trauma experienced during childhood and adolescence may be relevant for understanding breast cancer etiology.

1.6 Early Life Trauma and Glucocorticoid Receptor Gene (NR3C1) Methylation

The hypothalamus-pituitary-adrenal axis and the glucocorticoid receptor

The hypothalamic-pituitary-adrenal (HPA) axis is one of the primary stress response mechanisms in the body and functions via a series of biochemical feedback pathways between the hypothalamus, anterior pituitary gland, and adrenal glands. Glucocorticoids are one of the key hormone signals utilized in the HPA axis in stress reactivity. They are released from the adrenal glands and serve as the primary negative feedback mechanism to discontinue stress

response along the HPA axis[90]. Glucocorticoid regulation, which is managed by genes within the HPA axis, modulates the impact of socioenvironmental stress on biologic pathways (e.g., growth and development, metabolism, immune function and inflammatory processes)[91]. Persistent activation of the HPA access due to exposure to chronic stress can decrease the functionality of the glucocorticoid negative feedback loop contributing to greater allostatic load and disease susceptibility[90, 92].

The glucocorticoid receptor is a member of the nuclear receptor superfamily and is commonly found in the cytoplasm before binding to glucocorticoids (forming a glucocorticoid receptor complex) and translocating to the cell nucleus[93]. While in the cell nucleus, the glucocorticoid receptor complex can act as a transcription factor by binding to glucocorticoid response elements of genes responsive to glucocorticoid signaling, upregulating the synthesis of immune- and metabolic-related proteins, or can interact with transcription factors necessary for additional gene expression to downregulate the synthesis of immunosuppressive and pro-inflammatory proteins[93-95]. Glucocorticoid receptors are present in all tissue types (including healthy breast tissue) and is the primary vehicle for glucocorticoid signaling, which regulates mammary physiology and development[96] in addition to stress-induced inflammatory and immune responses and cellular proliferation and apoptosis[97].

Epigenetics, DNA methylation, and glucocorticoid receptor gene (NR3C1)

Epigenetic modifications are durable characteristics that alter gene expression without changing underlying genetic code, thus disrupting DNA transcription and preventing gene expression. Genes are read through the process of transcription, during which DNA uncoils and the protein RNA polymerase scans each DNA base pair to create messenger RNA and eventually proteins that terminate in phenotypic manifestations.

One of the primary mechanisms of epigenetic modification is DNA methylation, in which a methyl group is added to the 5 position of a cytosine (C) base to create 5-methylcytosine. DNA methylation frequently occurs at cytosine-guanine (G) dinucleotides, which consist of a cytosine nucleotide (C:G) followed by a guanine nucleotide (G:C) separated by a single phosphate group in the 5' to 3' direction of the sequence of base pairs, also known as CpG sites[31]. CpG islands are areas that are rich in CpG sites and are commonly found in gene promotor regions. When the CpG islands are methylated, RNA polymerase is unable to bind to the DNA, preventing messenger RNA (mRNA) production and subsequent gene expression, thus silencing the gene and prohibiting any physiological functions to which it may contribute. Conversely, when CpG islands located in the gene promoter region are unmethylated, RNA polymerase can bind to the DNA and proceed with mRNA production and gene expression. Abnormal methylation patterns can result in the expression or silencing of genes responsible for vital biological mechanisms (e.g. immune response, inflammatory pathways, DNA repair, cell apoptosis, etc.), contributing to disease risk and negative health impacts[31].

The glucocorticoid receptor gene, (i.e., nuclear receptor subfamily 3, group C, member 1 (*NR3C1*)), is located on chromosome 5q31-q32 and is composed of eight coding exons (2-9) and 9 tissue-specific alternative first exons (1A-1J, excluding G), which each have their own promoter immediately upstream (Figure 1.2). The promotor region consists of the distal promotor (exons 1A and 1I) and the proximal promotor (exons 1D, 1J, 1E, 1B, 1F, 1C, and 1H); the latter of which is located in a 3 kilobase pairs (kbp) CpG island located 5kbp upstream from the translation start site[98]. *NR3C1* regulates genes associated with development, metabolism, inflammation and immune function as well as glucocorticoid receptor expression across multiple tissues[94]. The single glucocorticoid receptor gene is responsible for generating multiple

glucocorticoid receptor isoforms, which are differentially distributed within different tissues, via alternative splicing and alternative translation initiation[99].

Trauma and NR3C1 methylation

Early life trauma has been associated with lower *NR3C1* expression in leukocytes[100] and hippocampal tissue[101]; differential methylation may contribute to this association by altering *NR3C1* gene transcription. Methylation of genes within the HPA axis has been hypothesized to serve as potential markers of stress embodiment and disease risk[29]. Negative socioenvironmental conditions, such as low childhood SEP[102] and childhood adversity (e.g., trauma, neglect, and abuse)[103, 104] have been associated with modified hormonal exposures[74] and earlier pubertal onset[102], as well as methylation of genes within the HPA axis[105-107].

One of the HPA genes commonly studied is *NR3C1*, due to the prominent role of glucocorticoid receptors in the stress response process. Methylation of *NR3C1* has been attributed to altered stress management and inflammatory response and has been associated with increased childhood adversity[38, 39, 108], as well as tissue-specific glucocorticoid receptor expression[94], contributing to increased disease susceptibility[29, 107, 109]. Hypermethylation of *NR3C1* fundamentally results in decreased glucocorticoid receptor expression and down regulates glucocorticoid receptor-regulated pathways related to stress management, development, metabolism, inflammation and immune function, and cell differentiation, and increasing the potential to alter risk for psychopathologies, immune dysfunction, and tumorigenesis[29, 110].

Much of the existing literature studying *NR3C1* methylation has focused on methylation of CpG sites within the 3kpb CpG island that houses the proximal promotor, due to its potential to affect *NR3C1* transcription, with less research conducted on the tissue-specific first exons and whose functional roles are currently unknown[111]. However, findings from much of the previous

literature have been mixed. Hypermethylation of exon 1F and its promotor, located within the *NR3C1* proximal promoter CpG island, in peripheral blood has been associated with increased childhood adversity and adult mental health outcomes (e.g., post-traumatic stress disorder, depression, and borderline personality disorder)[106, 111, 112]. Additional studies have reported increased levels of *NR3C1* methylation overall and within promoter regions associated with number of traumas[38, 39] or specific domains of childhood/adolescent adversity (e.g., physical abuse and emotional neglect)[113, 114], while other studies have reported decreased methylation around promoter regions and at individual CpG sites associated with different measures early life trauma[42, 114, 115]. Still other studies report no association between early life adversity and *NR3C1* methylation[43, 116, 117]. The variation in these results may be in part to the heterogeneity of measures used to assess childhood and/adolescent trauma, the period during which trauma data were ascertained, the *NR3C1* CpG sites included in the analyses, the method used to capture *NR3C1* methylation data, whether the studies corrected for multiple testing, as well as the study populations represented in previous studies, which have differed by age, sex, and proportion of participants selected for known psychiatric and mental health disorders. In a recent review article by Palma-Gudiel et al., the authors call for methodological consensus and a more methodical selection of CpG methylation sites in studies of early life trauma[59].

1.7 Early Life Trauma and Adult Leukocyte Telomere Length

Telomere length and cellular aging

Telomeres are repeating DNA protein complexes consisting of tandem 5'-TTAGGG-3' sequences found at the end of chromosomes that range from about 10 to 15 kb[118] and protect chromosomal stability and integrity[28]. Telomeres gradually shorten by approximately 50 to 200 bp with each cell replication[119] and continues until cell cycle arrest occurs, leading to apoptotic

cell death or cellular senescence-the state in which telomeres reach a critical length and no longer have the capacity to divide[33]. Telomeres can also shorten due to direct DNA damage as a result of oxidative stress related negative health behaviors such as high BMI or smoking[120, 121]. Although telomere shortening can be reversed with the addition of new repeats on chromosome ends by telomerase proteins, this process is only observed in progenitor cells of tissues with high turnover rates, with most tissues characterized by shortening telomeres with age[122-124]. Therefore, telomeres can serve as mitotic clocks or an indicator of potential cellular division and aging.

Leukocyte telomere length reflects the telomere length of hematopoietic stem cells[125] and is often studied due to its accessibility. Due to the constant proliferation of leukocytes, leukocyte telomere length may not be reflective of other tissues that are less proliferative (e.g., neurons, skeletal muscle, etc.)[126-128]. However, leukocyte telomere length is strongly correlated with telomere lengths in other somatic tissues, such that an individual with shortened telomeres in one tissues will also have shortened telomeres in another tissue[129]. Shortened leukocyte telomere length has been associated with age-dependent processes[130], the development of chronic diseases[131] including cancer[132-135], obesity[136, 137], diabetes[138, 139], and cardiovascular disease[138, 140], and mortality[141].

Trauma and telomere length

Due to the growing evidence linking telomere length and disease development, telomeres have been proposed as a pathway to describe how trauma and other adversities are biologically embodied to influence adverse health outcomes[28]. In 2010, Tyrka et al. first reported that childhood maltreatment related to physical neglect and emotional neglect was associated with significantly shorter telomeres in adults after controlling for age, sex, smoking, BMI, and other demographic characteristics[142]. Since then, research has supported the association between

childhood and adolescent trauma and shorter telomeres in both children[55, 58] and adults[54, 55, 57]. The type of traumatic adversity[55, 56, 143, 144], number of traumatic exposures[52, 58, 145], and timing of exposure[143, 145] have been shown to have differential or compounding effects on telomere length with increased trauma severity or chronic exposures contributing to shorter telomere length. Recent life course research has further suggested that exposure during early life may be particularly damaging, revealing cumulative adversity during early life predicted shorter telomere length in adulthood[57] in addition to shorter baseline adult telomere length and greater telomere attrition in mid-life[145]. Only one study at the time of this dissertation, has used structural equation modeling to evaluate different life course models of childhood stressful life events before 18 years, and was conducted by evaluating telomere length in saliva[146]. Using financial and social/traumatic adversity to create an adversity score first constructed in Puterman et al.[57], Wallis et al. reported that there was no direct effect of childhood stressful life events on adult telomere length, with all effects mediated through adult stressful life events[146].

Despite these findings, other studies have reported more complex results, suggesting temporal impacts of stress on telomere length. Studies have suggested that the timing between the exposure and measurement of telomere length may play a role in the observed association between early life stress and adult telomere length. McFarland et al. reported that the association between exposure to stressful life events in early life and shortened telomere length was only observed in adults aged 22 to 44 years, but not in adults over the age of 45 years[52]. Results from Verhoeven et al. did not find an association between psychosocial stress that occurred more than six years prior to telomere length measurement[53]. Conversely, a study by Parks et al. reported that current perceived stress was not significantly associated with shorter telomere length, but telomeres were significantly shorter with higher perceived stress in women over 55 years[147].

These studies fail to report how the occurrence of different early life traumatic events may differentially impact telomere length in adulthood.

1.8 Measurement of Early Life Trauma

There is currently no consensus on how early life psychosocial stress is measured. Early life stress is commonly described using terms such as "stressful life events" or "adverse events" (i.e., circumstances that may negatively impact physical or psychological well-being)[148], "childhood adversity" (i.e., "experiences that are likely to required significant adaptation by an average child and that represent a deviation from the expectable environment")[149], "adverse childhood experiences" (i.e., often limited to those experiences identified in the ACE Study)[35], "maltreatment" (i.e., all forms of physical and/or emotional ill-treatment that result in "actual or potential harm to the child's health, survival, development or dignity in the context of a relationship of responsibility, trust or power")[150], and "trauma" (i.e., circumstances that are perceived as extremely threatening or harmful to the physical and/or psychological safety of the individual or individuals close to them; often times it is inclusive of ACEs, but is not limited to those experiences)[149]. The nuances between these terms are not often addressed when describing the relationship between early life stress and adult health outcomes[149, 151]. The use of one term over another is based on the measure that is used to capture psychosocial stress, such as the ACE Questionnaire[35], a lifetime trauma survey[152], Brief Betrayal Trauma Survey[153], childhood trauma questionnaire[154], as well as the abstraction of individual events such as death of a parent or experience of a specific natural disaster.

Most studies assessing the relationship between early life adversity and breast cancer, HPA axis gene methylation, and leukocyte telomere length have relied on summative measures[36-40, 155], distinct traumatic events (e.g., loss of a parent) [41, 42, 44, 156], or trauma domains[43,

[46-49] as measures for early life adversity. However, early life trauma does not occur in isolation. Dong et al. reported that between 81-98% of individuals who reported experiencing at least one ACE reported at least one additional ACE and the report of one ACE increased the odds of reporting additional ACEs from 2 to 17.2 times when compared to individuals who did not report any ACEs[157]. Furthermore, early life trauma may also influence the adoption of health risk behaviors, additional stress or trauma in adulthood, or future socioeconomic environments and opportunities, which may mediate the pathway to poor health[158, 159]. Life course models that assess different profiles of early life trauma are therefore needed in order to better understand the relationship between the cooccurrence of different early life traumatic events and adult health outcomes and measures of biological embedding.

1.9 The Sister Study

The Sister Study is a prospective cohort study of women residing in the U.S. or Puerto Rico (ages 35 to 74 years old; N = 50,884 overall; n = 42,558 non-Hispanic White) identified between 2003-2009[160]. All participants had a sister who had been diagnosed with breast cancer but had not been diagnosed with breast cancer themselves at baseline. Participants completed a telephone interview, as well as anthropometric measurements and blood sample collection at baseline and continue to complete annual health updates and detailed follow-up visits every 2-3 years.

The Sister Study serves as a unique resource available to address the paucity of research examining epigenetic pathways by which childhood adversity and socioeconomic disadvantage influence incident breast cancer risk. The cohort design and the intentional enrichment of the cohort allows one to more easily explore breast cancer incidence among this population. The

robust collection of traumatic events independent of SEP data over the life course allows one to evaluate the effects of adversity and SEP during potential sensitive periods, such as childhood.

1.10 Social patterning of trauma

Socioeconomic position (SEP) and the experience of trauma over the life course are highly correlated; as such, early life trauma may impact health through adult SEP, which can serve as an intermediate confounder. The experience of early life trauma is socially patterned with higher rates of reported early life trauma and child maltreatment associated with low childhood SEP[161, 162], including receipt of public assistance[163], poverty[164], homelessness[165], and neighborhood disadvantage[166]. On an ecological level, counties in the U.S. with higher income inequality were also reported as having higher county rates of child maltreatment[167]. Although rates of child maltreatment are higher among children of racial and ethnic minorities, these differences disappear after controlling for individual, family, or environmental factors[168-170]. Early life SEP has also been associated with chronic disease[171] and poor health outcomes[172-174], and therefore serves as an important confounder in our assessment of the relationship between early life trauma and adult health. Adult SEP may exacerbate or buffer the effects of early life trauma on chronic illness. Mock and Arai reported that cumulative disadvantage following early life trauma was associated with greater risk of chronic illness[175]. Furthermore, Turner et al. found that the relationships between childhood adversity and adult health were mostly mediated by adult SEP and stress, except for the association between childhood adversity and allostatic load, which was not attenuated based on adult SEP and adult stress exposures[158].

Psychosocial theory of disease distribution posits that socially patterned stressors (e.g., environmental or neighborhood factors, social structures—such as race and gender, and SEP) can become internalized and embodied to negatively affect health, contributing to pathophysiological

and behavioral coping mechanisms in the form of altered neuroendocrine functioning and/or promoting the development of negative health behaviors[176]. The distribution of social and structural stressors are socially and economically patterned; persons with low SEP are not only more likely to experience social stressors and adverse events, but may also be more vulnerable to social stress and adversity over the life course due to restricted access to physical resources that would normally aid in developing healthy coping methods[177, 178].

1.11 Public Health Significance

Approximately half of women in the U.S. report experiencing at least one early life trauma in their lifetime[47, 60], and up to 98% of those women are likely to report at least one additional early life trauma[157]. The impacts of early life trauma on adult health are estimated to cost between $448 billion to over $1 trillion in North America annually (approximately 2.13-4.90% of the gross domestic product), mostly attributable to costs related to long term mental illness and chronic disease[179]. Early life trauma has the potential to affect adult health through the process of biological embedding during which the experience of traumatic events can get "under the skin" to disrupt normal physiologic processes and contribute to poor health[1, 2]. Adult health outcomes, such as breast cancer, or adult measures of biological embedding, such as DNA methylation of HPA axis genes (e.g., *NR3C1*) and leukocyte telomere length, can be used to assess potential pathways through which early life trauma can affect health. The research linking early life trauma and these outcomes have been mixed[23, 36, 37, 40, 47, 50-59]. The heterogeneity of these results may stem in part from the lack of consistency regarding how early life trauma is measured and assessed in adulthood. Previous studies have failed to evaluate the impact of potential clusters of co-occurring early life traumatic experiences, instead assessing summative scores, individual trauma domains, or specific traumatic events. Furthermore, they often fail to

incorporate a life course perspective in their analyses and address the ways in which adult stress/trauma, adult SEP, and adult health and lifestyle factors may mediate the relationship between early life trauma and adult measures of biological embedding and health outcomes. As such, it is unclear whether early life trauma independently affects adult health or if its impact lies solely through mediating pathways.

This dissertation proposes three aims to assess the relationship between early life trauma and adult health. The first aim of this dissertation investigates the association between early life trauma and incident breast cancer, using both traditional and latent variable measures of early life trauma. The second aim of this dissertation uses a life course approach to examine the association between early life trauma measures identified in Aim 1 and patterns of DNA methylation of the HPA axis gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) and to assess whether adult trauma mediates the relationship between early life trauma and *NR3C1* methylation. The third aim of this dissertation also uses a life course approach assess the relationship between early life trauma, using measures identified in Aim 1, and leukocyte telomere length, a biomarker of cellular aging. The results from this research will benefit public health by clarifying how various patterns of early life trauma may affect different measures of adult health and stress embodiment and how subsequent trauma may impact those outcomes. If certain clusters of early life traumatic experiences exacerbate risk for incident breast cancer or mechanisms of biological embedding of stress, then targeted programs and policies that may be more feasible than wide-sweeping policies can be implemented to reduce adult morbidity. Furthermore, identification of direct or mediating pathways between early life trauma and adult outcomes can support timing of interventions to best mitigate the effects of early life trauma over the life course. For example, if early life trauma primarily affects adult health via direct

pathways, then prevention strategies implemented to address youths will be necessary to fully negate the effects of early life trauma. Conversely, if the effect of early life trauma is largely mediated through adult stress/trauma, SEP, or health and lifestyle factors, then interventions in adulthood that target these factors may be most important in limiting the effect of early life trauma on adult health. Thus, this work has important implications for how early life trauma should be studied and addressed in order to limit the burden of early life trauma on adult health.

1.12 Figures

Figure 1.1 Life course model of early life trauma on adult measures of biological embedding and health outcomes. Dashed line represents direct effects of early life trauma on adult measures of biological embedding or health outcomes independent of mediating pathways.

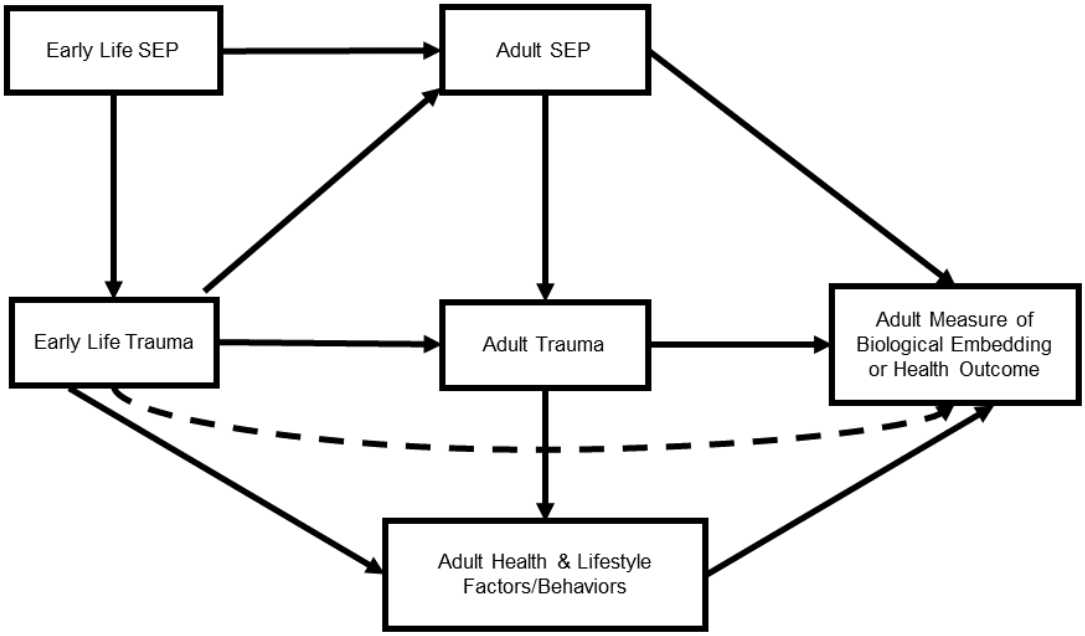
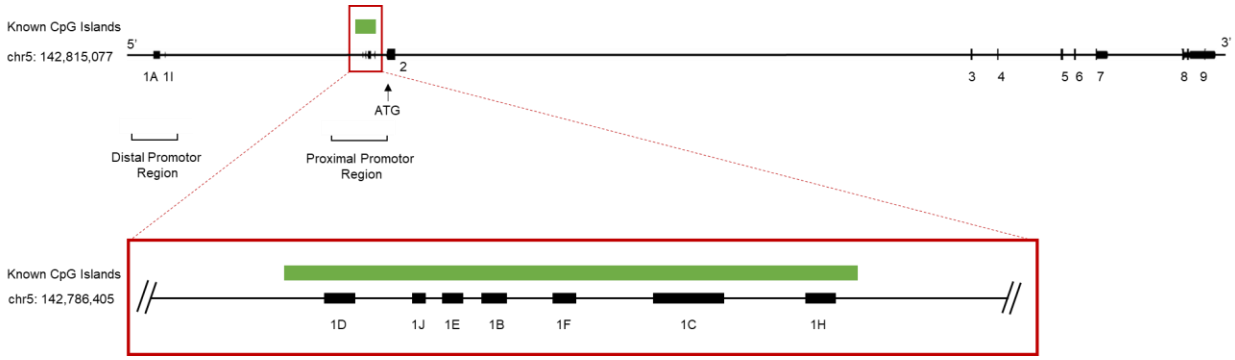


Figure 1.2 Nuclear receptor subfamily 3, group C, member 1 (NR3C1) gene structure. The NR3C1 gene is located on chromosome 5 and spans approximately one half mega base pairs. It is composed of eight coding exons (numbered 2-9) and nine alternative first exons (A-J, excluding G). A 3kbp CpG island located in the proximal promotor spans exons 1B-1H (green bar).



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CHAPTER 2

Early Life Trauma and Incident Breast Cancer

2.1 Introduction

The experience of psychosocial stress over the life course has the potential to impact current as well as future health outcomes. Adversity and trauma experienced during childhood and adolescence is an example of one type of stress that can have a lasting impact on adult health and well-being. Indeed, numerous studies have reported on the observed association between adverse childhood experiences (ACE) and health risk behaviors[1-4] (e.g., alcohol use, drug abuse, smoking, physical inactivity, and poor diet), mental health conditions[5-8], chronic disease[9, 10] (e.g. heart disease[10], autoimmune disease[11], and different cancers[12-14]), and overall poor health[15, 16].

Breast cancer is one of the most commonly diagnosed cancers among women in the U.S.[17, 18], affecting approximately 12% of women during their lifetime[19]. Psychosocial stress experienced over the life course has been associated with the development of breast cancer[20-22] and stress experienced at potentially vulnerable periods may differentially affect breast cancer risk via various behavioral and biological pathways. Both childhood and adolescence represent potential sensitive periods of breast development during which breast tissue is more vulnerable to pre-tumorigenic mechanisms compared to other life periods that are characterized by minimal changes in the breast tissue[23]. Indicators of early life trauma (e.g., cumulative adverse childhood experiences, childhood physical abuse, childhood sexual abuse, etc.) have been associated with early menarche and onset of menopause[24-27], obesity[28], and alcohol use[28, 29], all of which are reported breast cancer risk factors[30-32].

However, prior studies of stress and breast cancer risk have focused on either the years proceeding a woman's breast cancer diagnosis (i.e., 1 to 10 years)[14, 21, 22, 33, 34] or cumulative

adversity over the life course[20]. Stress is commonly measured by summary ACE scores[33, 35], singular events (e.g., death of a parent)[36], or specific types of trauma-such as sexual or physical abuse during childhood[37, 38]—as sole measures of early life adversity. There is a paucity of research on whether the patterning of concurrent traumas in early life may differentially affect breast cancer risk.

Using data collected from The Sister Study, a prospective cohort study of women free of breast cancer but with a biological sister diagnosed with breast cancer, the current study aims to investigate the relationship between early life trauma (childhood and adolescence) and incident breast cancer. We assessed whether cumulative early life trauma and traditional domains of early life trauma, were associated with increased risk for incident breast cancer during follow-up. Furthermore, since adverse experiences and traumas do not commonly occur alone, we evaluated whether the co-occurrence of different early life traumas is associated with breast cancer incidence.

2.2 Methods

Study population

The Sister Study is a prospective cohort study was designed to assess environmental and genetic risk factors for breast cancer and other conditions[39]. Participants are U.S. and Puerto Rican women (ages 35 to 74 years old; N = 50,884) who have a biological sister previously diagnosed with breast cancer and had not been diagnosed with breast cancer themselves by the time of enrollment (2003-2009). During the baseline visit, participants completed a telephone interview as well as anthropometric measurements and blood sample collection; annual health updates and detailed follow-up visits are completed every 2-3 years. Data for the current study used Sister Study data release 7.0, which included follow-up data collected through September

15, 2017. Requests for Sister Study data can be submitted by following directions at http://sisterstudy.niehs.nih.gov/English/coll-data.htm.

All participants provided written informed consent. The study was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences and the Copernicus Group. The current analysis using coded data was considered exempt from human subject research by the Institutional Review Board at the University of Wisconsin-Milwaukee.

We excluded 65 participants who either had withdrawn from the study at the time of analysis (n = 2), were missing date of breast cancer diagnosis (n = 6), or were diagnosed with breast cancer prior to completing enrollment procedures (n = 57). An additional 4,221 participants were excluded who did not complete the stress and trauma questionnaire at the first follow-up biennial interview. Thus, 45,973 participants were included in the present study.

Traumatic events during childhood and adolescence

Adverse experiences and traumatic events were collected during biennial follow-up study interviews. Questions were based on the revised 14-item Brief Betrayal Trauma Survey[40] and included an additional 13 questions developed for The Sister Study, for a total of 26 items; of these, 20 items were utilized to assess early life traumatic experiences. Participants were asked whether they had ever experienced each traumatic event in their lifetime (Possible responses: Yes, No, Don't know, Refuse to answer). Participants who answered affirmatively, were asked if they experienced the trauma before age 13 (childhood) and/or between the ages of 13 and 17 (adolescence). Due to the low reporting of some types of childhood trauma in this cohort (Supplemental Table 1), cumulative trauma was assessed by the number of traumatic experiences reported for childhood and/or adolescence. Trauma types not included in these scores were: death of a child, death of a spouse, and witnessed or learned of a child's experience

of unwanted sexual contact, or sexual, physical, or psychological abuse; these experiences were unlikely to be reported for early life. Summative trauma scores were calculated by summing the number of early life experiences reported for childhood and/or adolescence. In addition, the 20 trauma types were classified into 7 domains: 1) natural disasters, 2) major accidents, 3) household dysfunction, 4) sexual abuse, 5) physical abuse, 6) emotional or psychological abuse, and 7) major illness before age 18 (Figure 2.1).

Incident breast cancer

Breast cancer status was assessed at baseline (women with positive breast cancer diagnosis were excluded from the study) and during annual health updates. In addition, breast cancer status was also obtained during the triennial detailed follow-up questionnaires or communication with The Sister Study staff. Self-reported details about the participant's diagnosis was captured approximately six months after her diagnosis via a Breast Cancer Follow-Up questionnaire, which was self-administered from a mailed survey or completed via telephone with the Sister Study staff. Complementary information, such as estrogen receptor (ER) status, was requested form the participant's medical record and corresponding pathology reports when permission was granted by the participant. Response rates for were more than 94% for follow-up[39]; medical record-documented breast cancer diagnosis was available for 86% of cases. A previous study reported a positive predictive value of 99% between medical records and self-report among Sister Study participants with medical records[41]. There was also high agreement between self-reported ER status and abstracted data from medical records (95%)[42].

Covariates

Covariates were race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, other) and measures of early life socioeconomic position (SEP): highest level of education in the

household at age 13 (Less than high school, High school graduate, Some college, College degree or higher), a subjective measure of family income while growing up (Poor, Low income, Middle income, Well off), and as a child, whether there were times when the family did not have enough food to eat (Yes, No). Due to the timing of the exposure of interest (early life trauma during childhood and/or adolescence) and incident breast cancer risk, common breast cancer risk factors (e.g., parity, age at menarche, thelarche, age at first birth, etc.), were not included as potential confounders in the models, as they instead represent potential mediators along the early life trauma – breast cancer pathway.

Statistical analysis

Descriptive statistics were calculated using means and standard deviations (SD) for continuous variables and frequency counts for categorical variables.

Two latent class analyses were conducted to evaluate potential relationship between combinations of 1) concurrent trauma types or 2) trauma domains and incident breast cancer. This model assumes that there is a finite number of mutually exclusive latent clusters that represent the associations between the set of categorical trauma types and domains. Latent class analysis does not assume that the categorical indicators (i.e., traumatic experiences or trauma domains) are independent; as some trauma types reflect different aspects of similar traumas (e.g., unwanted sexual contact by someone close or unwanted sexual contact by someone not close), we would not expect these traumas to be independent. The number of clusters of individual trauma types was determined by estimating models with two to six clusters. Models were compared using model fit (Bayesian Information criterion - BIC)[43], entropy, and substantive groupings of trauma types and domains. There were no *a priori* assumptions made for the number of latent classes and models with successive number of clusters were identified until

further models were not justified by model fit criteria or substantive clusters. This process was repeated with the seven trauma domains in models with two to five clusters.

Cox proportional hazards regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for incident breast cancer risk; age was used as the time scale with person-time accrued from age at enrollment until the age at which the woman received a breast cancer diagnosis or the age on the date of completion of the last follow-up. Models were run using cumulative trauma scores, individual traumas (separate model for each trauma), trauma domains (separate models for each trauma domain), and the two latent class variables for early life trauma (trauma type and trauma domains) as the primary exposures. Models were adjusted for the noted covariates. In analyses of breast cancer subtypes based on ER status, women with competing or missing ER status (i.e., ER negative breast cancer when assessing risk for ER positive breast cancer) were censored from follow-up at time of breast cancer diagnosis

Test for the proportional hazard assumption was conducted by assessing the interaction between age (i.e., time from age at baseline to age at end of follow-up) and the main exposure of interest. For pre-menopausal breast cancer risk, Cox proportional hazards models were conducting using time from age at baseline to age of onset of menopause, breast cancer onset, or loss to follow-up, which ever occurred first. For post-menopausal breast cancer risk, Cox proportional hazards models were conducted using time from age at baseline or onset of menopause, which ever came second, until age of breast cancer onset or loss to follow-up.

Participants with missing data for any early life trauma variables or covariates were excluded from the given analysis. Missing data was <5% for each covariate and 3.3% overall for participants who had complete trauma data. All tests were two-sided with $p < 0.05$ considered

statistically significant. Analyses were conducted using Mplus version 8.3[44] and SAS version 9.4 (SAS Institute Inc., Cary, NC)[45].

2.3 Results

Characteristics of study participants

For this study, 45,973 women contributed 435,220.05 person-years of follow-up (mean (SD): 9.47 (2.13) years), with 3,082 women developing incident invasive breast cancer (n = 2,397), *in situ* breast cancer (n = 681), or tumors of unknown stage (n = 4). Approximately 50% of Sisters Study participants reported experiencing at least one early life trauma before age 18, similar to other national surveys that reported between 48 - 51% of women reporting the experience of at least one lifetime trauma[37, 46]. Women who reported at least one early life trauma were slightly younger, more often reported growing up in poor or low income households, and recalled periods when their families did not have enough to eat compared to women who reported no early life traumas (Table 1). Furthermore, women who reported at least one category of early life trauma reported earlier menarche and thelarche, more often had at least one pregnancy, were younger at the birth of their first child, more often consumed alcohol before age 20, as well as over their lifetime, and more often smoked before age 20, as well as over their lifetime (Table 1).

Over one third of participants reported early life psychological or emotional trauma, with 17% of women reporting being emotionally or psychologically mistreated by someone close before age 18. Other commonly reported domains of early life trauma reported included sexual trauma (16%) and household dysfunction (12%). Prevalence of sexual trauma in which the perpetrator was someone close and someone not close was similar (9.7 % and 8.4%, respectively). The most commonly reported source of household dysfunction in early life was

attributed to experiencing serious family problems related to alcohol, drug, or other substance abuse, or mental illness before the participant was age 18 (11.8%) or witnessing the attack of a family member by another family member (7.7%).

Early life trauma types, trauma domains, and cumulative trauma score and incident breast cancer

The report of different domains of early life trauma were similar among women who developed breast cancer and those who remained breast cancer free at the end of follow-up/ Approximately 39% (n = 1,052) of women who developed breast cancer reported experiencing at least one type of emotional or psychological trauma before age 18, with 15% (n = 462) and 12% (n = 361) of women who developed breast cancer reporting sexual trauma and household dysfunction in early life, respectively. We found no overall association between number of early life traumas and incident breast cancer, before and after adjusting for highest level of education attained in the household, family income while growing up, and childhood food insecurity (HR = 0.98; 95% CI = 0.96, 1.01 vs. HR = 0.98; 95% CI = 0.96, 1.01, respectively, Table 2). Incident breast cancer was not associated with any of the domains of early life trauma (natural disasters, major accidents, household dysfunction, sexual trauma, physical trauma, psychological or emotional trauma, or experiencing a major illness). We did not observe any associations between individual types of trauma and incident breast cancer (Table 2). There were no statistically significant associations between any of the domains of early life trauma and risk of ER positive or ER negative breast cancer.

Co-occurrence of early life trauma types and incident breast cancer risk

Latent class analysis was used to create a measure that better captures the co-occurrence of early life traumas in The Sister Study cohort. To identify profiles of early life trauma, we

estimated five LCA models with 2 to 6 latent clusters using the 20 trauma types reported for early life and four LCA models with 2 to 5 latent clusters using the 7 domains of early life trauma. Within each model, we assigned substantive classifications to each cluster based on conditional response probabilities. Entropy values, which represent the level of certainty with which participants are assigned to the correct clusters, ranged from moderate to high (0.67 to 0.82, ideally greater than 0.8) for models using trauma types and were low (0.53 to 0.63) for models using trauma domains. Although the estimated models differ in model fit and substantive clustering, all models using trauma types had clusters that distinguished between high and low early life trauma. The trauma type models with 4 or more clusters further distinguished between moderate levels of early life trauma, including unique clusters for moderate early life trauma overall and moderate early life trauma specifically related to traumas with high levels of perceived betrayal (sexual, emotional, and physical trauma perpetrated by someone close) and family drug and/or alcohol use or mental health issues (Table 3). The latent class model with six clusters had the best model fit of the models tested while still maintaining moderate to high level certainty that participants were placed in the correct clusters (entropy = 0.72); the six clusters were classified as 1) Low early life trauma, 2) Moderate early life trauma: Family health issues, 3) Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues, 4) Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues, 5) Moderate early life trauma: Overall, and 6) High early life trauma (Table 4). Latent class models using the seven trauma domains had poor separation between clusters with entropy less than 0.65 for all models with 2 to 5 clusters (data not shown).

In latent class models, there were no statistically significant associations between clusters of early life trauma and incident breast cancer overall or by ER status after adjusting for age,

race/ethnicity, and early life SEP. However, women classified as reporting moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues had higher risk of breast cancer, albeit not statistically significant, overall (HR=1.11; 95% CI: 0.97, 1.29), as well as when stratified by menopausal status (pre-menopausal breast cancer: HR=1.19; 95% CI: 0.89, 1.59; post-menopausal breast cancer: HR=1.09; 95% CI: 0.93, 1.29) when compared to women classified as low early life trauma, after controlling for early life socioeconomic position (Table 4). Violations of the proportional hazard assumption were observed for breast cancer risk models and in post-menopausal breast cancer models in our latent class analysis. Exploratory analyses indicated that the relationship between early life trauma, as measured as latent classes, and breast cancer risk varied over time, such that the magnitude of association increased over time. As a result, we stratified models by menopausal status at censoring, as pre- and post-menopausal breast cancer are associated with differential risk patterns, to account for potential time effects. Stratification eliminated the time effect within the pre-menopausal group, however, the magnitude of risk continued to increase over time within the post-menopausal group.

Sensitivity analyses

Additional studies looking at pre- and post- menopausal breast cancer risk using cumulative trauma scores, individual traumas, and trauma domains resulted in similar findings as noted above for the full cohort.

2.4 Discussion

This is the first study to comprehensively assess the relationship between early life trauma and risk of incident breast cancer. In addition to evaluating the effects of cumulative number of traumatic or adverse events, singular traumatic events, or commonly evaluated trauma

domains (e.g., sexual and physical abuse), we attempted to capture a more nuanced measure of early life trauma by assessing how individual traumas and trauma domains may work together in affecting incident breast cancer risk in adulthood. We did not find any statistically significant associations between early life trauma and incident breast cancer risk using traditional measures of early life adversity. The present study identified six profiles of early life trauma and observed patterns that suggest that compared to women who report low early life trauma, women who reported moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues were at higher risk of developing pre- and post-menopausal breast cancer. Women reporting other early life trauma profiles also had a higher risk of developing post-menopausal breast cancer compared to women with low early life trauma, except for those with the highest profile of early life trauma. These findings suggest that the role of early life trauma on breast cancer risk may be more nuanced than a cumulative trauma score, a single traumatic event, or a domain of trauma can capture.

Previous studies of stress and adversity over the life course and incident breast cancer risk have been inconsistent, with studies reporting positive[33, 47], negative[48], and null[22, 35, 37] associations; the observed variation in results may in part be due to the heterogeneous nature of the disease, as well as methodological differences in how stress and adversity have been operationalized and the periods of trauma exposure captured in these studies. A majority of the previously published literature has not evaluated the potential role of early life adversity outside of the scope of cumulative adversity over the life course. We found four studies that reported breast cancer risk associated with childhood and/or adolescence adversity within their assessments of life course adversity; all of them differ in how early life adversity was captured[35, 37, 47, 49]. Our results for cumulative trauma counts, individual traumas, and trauma domains are

consistent with the findings of Wise et al., who found no significant associations between childhood sexual or physical abuse victimization and breast cancer risk among the prospective cohort of Black women enrolled in the Black Women's Health Study.[37] and Surtees et al., who reported no significant associations between difficulties reported in childhood and incident breast cancer after adjustment for common breast cancer risk factors in the European Prospective Investigation into Cancer (EPIC) cohort [35]. Conversely, Eskelinen and Ollonen reported a weak association between losses and deficits in childhood and adolescence and breast cancer risk in a prospective case-control study of Finnish women[47] and Jacobs and Bovasso reported increased odds of breast cancer associated with maternal death during childhood[50], which we did not observe.

The mechanisms through which trauma and adversity during sensitive periods of breast development may affect breast cancer risk are poorly understood. One potential biologic pathway though which early life trauma could affect breast cancer risk are modification of stress response mechanisms. The hypothalamus-pituitary-adrenal (HPA) axis is one of the primary stress response mechanisms and functions via a series of biochemical feedback pathways. Persistent activation of the HPA axis resulting from exposure to chronic stress can decrease its functionality, contributing to greater allostatic load and disease susceptibility[51, 52]. Experiences of early life trauma may predispose women to a chronic dysregulation of stress management mechanisms including immune function and inflammatory processes[53-55]. Early life trauma could also affect breast cancer risk through the alteration of circulating hormone milieus that affect pubertal changes and early breast development and subsequent reproductive behaviors. Younger age at menarche[56], an indicator of accelerated breast development, has been associated with increased breast cancer risk, suggesting that the period of breast duct development may serve as

a sensitive period for carcinogenesis possibly due to rapid cellular differentiation in the breast ducts. Previous studies have reported an association between childhood sexual abuse and lower age at menarche[24], but less is known about the potential relationship between early life trauma and other known breast cancer risk factors, including delayed age at first birth, parity, and time between pregnancies[57-59].

Strengths of this study include the prospective design of The Sister Study cohort and the comprehensive nature in which traumas were collected to reflect periods over the life course. Trauma in early life was assessed prior to breast cancer diagnosis, limiting potential recall bias. For this study, experiences of early trauma were collected separately for childhood (before age 12) and adolescence (ages 13-17). Prior studies that have assessed the association between early life adversity in breast cancer have focused primarily on childhood adversity, however, studies that limit their scope to childhood may overlook the impact of trauma on pubertal development and subsequent reproductive pathways associated future breast cancer risk. Furthermore, although other studies have evaluated different methods of assessing adverse childhood experiences[60], this is the first study to utilize and compare different methods of evaluating breast cancer risk in a single cohort.

This study also presents some limitations. First, although we capture the number and type of early life traumas reported during either childhood or adolescence, we do not know the duration or the magnitude of these exposures over these two periods. Some trauma types tend to have shorter exposure periods (e.g., natural disasters) whereas other trauma types may be more persistent (e.g., emotional or psychological mistreatment). The duration and repetition of trauma over potentially sensitive time periods over the life course may play an important role in the association between early life trauma and breast cancer risk. Furthermore, although The Sister

Study collected information on a comprehensive list of traumas, we cannot exclude the possibility that there may be other types of stress that may be more likely to be biologically embedded and subsequently play a larger role in the risk of developing breast cancer.

Second, due to the low frequency of reported early life traumas before age 13 when assessed by breast cancer status, we combined reporting during childhood and adolescence (younger than 18 years of age) to represent the window of risk during which trauma-related hazards could influence biological pathways associated with pubertal changes and early breast development. This may not be ideal as childhood and adolescence may represent potentially distinct periods of breast cancer risk due do unique developmental processes that occur during each period. Additional sensitivity analyses should be completed to potentially elucidate the unique effects of traumatic events experienced during childhood versus adulthood.

Third, The Sister Study does not capture adverse childhood experiences as presented by the ACE study, instead uses a trauma framework to collect information on early life adversity; as a result, we cannot not directly compare our results to other studies that have used the traditional categories for ACEs. However, Bethell et al. demonstrated that although variability exists among measures of adverse childhood experiences, most measures remain similar and cumulative scores from these measures reflect consistent association with poor health outcomes[60].

Fourth, The Sister Study used an enriched sample of women who had at least one biological sister diagnosed with breast cancer, but was not diagnosed with breast cancer herself at enrollment, in order to maximize the likelihood of being able to identify potential risk factors for incident breast cancer as women who have a sister diagnosed with breast cancer are twice as likely to develop breast cancer themselves[61]. In addition, the Sister Study population are generally older, more educated, and more likely to be healthy and economically sound, which

may be masking the effects of early life trauma in more vulnerable populations. However, the distribution of breast cancer risk factors in The Sister Study cohort resembles that of the general population, which suggests that the results observed in this study may still be generalizable and internally valid[62].

Finally, breast cancer has a relatively long latency period between potential exposures, triggering events, and clinical manifestation. We do not adjust for intervening factors or subsequent stressors, which may serve as mediators in the relationship between early life trauma and incident breast cancer. Furthermore, there were 841 women who were diagnosed with breast cancer prior to completion of the biennial stress update, approximately 27% of women diagnosed with breast cancer who completed a stress questionnaire, which could contribute to differential recall among women diagnosed versus not diagnosed with breast cancer. Sensitivity analyses did not show significant differences in the results restricted to women who completed the stress questionnaire before any breast cancer diagnosis (data not shown). Although we can assure temporality between reported early life trauma and breast cancer diagnosis, non-differential exposure misclassification may exist as a result of the long period between experienced trauma and reporting with potential underreporting of early life trauma. Previous studies have reported between 60 and 81 percent of study participants accurately disclosing previous experiences of sexual and physical trauma[63-65]. This underreporting has been attributed to inability to recall, as well as avoidance of potential stigmas associated with disclosing early life trauma. Concurrent mental health issues (e.g., psychological distress) can contribute to higher recall of and reporting of childhood traumatic experiences[66].

In conclusion, we did not find any statistically significant associations between early life trauma and breast cancer risk in this large cohort of U.S. and Puerto Rican women using

traditional measures of early life adversity, including cumulative traumatic experiences, singular trauma types, and types of trauma. However, nuanced measures of early life trauma that incorporate the co-occurrence of different types of trauma may be more meaningful in defining breast cancer risk.

2.5 Tables and Figures

Table 2.1 Characteristics of the 45,973 Sister Study participants based on experience of early life traumas before age 18

	No Early Life Trauma N = 23,076 (50%)		**Any Early Life Trauma N = 22,897 (50%)**		**p-value**
	n	**%**	**n**	**%**	
Age at baseline, mean (SD)	56.54 (9.02)		54.93 (8.70)		<.0001
Race/Ethnicity, %					<.0001
Non-Hispanic White	20,662	85.98	18,547	84.52	
Non-Hispanic Black	1,830	7.62	1,821	8.3	
Hispanic	1,043	4.34	917	4.18	
Other	495	2.06	658	3	
Highest level of education in the household at age 13, %					<.0001
Less than high school	4,170	17.34	4,186	19.08	
High school graduate	8,574	35.68	7,856	35.8	
Some college	4,473	18.61	4,252	19.38	
College degree or higher	6,813	28.35	5,649	25.73	
Family income while growing up, %					<.0001
Poor	1,349	5.61	2,148	9.79	
Low income	5,601	23.31	6,210	28.3	
Middle income	15,477	64.41	12,217	55.68	
Well off	1,603	6.67	1,368	6.23	
As a child, were there times when your family did not have enough food to eat?, %					<.0001
Yes	1,336	5.56	2,781	12.67	
No	22,694	94.44	19,162	87.33	
Ever consumed alcohol, %					<.0001
Missing	3	0.01	3	0.01	
No	1,033	4.30	689	3.14	
Yes	22,994	95.69	21,251	96.85	
Smoking status at baseline, %					<.0001
Missing	3	0.01	5	0.02	
Never smoked	14,330	59.63	11,739	53.50	
Past smoker	8,128	33.82	8,263	37.66	
Current smoker	1,569	6.53	1,936	8.82	
Age at menarche, mean (SD)	12.69 (1.50)		12.60 (1.55)		<.0001
Age at thelarche, mean (SD)	12.27 (1.65)		12.16 (1.71)		<.0001
Parity, mean (SD)	1.99 (1.35)		1.88 (1.34)		<.0001
Age at first pregnancy, mean (SD)	24.89 (5.12)		24.57 (5.36)		<.0001
Menopause status at censoring					<.0001
Missing	38	0.16	49	0.22	
Pre-menopausal	2,130	8.86	2,285	10.41	
Post-Menopausal	21,862	90.98	19,609	89.36	
Age at menopause onset, mean (SD)	53.29 (3.11)		53.16 (3.31)		0.0193

Figure 2.1 Traditional trauma domains (dark blue) and types of early life trauma (light blue)

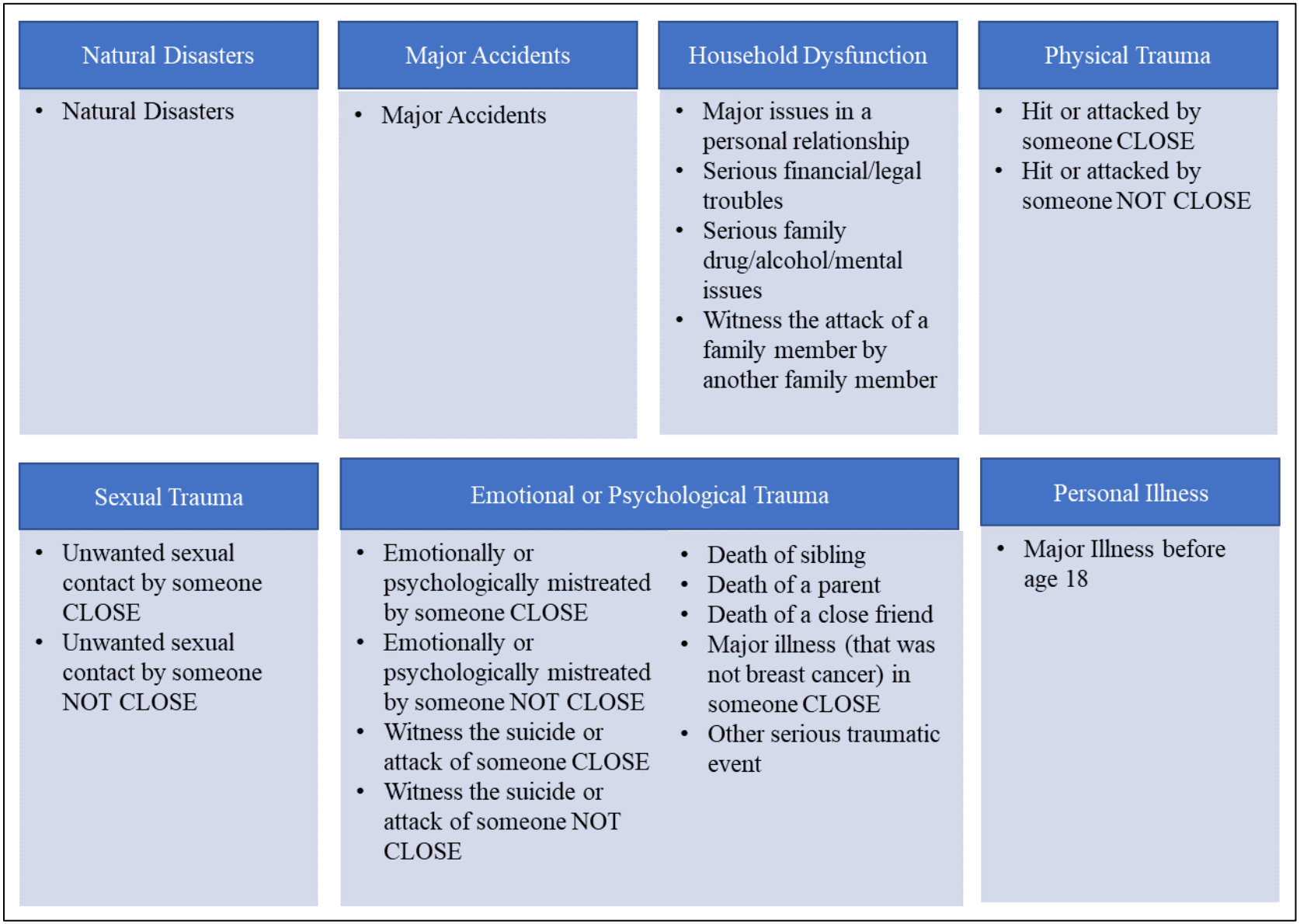

Table 2.2 Association between early life traumas and incident breast cancer risk

	Cases	Person-years	Age-adjusted HR (95% CI)	Multivariable HR (95% CI)[a]
Number of early life traumas	3082	435,176	0.98 (0.96, 1.01)	0.98 (0.95, 1.01)
No early life trauma	1675	226,972	1.00 (ref)	1.00 (ref)
Any early life trauma	1407	208,204	0.96 (0.89, 1.03)	0.96 (0.89, 1.03)
Natural disaster	85	14,346	0.94 (0.77, 1.16)	0.94 (0.77, 1.16)
Major accident	77	14,200	0.91 (0.73, 1.12)	0.91 (0.73, 1.12)
Household Dysfunction	314	52,947	0.98 (0.87, 1.10)	0.97 (0.87, 1.09)
Witness attack of a family member by another family member	208	33,240	1.10 (0.97, 1.26)	1.10 (0.97, 1.26)
Major issue in personal relationship	24	4,440	0.87 (0.59, 1.29)	0.87 (0.59, 1.29)
Serious financial/legal issues	21	2,795	1.29 (0.86, 1.93)	1.28 (0.86, 1.92)
Serious family drug/alcohol/mental illness	301	51,787	0.97 (0.87, 1.08)	0.97 (0.86, 1.08)
Sexual Trauma	373	52,947	0.93 (0.84, 1.04)	0.93 (0.84, 1.03)
Unwanted sexual contact by someone close	218	42,288	0.92 (0.81, 1.04)	0.92 (0.81, 1.05)
Unwanted sexual contact by someone not close	201	36,787	0.99 (0.87, 1.13)	0.99 (0.87, 1.13)
Physical Trauma	151	52,947	1.05 (0.90, 1.23)	1.05 (0.90, 1.23)
Hit/attacked by someone close	132	21,584	1.05 (0.89, 1.24)	1.05 (0.89, 1.24)
Hit/attacked by someone not close	26	5,667	1.00 (0.72, 1.38)	1.00 (0.72, 1.38)
Psychological Trauma	889	52,947	0.98 (0.90, 1.06)	0.97 (0.90, 1.06)
Emotionally/psychologically mistreated by someone close	433	74,889	0.98 (0.89, 1.08)	0.98 (0.89, 1.08)
Emotionally/psychologically mistreated by someone not close	56	10,716	0.92 (0.73, 1.17)	0.92 (0.73, 1.17)
Witness suicide/attack of someone close	75	14,621	0.88 (0.71, 1.09)	0.88 (0.71, 1.09)
Witness suicide/attack of someone not close	14	3,851	0.62 (0.38, 1.02)	0.63 (0.38, 1.02)
Death of a sibling	87	13,909	0.99 (0.81, 1.21)	0.99 (0.81, 1.21)
Death of a parent	172	30,004	0.92 (0.80, 1.07)	0.93 (0.80, 1.07)
Death of a close friend	77	16,226	0.81 (0.65, 1.00)	0.81 (0.65, 1.00)
Major illness (not BC) in someone close	120	19,578	1.00 (0.84, 1.19)	1.00 (0.84, 1.19)
Major illness before age 18	54	9,850	0.91 (0.71, 1.16)	0.91 (0.71, 1.16)

[a] Adjusted for race/ethnicity, highest level of education in the household at age 13, family income while growing up, and food security as a child.

BC: Breast cancer; CI: Confidence interval; HR: Hazard ratio

Table 2.3 Fit indices, cluster description, and cluster population share for the latent class analyses of early life trauma types (n = 20)

# of clusters	Cluster description	n	Estimated cluster population share (%)	BIC	Entropy
2	Low early life trauma	40090	87.21	308195.1	0.819
	High early life trauma	5877	12.79		
3	Low early life trauma	35832	77.95	306561.8	0.673
	Moderate early life trauma: Sexual trauma, physical trauma, and family drug/alcohol/mental issues	7989	17.38		
	High early life trauma	2146	4.67		
4	Low early life trauma	35550	77.34	305796.7	0.718
	Moderate early life trauma: Sexual trauma, physical trauma, and family drug/alcohol/mental issues	7180	15.62		
	Moderate early life trauma: Overall	2409	5.24		
	High early life trauma	828	1.80		
5	Low early life trauma	35674	77.61	305147.3	0.709
	Moderate early life trauma: Family health issues	1943	4.23		
	Moderate early life trauma: Sexual trauma, physical trauma, and family drug/alcohol/mental issues	5077	11.04		
	Moderate early life trauma: Overall	2432	5.29		
	High early life trauma	841	1.83		
6	Low early life trauma	35292	76.78	304932.1	0.722
	Moderate early life trauma: Family health issues	1234	2.68		
	Moderate early life trauma: Sexual trauma	2901	6.31		
	Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues	3357	7.30		
	Moderate early life trauma: Overall	2318	5.04		
	High early life trauma	865	1.88		

Figure 2.2 Latent class plot of early life trauma (6 clusters)

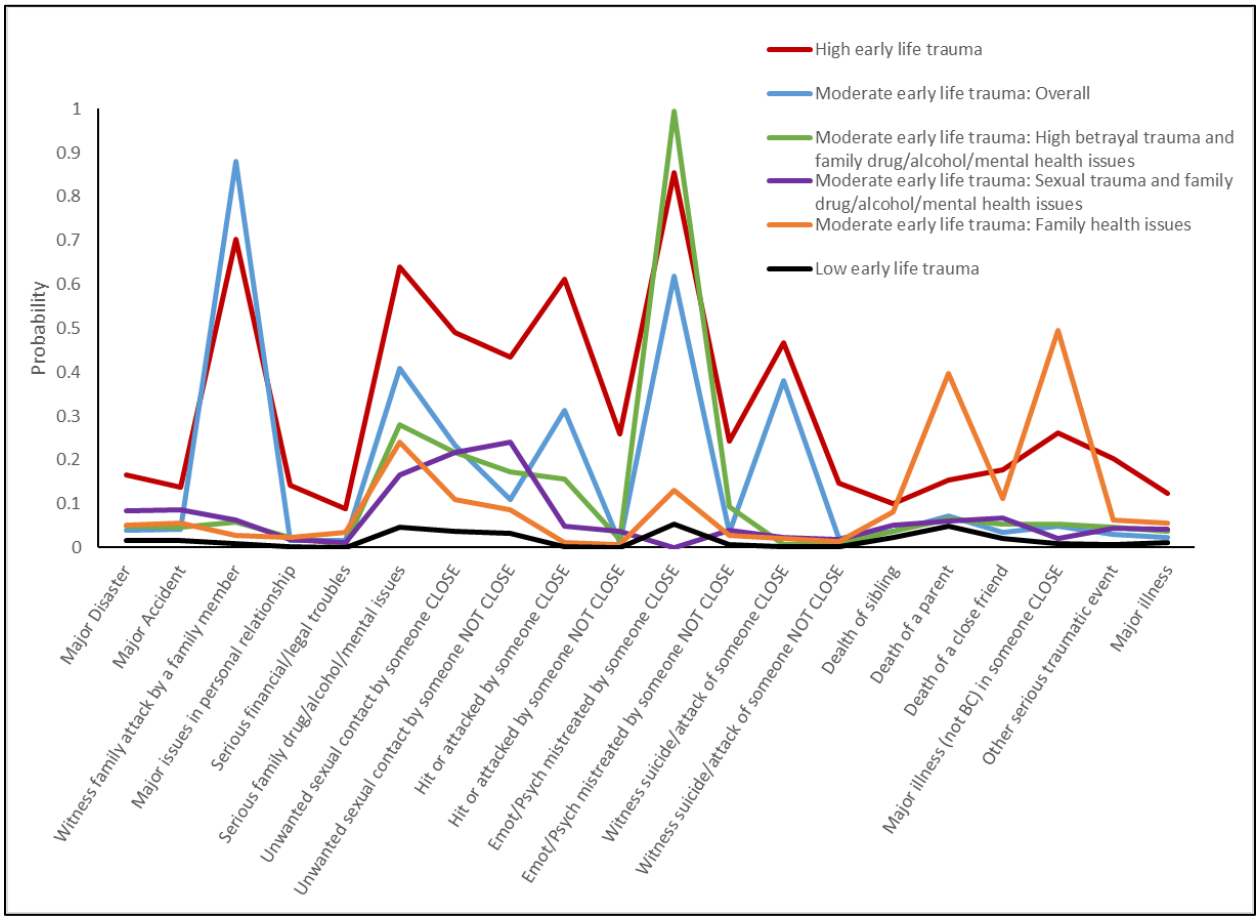

Table 2.4 Association between latent classes of early life trauma and incident breast cancer risk by pre/post-menopausal breast cancer

		Total Breast Cancer			Pre-menopausal Breast Cancer			Post-menopausal Breast Cancer		
	Person-years	Cases	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Cases	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Cases	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)
Early life trauma (Latent class model)										
Low early life trauma	334,144	2374	1.00 (Ref)	1.00 (Ref)	401	1.00 (Ref)	1.00 (Ref)	1973	1.00 (Ref)	1.00 (Ref)
Moderate early life trauma: Family health issues	11,791	77	0.98 (0.78, 1.23)	0.98 (0.78, 1.23)	14	0.77 (0.45, 1.31)	0.77 (0.45, 1.31)	63	1.03 (0.80, 1.32)	1.04 (0.81, 1.33)
Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues	27,308	206	1.11 (0.97, 1.28)	1.11 (0.97, 1.29)	51	1.19 (0.89, 1.59)	1.19 (0.89, 1.59)	155	1.09 (0.93, 1.29)	1.09 (0.93, 1.29)
Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues	31,923	218	0.98 (0.86, 1.13)	0.98 (0.86, 1.13)	30	0.72 (0.49, 1.04)	0.71 (0.49, 1.03)	188	1.04 (0.90, 1.21)	1.04 (0.90, 1.21)
Moderate early life trauma: Overall	21,936	158	1.08 (0.92, 1.26)	1.07 (0.91, 1.26)	25	0.82 (0.55, 1.23)	0.82 (0.54, 1.23)	133	1.13 (0.95, 1.35)	1.13 (0.94, 1.34)
High early life trauma	8,074	39	0.73 (0.53, 1.00)	0.73 (0.53, 1.01)	8	0.64 (0.32, 1.28)	0.63 (0.31, 1.28)	31	0.76 (0.53, 1.09)	0.76 (0.53, 1.09)

[a]Age-adjusted (time-scale)

[b]Adjusting for age (time scale), race, childhood household education, childhood household income, and childhood food security

2.6 Supplemental Materials

Supplemental Table 2.1 Distribution of trauma types by childhood, adolescence, and childhood and/or adolescence

	No Breast Cancer (n = 42,891)			Breast Cancer (n = 3,082)		
	Missing	No	Yes	Missing	No	Yes
	n	n	n	n	n	n
Natural Disaster						
Childhood	386	41,488	1,017	36	2,986	60
Adolescence	386	42,051	454	36	3,007	39
Childhood and/or adolescence	386	41,077	1,428	36	2,950	96
Major Accident						
Childhood	525	41,740	626	45	2,993	44
Adolescence	525	41,559	807	45	2,991	46
Childhood and/or adolescence	525	40,958	1,408	45	2,948	89
Hit or attacked by someone CLOSE						
Childhood	409	40,952	1,530	35	2,933	114
Adolescence	409	41,289	1,193	35	2,975	72
Childhood and/or adolescence	409	40,330	2,152	35	2,893	154
Hit or attacked by someone NOT CLOSE						
Childhood	237	42,376	278	23	3,037	22
Adolescence	237	42,302	352	23	3,040	19
Childhood and/or adolescence	237	42,076	578	23	3,022	37
Unwanted sexual contact by someone CLOSE						
Childhood	335	39,473	3,083	36	2,853	193
Adolescence	335	41,007	1,549	36	2,950	96
Childhood and/or adolescence	335	38,344	4,212	36	2,779	267
Unwanted sexual contact by someone NOT CLOSE						
Childhood	364	40,440	2,087	30	2,900	152
Adolescence	364	40,841	1,686	30	2,945	107
Childhood and/or adolescence	364	38,912	3,615	30	2,799	253
Psychologically mistreated by someone CLOSE						
Childhood	1,035	35,825	6,031	90	2,568	424
Adolescence	1,035	36,765	5,091	90	2,642	350
Childhood and/or adolescence	1,035	34,478	7,378	90	2,480	512
Psychologically mistreated by someone NOT CLOSE						
Childhood	732	41,458	701	48	2,993	41
Adolescence	732	41,489	670	48	2,988	46
Childhood and/or adolescence	732	41,094	1,065	48	2,964	70
Witness suicide/attack of someone CLOSE						
Childhood	472	41,325	1,094	26	2,991	65
Adolescence	472	41,643	776	26	3,008	48
Childhood and/or adolescence	472	40,947	1,472	26	2,969	87
Witness suicide/attack of someone NOT CLOSE						
Childhood	483	42,246	162	30	3,046	6
Adolescence	483	42,139	269	30	3,040	12
Childhood and/or adolescence	483	42,013	395	30	3,036	16

Supplemental Table 2.1 (*continued*)

	No Breast Cancer (n = 42,891)			Breast Cancer (n = 3,082)		
	Missing	No	Yes	Missing	No	Yes
	n	n	n	n	n	n
Witness family attack by a family member						
Childhood	548	39,789	2,554	43	2,856	183
Adolescence	548	40,731	1,612	43	2,912	127
Childhood and/or adolescence	548	39,048	3,295	43	2,793	246
Death of sibling						
Childhood	1,444	40,570	877	90	2,924	68
Adolescence	1,444	40,906	541	90	2,960	32
Childhood and/or adolescence	1,444	40,070	1,377	90	2,894	98
Death of a parent						
Childhood	2,864	38,425	1,602	189	2,789	104
Adolescence	2,864	38,616	1,411	189	2,800	93
Childhood and/or adolescence	2,864	37,059	2,968	189	2,696	197
Death of a close friend						
Childhood	2,258	40,282	351	164	2,899	19
Adolescence	2,258	39,313	1,320	164	2,845	73
Childhood and/or adolescence	2,258	39,026	1,607	164	2,831	87
Major illness						
Childhood	915	41,260	716	82	2,958	42
Adolescence	915	41,651	325	82	2,972	28
Childhood and/or adolescence	915	40,997	979	82	2,934	66
Major illness (not BC) in someone CLOSE						
Childhood	1,816	40,051	1,024	120	2,899	63
Adolescence	1,816	39,800	1,275	120	2,871	91
Childhood and/or adolescence	1,816	39,139	1,936	120	2,828	134
Major issues in personal relationship						
Childhood	1,116	41,526	249	54	3,013	15
Adolescence	1,116	41,477	298	54	3,013	15
Childhood and/or adolescence	1,116	41,333	442	54	3,003	25
Serious financial/legal troubles						
Childhood	880	41,886	125	56	3,016	10
Adolescence	880	41,808	203	56	3,006	20
Childhood and/or adolescence	880	41,740	271	56	3,002	24
Serious family drug/alcohol/mental issues						
Childhood	1,453	37,692	3,746	86	2,753	243
Adolescence	1,453	37,559	3,879	86	2,727	269
Childhood and/or adolescence	1,453	36,341	5,097	86	2,650	346
Other serious traumatic event						
Childhood	751	41,559	581	47	2,996	39
Adolescence	751	41,656	484	47	3,011	24
Childhood and/or adolescence	751	41,162	978	47	2,981	54

2.7 References

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CHAPTER 3

Early Life Trauma and Methylation of the Glucocorticoid Receptor Gene (NR3C1)

3.1 Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is one of the main stress response mechanisms in the body and functions via a series of biochemical feedback pathways between the hypothalamus, anterior pituitary gland, and adrenal glands. Glucocorticoid receptors play a dual role in the stress management by binding to circulating cortisol, a stress hormone, and regulating the negative feedback of the HPA stress response, thus modulating the impact of socioenvironmental stress on biologic pathways (e.g., growth and development, metabolism, immune function and inflammatory processes)[1, 2]. Persistent activation of the HPA axis due to exposure to chronic stress can decrease the functionality of the glucocorticoid negative feedback loop contributing to greater allostatic load and disease susceptibility[2, 3].

Early life trauma has been shown to have lasting effects on health[4, 5], but the mechanisms by which adverse early life exposures get under the skin are still being elucidated. Studies of the effect of adversity and trauma over the life course on stress response mechanisms and HPA axis dysregulation have largely focused on the epigenetic modification, and primarily DNA methylation, of HPA axis genes[6, 7]. Epigenetic modifications are durable, but sometimes reversible, characteristics that alter gene expression by regulating chromatin structure and DNA accessibility to DNA transcription factors, without changing the underlying genetic code[8]. Cytosine-phosphate-guanine (CpG) sites, which are found along a single strand of DNA, serve as the primary site of DNA methylation during which a methyl group, --CH_3, is added to the 5' position of the cystosine base. Changes in methylation patterns can result in the expression or silencing of genes responsible for vital biological mechanisms (e.g. immune response,

inflammatory pathways, DNA repair, cell apoptosis, etc.), contributing to disease risk and negative health impacts[9]. Furthermore, methylation of genes within the HPA axis has been hypothesized to serve as a potential marker of stress embodiment and disease risk[10].

One such gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*), is responsible for glucocorticoid receptor expression across multiple tissues, as well as regulation of genes associated with development, metabolism, inflammation and immune function[11]. Much of the existing literature studying *NR3C1* methylation has focused on methylation of CpG sites within the 3kpb CpG island that houses the proximal promoter, including seven non-coding first exons, due to its functional role in *NR3C1* transcription. Hypermethylation of *NR3C1* promoter region results in decreased glucocorticoid receptor expression and inhibits glucocorticoid receptor-regulated pathways related to stress management, development, metabolism, inflammation and immune function, and cell differentiation, increasing the potential risk for psychopathologies (e.g., post-traumatic stress disorder, depression, and borderline personality disorder)[12-14], immune dysfunction, and tumorigenesis[10, 15].

Early life trauma has been associated with lower *NR3C1* expression in leukocytes[16]; differential methylation may contribute to this process by altering *NR3C1* transcription. However, reports on the association between early exposure to stressors and traumatic events on *NR3C1* methylation have been mixed due to differences in study design, measures used to assess stressors and traumatic events, and CpG sites selected for investigation[6]. Studies that have evaluated the relationship between early life adversity and *NR3C1* methylation have utilized summative trauma scores[17, 18], distinct traumatic events (e.g., loss of a parent)[19], or trauma domains[20]. Types of trauma may differ in duration and intensity (i.e., experience of a natural disaster versus chronic physical abuse throughout childhood and adolescence), which may affect

how they may in turn impact health later in life. Few studies have looked at how different patterns of concurrent trauma may affect *NR3C1* methylation nor have they taken a life course approach to assess potential mediating pathways in adulthood that may explain the relationship between early life trauma and adult levels of *NR3C1* methylation.

Using data collected from women enrolled in the Sister Study, the current analysis aims to investigate the relationship between early life (i.e., during childhood and adolescence) exposure to traumatic events and methylation of *NR3C1* in adulthood.

3.2 Methods

Study population

The Sister Study is a prospective cohort study designed to assess environmental and genetic risk factors for breast cancer and other conditions[21]. Participants are women residing in the U.S. and Puerto Rico (ages 35 to 74 years old; N = 50,884) who have a biological sister previously diagnosed with breast cancer but had not been diagnosed with breast cancer themselves at time of enrollment (2003-2009). During the baseline visit, participants completed a telephone interview, as well as anthropometric measurements and blood sample collection. Health updates are completed annually, and detailed follow-up questionnaires have been completed every 2-3 years since baseline. Requests for Sister Study data can be submitted by following the directions at http://sisterstudy.niehs.nih.gov/English/coll-data.htm.

All participants provided written informed consent. The study was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences and the Copernicus Group. The current analysis using coded data was considered exempt from human subject research by the Institutional Review Board at the University of Wisconsin-Milwaukee.

Women eligible for this analysis were included in a previously described case-cohort study of methylation and breast cancer risk[22]. A random sample of 1,336 non-Hispanic White women in the Sister Study cohort for whom a blood sample had been collected at time of study enrollment was selected. Cases included the 1,542 non-Hispanic White women who were diagnosed with incident invasive breast cancer or ductal carcinoma *in situ* (DCIS) between study enrollment and time of sampling in March 2015, (100 of whom had been selected as part of the random cohort sample)[23] (data release 7.0).

We excluded 102 participants whose sample did not meet methylation-related quality control thresholds[22] and 175 participants who did not complete the stress and trauma questionnaire at the first biennial follow-up interview. A total of 2,678 participants were included in the present study.

Trauma measurement

Data on traumatic experiences were collected during the first follow-up interview (two to three years after enrollment when the blood sample used for these analyses were collected). Questions were based on the revised 14-item Brief Betrayal Trauma Survey[24] and included an additional 13 questions developed for the Sister Study, for a total of 26 items. Of these, 20 items were retained to assess early life traumatic experiences. Participants were asked whether they had ever experienced each type of trauma in their lifetime (Possible responses: Yes, No, Don't know, Refuse to answer). Participants who answered affirmatively, were asked if they experienced the trauma before age 13 (childhood), between the ages of 13 and 17 (adolescence), from age 18 to one year prior to survey completion (adult), and/or in the past 12 months (recent adulthood). Traumatic experiences in the past 12 months were excluded from these analyses as

they would have occurred after *NR3C1* methylation was measured in the blood sample collected at baseline.

Early life trauma

Women were dichotomized based on experience of early life trauma and were considered to have exposure to traumatic events in early life if they reported experiencing at least one event in childhood or adolescence. In addition, a latent class variable was created to group women according to the co-occurrence of early life traumas among all Sister Study participants who completed the trauma questionnaire (n = 45,973) in order to capture the distribution of trauma profiles in the underlying Sister Study cohort. We estimated five latent class models with 2 to 6 latent clusters using the 20 types of early life trauma as indicators. Within each model, we assigned substantive labels to each latent class based on the pattern of conditional response probabilities within each cluster. The latent class model with six clusters had the best model fit of the models tested while still maintaining moderate to high level certainty that participants were placed in the correct clusters, which is evaluated using entropy on a scale of 0 to 1 (entropy = 0.72). We identified six latent classes based on the proportion of women who reported each early life trauma within each cluster: 1) low early life trauma (average probability of reporting any type of early life trauma was less than 2% across all possible traumatic events; referent), 2) family health issues (highest proportions for death of a parent and major illness (not breast cancer) in someone close, 40% and 49%, respectively. Average across all other traumas = 6%), 3) sexual trauma and family drug/alcohol/mental health issues (highest proportions for unwanted sexual contact by someone close (22%) or someone not close (22%) and family drug, alcohol, and/or mental health issues (17%). Average across all other traumas = 4%), 4) high betrayal trauma and family drug/alcohol/mental health issues (highest proportions for traumas perpetrated

by someone close – unwanted sexual contact (22%), hit or attached (16%), emotionally or psychologically mistreated (99%) and family drug, alcohol, and/or mental health issues (28%). Average across all other traumas = 5%), 5) moderate early life trauma: overall (average probability of reporting each early life traumatic event = 17%), and 6) high early life trauma (average probability of reporting each early life traumatic event = 32%).

Adult trauma

Adult trauma (occurring between age 18 and 12 months before completion of the stress and trauma questionnaire) was evaluated as a potential mediator of the relationship between early life trauma and *NR3C1* methylation. A continuous latent variable representing trauma experienced in adulthood was created using indicators for the same 20 traumatic events in adulthood that were used in the early life trauma latent class model (RMSEA = 0.025, CFI = 0.919).

DNA methylation of NR3C1

Blood samples were obtained at baseline prior to any diagnoses of breast cancer. DNA extraction and processing are described elsewhere[22]. Briefly, genomic DNA was extracted from whole blood samples using an automated system (Autopure LS, Gentra Systems) housed at the Molecular Genetics Core Facility at the National Institute of Environmental Health Sciences or using DNAQuick at BioServe Biotechnologies LTD (Beltsville, MD). DNA was analyzed using the Illumina Infinium HumanMethylation450 BeadChip following the manufacturer's protocol. Preprocessing and quality control of methylation data were previously described[25]. Methylation results included 40 CpG sites located on *NR3C1* and were included in the present study (Figure 3.1).

Methylation values were originally reported on the Beta-value scale (i.e., the ratio of the methylated probe intensity compared to the sum of the methylated and unmethylated probe intensities), which represents the percentage of methylated copies of a CpG site ranging from 0% (no copies methylated) to 100% (all copies methylated)[26]. These values were logit-transformed to calculate *M* values (i.e., the natural log ratio of methylated probe intensity and unmethylated probe intensity), with positive values indicating more methylated copies than unmethylated copies and vice versa for negative values. *M* values were used for statistical analyses in order to address homoscedastic assumptions of data for linear models. In addition, we calculated the mean *M* value across all 40 *NR3C1* CpG sites as well as subsets of CpG sites based on genomic function, either promoter-associated (n = 24) or within the gene body (n = 7).

Covariates

Covariates including age at blood draw (continuous); measures of early life socioeconomic position (SEP): highest level of education in the household at age 13 (Less than high school, High school graduate, Some college, College degree or higher), family income while growing up (Poor, Low income, Middle income, Well off), and as a child, whether there were times when the family did not have enough food to eat (Yes, No); adult educational attainment (continuous) as a measure of adult SEP; body mass index (continuous; kg/m^2); and smoking history - number of pack years smoked (continuous) were adjusted for in our analyses, as well as proportions of white blood cell types (CD8 T cells, CD4 T cells, natural killer cells, B cells, monocytes, or granulocytes versus other), which were estimated using the methods outlined by Houseman et al.[27]. Although no participants were diagnosed with breast cancer at time of blood draw, all models were adjusted for breast cancer case status at end of follow-up

(dichotomous; case vs. non-case) to account for potential pre-clinical effects of breast cancer on *NR3C1* methylation.

Statistical analysis

Descriptive characteristics were evaluated using means and standard deviations for continuous variables and frequency counts for categorical variables. Multivariable robust linear regression analysis was conducted to evaluate the association between measures of early life trauma and *M* values for 40 individual CpG sites located on *NR3C1*, as well as overall methylation and methylation based on CpG location. Models were adjusted for age, breast cancer status, smoking history, body mass index, and blood cell types. Although smoking history and body mass index are potential mediators of the early life trauma and *NR3C1* methylation pathway, they are often included as covariates in the literature as strong predictors of DNA methylation. They were included as confounders in robust linear regression models to contribute to the robustness of *NR3C1* methylation measures despite the potential to attenuate effect sizes as mediating factors. To correct for multiple testing across the 40 *NR3C1* CpG sites, a false discovery rate (FDR) was applied using the Q-value method[28] and CpG sites with FDR $Q < 0.05$ were considered differentially methylated in relation to early life trauma. Analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC)[29].

We carried out structural equation modeling (SEM) in Mplus version 8.3[30] to assess the relationship between early life exposure to traumatic events and different measures of *NR3C1* methylation in adulthood and to evaluate potential mediation through adult trauma. Figure 3.2 outlines the three models used to assess this relationship: model 1 adjusted for age, breast cancer status, smoking history, body mass index, and blood cell types (black); model 2 additionally adjusted for early life and adult SEP, with adult SEP acting as a potential intermediate

confounder (blue); and model 3 allowed for mediating pathways via adult health and lifestyle factors (smoking and BMI), which were previously included as covariates in models 1 and 2 (green).Path coefficients were estimated using full information maximum likelihood estimation to calculate the total effect, the direct effect (pathway A) between early life trauma and adult *NR3C1* methylation, the total indirect effect, and the indirect effect mediated specifically by adult trauma (model 1: B-C; model 2: additionally D-E-C; model 3: additionally D-E-F-G and B-F-G). Goodness of fit was evaluated using root mean square error of approximation (RMSEA) and the Comparative Fit Index (CFI), with RMSEA < 0.02 and CFI > 0.95 as indicators of good fit[31].

Change in percent methylation was calculated on the β-value scale from *M* value robust linear regression estimates or SEM pathway coefficients and mean *M* values using the 'M-model-M-mean' model described by Xie et al.[32]

3.3 Results

Sample characteristics

Characteristics of Sister Study participants with DNA methylation and early life trauma data are shown in Table 3.1. Approximately 46% of women included in the sample reported at least one traumatic experience before age 18, which is slightly lower than the rate of early life traumas in the full Sister Study cohort (50%). The average age at baseline of women with at least one early life trauma was 56.5 (standard deviation: 8.5) years and was 57.8 (standard deviation: 8.9) years for women who experienced no early life trauma. Women who experienced one or greater early life trauma more often reported lower parental educational attainment, growing up in a poor or low-income household, and periods of food scarcity as a child compared to women

with no early life trauma. The distribution of body mass index and smoking status among the study sample was similar for all women.

Percent NR3C1 *methylation*

The mean DNA methylation across the 40 *NR3C1* CpG sites among women in the sample was 34.8% with approximately 10% methylation of promoter-associated CpG sites and 93% methylation of CpG sites located in the gene body (Table 3.2). The mean percent methylation for individual CpG sites ranged from 0.56% (cg26720913, located in exon 1A in the primary promoter region) to 98.17% (cg23273257, located in the 3' untranslated region) (Table 3.3).

Early life trauma and overall NR3C1 *methylation*

We assessed the relationship between latent classes of early life trauma and differential *NR3C1* methylation overall, as well as across promoter-associated CpG sites and CpG sites located within the gene body. Latent classes of moderate to high early life trauma were not associated with differential methylation overall or within promoter-associated CpG sites when compared to the latent class of low early life trauma after adjusting for age, breast cancer status, and blood cell types (data not shown) or additional adjustment for smoking history and body mass index (Table 3.4). The latent class of early life trauma consisting of personal sexual trauma and family drug, alcohol, and/or mental health issues was associated with a 0.08% decrease in methylation of gene body CpG sites compared to women with low early life trauma, after adjusting for age, breast cancer status, smoking history, body mass index, and blood cell types ($p = 0.036$). We did not observe any associations between other latent classes of early life trauma and methylation across *NR3C1* gene body CpG sites when compared to the low early life trauma group.

Early life trauma and differential NR3C1 *methylation at individual CpG sites*

We examined the association between latent classes of early life trauma and differential methylation at 40 individual CpG sites across *NR3C1* included on the Illumina Infinium HumanMethylation450 BeadChip. Ten CpG sites were differentially methylated when comparing latent classes of early life trauma with low early life trauma as the referent after controlling for age, breast cancer status, smoking history, body mass index, and cell types, but failed to remain statistically significant following FDR adjustment (Supplemental Table 3.1).

Although the associations between latent classes of early life trauma and methylation at individual CpG sites were not statistically significant after controlling for age, breast cancer status, smoking history, body mass index, and cell types, we explored potential trends in differential methylation based on latent class of early life trauma. Figure 3.3 shows the percent change in methylation between each latent class of moderate or high early life trauma compared to low early life trauma for each CpG site included in the study. Whereas most CpG sites hover around no differential methylation between latent classes of moderate to high early life trauma and low early life trauma, a few CpG sites suggest a patterning of differential methylation. Visual trends suggest that the percent methylation change increased with greater severity of moderate to high early life trauma compared to low early life trauma for a CpG site in the proximal promoter, cg12466613, and cg03857453, in the gene body. Conversely, for cg06968181 and cg18019515, both promoter-associated CpG sites, when compared to women who reported low levels of early life trauma, percent change in methylation appeared greatest among women who reported moderate levels of early life trauma consisting of either family health issues or sexual trauma and family drug, alcohol, or mental health issues (Figure 3.3).

Life course models of trauma and NR3C1 *methylation of gene body CpG sites*

We further examined whether the observed association between latent classes of early life trauma and mean hypomethylation of CpG sites in the *NR3C1* gene body was mediated by adult measures of trauma, SEP, or health and lifestyle factors. Table 3.5 presents results from three life course models that estimated the total, direct, total indirect, and indirect effects mediated by adult trauma of this association. In all models, we did not see an association between adult trauma and *NR3C1*methylation of gene body CpG sites (path C). We did not observe direct (pathway A) or indirect effects via adult trauma (path B-C) of latent classes of early life trauma on percent change in methylation of *NR3C1* in models that included age, breast cancer status, smoking history, BMI, and cell types (RMSEA = 0.030, CFI = 0.985); models that additionally adjusted for early life and adult SEP (RMSEA = 0.021, CFI = 0.889); and models that additionally allowed for mediating pathways through adult health and lifestyle factors associated with DNA methylation - BMI and smoking history (RMSEA = 0.017, CFI = 0.919).

Moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues was associated with hypomethylation of *NR3C1* gene body CpG sites when direct and indirect pathways were considered in the fully adjusted pathway model; moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues resulted in a 0.04% decrease in methylation of *NR3C1* gene body CpG sites holding all covariates constant ($p = 0.01$), which was attenuated from the effect estimated in the robust linear regression models (Table 3.5). This was primarily driven by the direct effect of moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues on methylation of *NR3C1* gene body CpG sites (% methylation change = 0.03, $p = 0.048$). The indirect effect of early life trauma on *NR3C1* methylation, mediated by all pathways that included adult trauma, was not statistically significant, suggesting that sexual trauma and family

drug, alcohol, and/or mental health issues is associated with methylation of *NR3C1* gene body CpG sites independent of adult trauma.

3.4 Discussion

This study used a candidate gene approach to assess the relationship between early life trauma and adult levels of *NR3C1* methylation, as a measure of epigenetic modification of HPA axis genes in response to early life trauma. To our knowledge, it is one of the largest studies to specifically assess this relationship. We utilized a latent class model of early life trauma, instead of traditional overall counts or trauma domains, to assess how various combinations of early life trauma may differentially affect *NR3C1* methylation. We found that moderate early life trauma consisting of sexual trauma and household dysfunction was associated with moderate decrease in methylation among CpG sites within the gene body. However, we did not observe any additional associations between other latent classes of trauma and differential *NR3C1* methylation overall, among promoter-associated CpG sites, or within the gene body. Although there were several CpG sites that were associated with different latent classes of early life trauma after controlling for age, breast cancer status, smoking history, body mass index, and blood cell types, these associations did not survive correction for multiple testing. Furthermore, moderate early life trauma consisting of sexual trauma and family drug, alcohol, and mental health issues was also associated with hypomethylation of *NR3C1* gene body CpG sites compared to low early life trauma when considering both direct and indirect effects in life course SEM models. We observed no mediating effects via adult trauma, adult SEP, nor adult smoking or BMI, which have been associated with differential DNA methylation[33, 34].

The glucocorticoid receptor plays a pivotal role in HPA axis functioning and stress response mechanisms. Most research on *NR3C1* methylation has focused on CpG sites within the

proximal promotor region of *NR3C1* due to its prominent role in *NR3C1* transcription; less studied, methylation changes in the bodies of genes have been reported to play a role in differential promotor usage, transcription elongation, or alternative splicing[35, 36]. Hypomethylation of CpG sites along the gene body, as seen with moderate early life trauma consisting of sexual trauma and family drug, alcohol, or mental health issues, may contribute to aberrant DNA transcription and may have implications for downstream health outcomes related to abnormal tissue-specific expression of glucocorticoid receptors and dysfunction of stress response regulation, however, these impacts have not yet been elucidated.

Findings from previous studies that have investigated the association between childhood or early life trauma and adult *NR3C1* methylation in peripheral blood have been mixed. Whereas some studies have reported increased levels of *NR3C1* methylation overall and within promoter regions associated with number of traumas[17, 18] or specific domains of childhood/adolescent adversity (e.g., physical abuse and emotional neglect)[37, 38], other studies have reported decreased methylation around promoter regions and at individual CpG sites associated with different measures early life trauma[19, 38, 39]. Still other studies report no association between early life adversity and *NR3C1* methylation[20, 40, 41]. The variation in these results may be in part to the heterogeneity of measures used to assess childhood and/adolescent trauma, the period during which trauma data were ascertained, the *NR3C1* CpG sites included in the analyses, the method used to capture *NR3C1* methylation data, whether the studies corrected for multiple testing, as well as the study populations represented in the study, which differed by age, sex, and proportion of participants selected for known psychiatric and mental health disorders.

The lack of associations between early life adversity and *NR3C1* methylation observed in this study, when previous studies have reported otherwise, may be attributable to several factors.

Our findings are consistent with two studies by Vangeel et al. that found no association between childhood trauma and emotional abuse and *NR3C1* methylation in women diagnosed with chronic fatigue syndrome, who had a mean age older than 40 years[20, 41]. The lack of associations observed in those studies, as well as the current study, could be attributed to a in part due to the long period between the reported early life trauma and the measurement of *NR3C1* methylation in adulthood. Although DNA methylation is thought to be a durable epigenetic modification, methylation levels can be modified over time and may not persist over the lifecourse[42, 43]. As such, different risk or protective factors experienced over the life course may mediate the association between the reported trauma and the observed methylation patterns. A comprehensive study by Marzi et al. looked at the association between childhood trauma and *NR3C1* methylation at age 18, thus eliminating the long period between exposure and measurement, and found no associations[40]. However, Marzi et al. cited a limitation of using commercial arrays to assess detailed methylation patterns among candidate genes commonly associated with stress and maltreatment as these regions are not well covered in the Illumina array[6, 40, 44].

The current study has some limitations. First, this study presents a single measure of DNA methylation of a single stress response gene, *NR3C1* captured in later adulthood (after 35 years of age). It is unknown when epigenetic modification becomes stabilized following exposures of stress and trauma and the degree to which these changes can be modified post exposure. Although *NR3C1* plays a pivotal part in the stress response due to the role of glucocorticoid receptors in HPA axis signaling, it is a single gene in a complex network of mechanisms that characterize the stress management process. In order to better assess the role of epigenetic modification in the association between early life trauma and stress response

mechanisms and subsequent health outcomes, it would be prudent to gain a more global picture by assessing additional HPA axis genes.

Second, although it can be assumed that experience of early life trauma occurred prior to measurement of *NR3C1* methylation in adulthood, the stress and trauma questionnaire was completed at the biennial follow-up interview, approximately two years after the blood sample was collected at baseline. Although adult trauma was assessed for the period between age 18 and a year before completion of the stress questionnaire, it is possible that reported adult traumas could have occurred after the blood sample was collected, thus attenuating the observed effect of adult trauma on *NR3C1* methylation.

Finally, our population was limited to non-Hispanic White women, who, due to the eligibility criteria of the parent Sister Study population, all have a family history of breast cancer, and were sampled in a case-cohort fashion. The Sister Study cohort is generally older, more educated, and more likely to be healthy and economically sound, which may underestimate the effects of early life trauma in more vulnerable populations. Although this would not affect the internal validity of our results, it may limit their application to other diverse populations.

This study also has several strengths and innovations. First, this is the first study to look at the relationship between early life trauma and *NR3C1* methylation using a life course framework within a structural equation model approach. Other studies that control for SEP measures over the life course, adult trauma, and/or adult health and lifestyle factors may inappropriately adjust for complex mediating pathways that affect the overall relationship between early life trauma and *NR3C1* methylation in adulthood, thus attenuating the effect of early life trauma on *NR3C1* methylation. Second, whereas previous studies have utilized summary measures, domains, or distinct events to assess life course trauma, we utilize a latent

class approach for early life trauma and a latent variable approach for adult trauma to capture potential substantive differences in the role of concomitant traumas on adult *NR3C1* methylation.

In summary, we did not find an association between latent classes of early life trauma and adult levels of *NR3C1* methylation from peripheral blood, either overall, based on functional location, or at specific CpG sites along the *NR3C1* gene. However, the small decrease in methylation among CpG sites along the gene body associated with a latent class of early life trauma representing moderate trauma associated with sexual trauma and family drug, alcohol, and/or mental health issues may suggest that the cooccurrence of these traumas may open the door for potential alternative transcription of the *NR3C1* variants and that various clusters of concurrent early life traumatic events should be explored to identify more nuanced patterns of epigenetic modification. Large studies that encompass multiple stress response genes may provide a better picture of the role epigenetic modification may play in the association between the experience of early life trauma and poor health outcomes in adulthood.

3.5 Tables and Figures

Figure 3.1 Cystine-phosphate-guanine (CpG) sites located on *NR3C1*

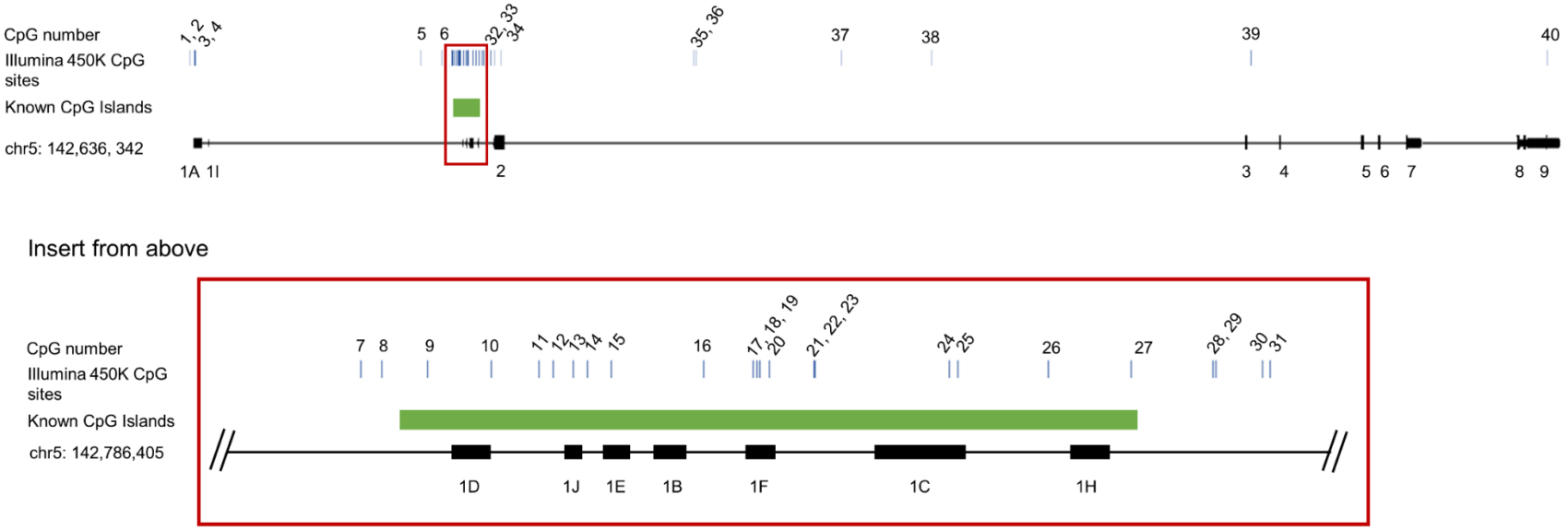
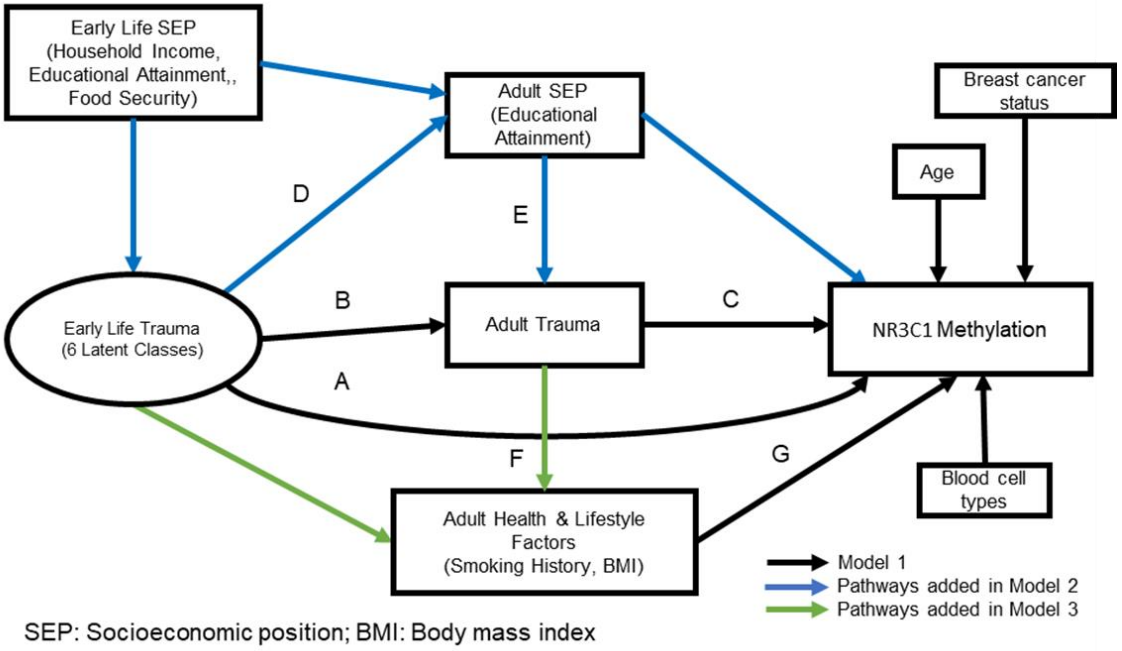


Figure 3.2 Life course model of *NR3C1* methylation with annotated pathways of interest



SEP: Socioeconomic position; BMI: Body mass index

Table 3.1 Characteristics of a sub-sample of 2,678 Sister Study participants based on experience of early life traumas before age 18

	No Early Life Trauma (n = 1,451)		Any Early Life Trauma (n = 1,227)		p-value
	n	%	n	%	
Breast cancer status					0.104
No breast cancer	591	40.73	538	43.85	
Invasive or in situ breast cancer	860	59.27	689	56.15	
Age at baseline, mean (SD)	57.81	8.86	56.47	8.53	<.0001
Highest level of education in the household at age 13, %					0.113
Less than high school	202	13.92	207	16.87	
High school graduate	533	36.73	441	35.94	
Some college	288	19.85	252	20.54	
College degree or higher	428	29.5	327	26.65	
Family income while growing up, %					<.0001
Poor	54	3.72	114	9.29	
Low income	336	23.16	365	29.75	
Middle income	976	67.26	676	55.09	
Well off	85	5.86	72	5.87	
As a child, were there times when your family did not have enough food to eat, %					<.0001
Yes	60	4.14	140	11.41	
No	1391	95.86	1087	88.59	
Body Mass Index					0.627
Underweight	19	1.31	10	0.81	
Normal weight	561	38.66	467	38.06	
Overweight	469	32.32	401	32.68	
Obese	401	27.64	349	28.44	
Smoking Status					0.072
Never smoked	789	54.38	613	49.96	
Past smoker	564	38.87	526	42.87	
Current smoker	98	6.75	88	7.17	

Table 3.2 *NR3C1* percent methylation by location

Location	% Methylated
Overall	34.83
Promotor-associated	10.05
Gene body	93.34

Table 3.3 *NR3C1* percent methylation by CpG site

Study number	CpG Site		% Methylated	Study number	CpG Site		% Methylated
	Name	Location			Name	Location	
1	cg12466613	TSS1500	96.04	21	cg18019515	TSS200/5'UTR	1.25
2	cg07589972	TSS1500	96.82	22	cg11152298	TSS200/5'UTR	1.36
3	cg26720913	1stExon/5'UTR	0.56	23	cg00629244	TSS200/5'UTR	0.61
4	cg08818984	1stExon/5'UTR	1.13	24	cg18146873	1stExon/5'UTR	0.99
5	cg07528216	5'UTR	97.82	25	cg20753294	1stExon/5'UTR	5.62
6	cg27345592	5'UTR	97.71	26	cg17617527	5'UTR	1.45
7	cg13648501	TSS1500/5'UTR	2.20	27	cg06521673	5'UTR	0.93
8	cg24026230	5'UTR	1.59	28	cg06952416	5'UTR	5.56
9	cg14558428	TSS1500/5'UTR	0.82	29	cg27122725	5'UTR	5.53
10	cg21702128	TSS1500/5'UTR	2.76	30	cg18998365	5'UTR	65.76
11	cg10847032	TSS1500/5'UTR	1.22	31	cg07733851	5'UTR	36.69
12	cg16335926	TSS1500/5'UTR	1.49	32	cg08845721	5'UTR	96.52
13	cg18849621	TSS1500/5'UTR	1.54	33	cg17342132	Body	94.11
14	cg06968181	TSS1500/5'UTR	4.76	34	cg06613263	Body	93.74
15	cg26464411	TSS1500	2.91	35	cg25535999	Body	96.34
16	cg18068240	TSS1500/5'UTR	0.63	36	cg16586394	Body	97.32
17	cg15645634	TSS1500/5'UTR	0.59	37	cg18484679	Body	95.88
18	cg15910486	TSS1500/5'UTR	5.07	38	cg03857453	Body	82.27
19	cg04111177	TSS1500/5'UTR	1.40	39	cg19457823	Body	94.46
20	cg17860381	TSS1500/5'UTR	1.54	40	cg23273257	3'UTR	98.17

Table 3.4. Associations between early life trauma and mean percent methylation across NR3C1 CpG sites or functional subgroups with early life trauma

		All locations			Promoter-Associated			Gene body		
Early life trauma Latent Classes	**n**	**Estimate[a]**	**p value**	**% Methylation Change**	**Estimate[a]**	**p value**	**% Methylation Change**	**Estimate[a]**	**p value**	**% Methylation Change**
Low early life trauma	1682	Ref			Ref			Ref		
Moderate early life trauma: Family health issues	54	-0.0061	0.4738	-0.06	-0.0063	0.5524	-0.01	-0.0204	0.2214	-0.07
Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues	149	0.0005	0.9287	0.01	0.0056	0.3841	0.01	**-0.0214**	**0.0357**	**-0.08**
Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues	171	-0.0016	0.7540	-0.02	-0.0036	0.5601	-0.01	-0.0018	0.8501	-0.01
Moderate early life trauma: Overall	120	0.0071	0.2286	0.07	0.0012	0.8698	0.00	0.0141	0.2184	0.05
High early life trauma	47	-0.0059	0.5404	-0.06	-0.0037	0.7527	-0.01	-0.0137	0.4633	-0.05

[a]Adjusted for age, breast cancer status, smoking history, body mass index, and cell types.

Figure 3.3 Percent change in NR3C1 CpG site methylation by latent class of early life trauma (compared to low early life trauma)

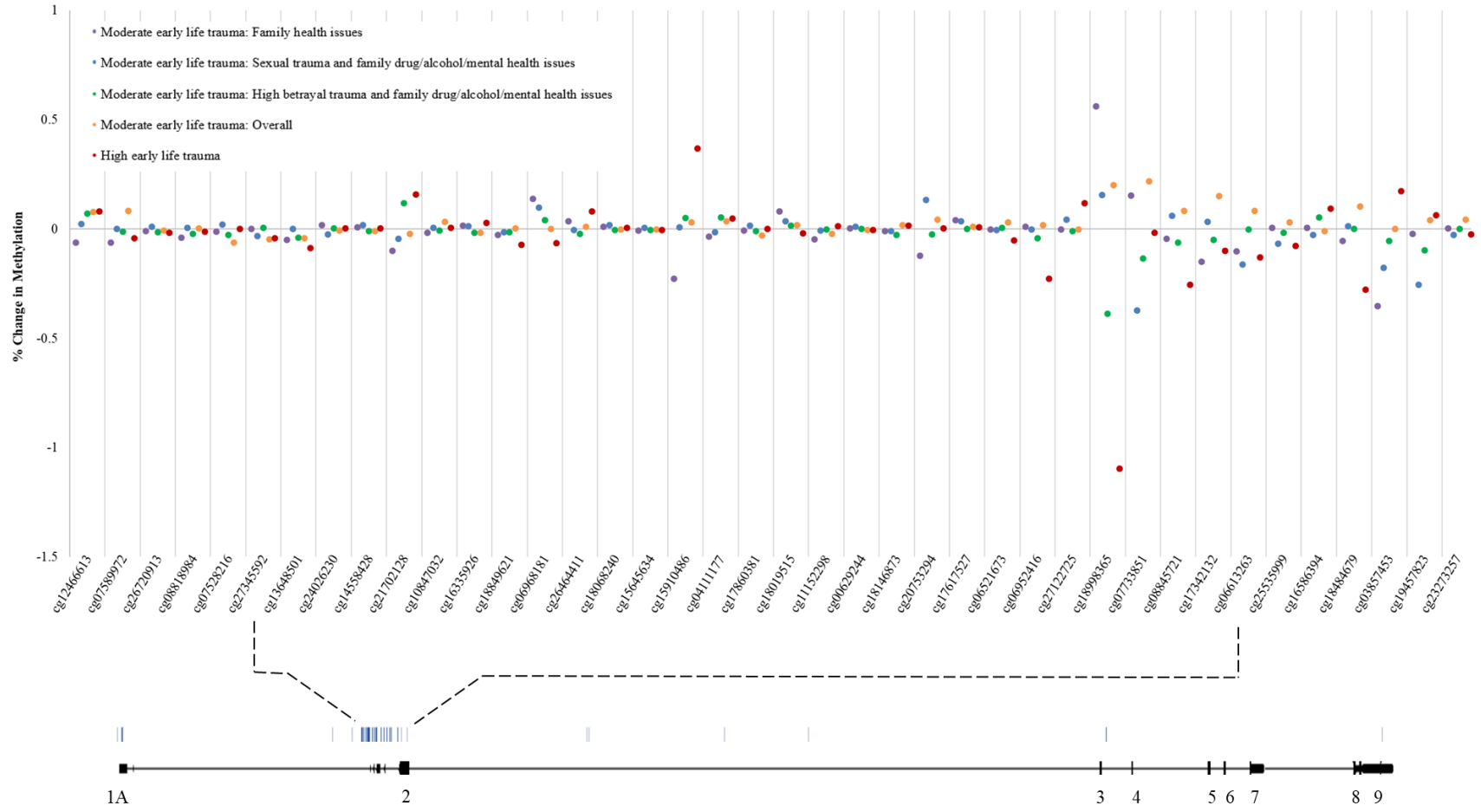


Table 3.5 Estimates of the association between latent classes of early life trauma (compared to low early life trauma) and NR3C1 methylation on the gene body

	Model 1[a]				Model 2[b]				Model 3[c]			
Pathway	**β**	**SE**	**p-value**	**Δ % mDNA**	**β**	**SE**	**p-value**	**Δ % mDNA**	**β**	**SE**	**p-value**	**Δ % mDNA**
Adult Trauma	0.004	0.016	0.807	0.01	0.021	0.016	0.194	0.07	-0.021	0.018	0.240	-0.07
Moderate early life trauma: Family health issues												
Direct	-0.018	0.016	0.276	-0.06	-0.007	0.006	0.286	-0.02	-0.009	0.008	0.254	-0.03
Total	-0.018	0.016	0.277	-0.06	-0.007	0.006	0.286	-0.02	-0.005	0.006	0.434	-0.02
Total Indirect	0.000	0.000	0.815	0.00	0.000	0.000	0.827	0.00	0.004	0.002	0.063	0.01
Indirect - Adult Trauma[d]	0.000	0.000	0.815	0.00	0.000	0.000	0.973	0.00	0.000	0.000	0.623	0.00
Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues												
Direct	**-0.022**	**0.010**	**0.032**	**-0.08**	**-0.011**	**0.005**	**0.014**	**-0.04**	**-0.009**	**0.005**	**0.048**	**-0.03**
Total	**-0.021**	**0.010**	**0.033**	**-0.07**	**-0.010**	**0.004**	**0.020**	**-0.04**	**-0.011**	**0.004**	**0.011**	**-0.04**
Total Indirect	0.000	0.001	0.807	0.00	0.001	0.001	0.214	0.00	-0.002	0.001	0.114	-0.01
Indirect - Adult Trauma[d]	0.000	0.001	0.807	0.00	0.001	0.001	0.220	0.00	-0.001	0.001	0.247	0.00
Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues												
Direct	-0.004	0.010	0.711	-0.01	-0.002	0.004	0.681	-0.01	0.001	0.004	0.873	0.00
Total	-0.003	0.010	0.740	-0.01	0.000	0.004	0.914	0.00	-0.001	0.004	0.762	0.00
Total Indirect	0.000	0.002	0.807	0.00	0.001	0.001	0.198	0.00	-0.002	0.001	0.123	-0.01
Indirect - Adult Trauma[d]	0.000	0.002	0.807	0.00	0.001	0.001	0.206	0.00	-0.001	0.001	0.248	0.00
Moderate early life trauma: Overall												
Direct	0.013	0.011	0.243	0.05	0.008	0.005	0.163	0.03	0.01	0.006	0.086	0.04
Total	0.014	0.011	0.225	0.05	0.009	0.005	0.107	0.03	0.008	0.005	0.147	0.03
Total Indirect	0.000	0.002	0.807	0.00	0.001	0.001	0.191	0.00	-0.002	0.001	0.126	-0.01
Indirect - Adult Trauma[d]	0.000	0.002	0.807	0.00	0.001	0.001	0.199	0.00	-0.001	0.001	0.264	0.00

Table 3.5 (*continued*)

	Model 1[a]				Model 2[b]				Model 3[c]			
Pathway	**β**	**SE**	**p-value**	**Δ % mDNA**	**β**	**SE**	**p-value**	**Δ % mDNA**	**β**	**SE**	**p-value**	**Δ % mDNA**
High early life trauma												
Direct	-0.011	0.019	0.553	-0.04	-0.005	0.007	0.495	-0.02	-0.001	0.008	0.915	0.00
Total	-0.010	0.018	0.574	-0.04	-0.003	0.006	0.622	-0.01	-0.005	0.006	0.457	-0.02
Total Indirect	0.001	0.003	0.807	0.00	0.002	0.001	0.209	0.01	-0.004	0.002	0.070	-0.01
Indirect - Adult Trauma[d]	0.001	0.003	0.807	0.00	0.002	0.001	0.216	0.01	-0.002	0.001	0.240	-0.01
	RMSEA = 0.030; CFI = 0.985				RMSEA = 0.021; CFI = 0.889				RMSEA = 0.017; CFI = 0.919			

[a] Adjusted for age, breast cancer status, cell types, smoking history, and BMI

[b] Additionally adjusted for early life socioeconomic factors (early life household income, household educational attainment, and food security) and allowing mediating pathway via adult socioeconomic position (educational attainment)

[c] Additionally allow for mediating pathways via adult lifestyle factors (smoking history and body mass index)

[d] Pathways from Figure 3.2 - Model 1: B-C; Model 2: Additionally D-E-C; Model 3: Additionally D-E-F-G and B-F-G

CFI: Comparative fit index; mDNA: Methylation; RMSEA: Root mean square error of approximation; SE: Standard error

3.6 Supplemental Materials

Supplemental Table 3.1 Latent classes of early life trauma and adult NR3C1 methylation by CpG site

	Moderate early life trauma: Family health issues				Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg12466613	-0.0235	0.4072	0.9676	-0.0618	0.0093	0.5887	0.9889	0.0243
cg07589972	-0.0285	0.3188	0.9676	-0.0609	-0.0003	0.9873	0.9971	-0.0006
cg26720913	-0.0228	0.4761	0.9889	-0.0086	0.0261	0.1805	0.8870	0.0100
cg08818984	-0.0518	0.1982	0.9008	-0.0385	0.0071	0.7721	0.9971	0.0054
cg07528216	-0.0077	0.7849	0.9971	-0.0113	0.0133	0.4396	0.9846	0.0194
cg27345592	0.0001	0.9952	0.9971	0.0002	-0.0213	0.1332	0.8034	-0.0331
cg13648501	-0.0336	0.3946	0.9676	-0.0486	-0.0002	0.9919	0.9971	-0.0004
cg24026230	0.0172	0.4845	0.9889	0.0186	-0.0224	0.1366	0.8034	-0.0238
cg14558428	0.0147	0.5174	0.9889	0.0083	**0.0313**	**0.0239**	0.6836	0.0178
cg21702128	-0.0559	0.2143	0.9237	-0.0992	-0.0247	0.3682	0.9676	-0.0443
cg10847032	-0.0194	0.5879	0.9889	-0.0158	0.0062	0.7776	0.9971	0.0051
cg16335926	0.0154	0.5985	0.9889	0.0156	0.0119	0.5058	0.9889	0.0120
cg18849621	-0.0263	0.4953	0.9889	-0.0270	-0.0151	0.5201	0.9889	-0.0156
cg06968181	0.0439	0.1907	0.8870	0.1378	0.0315	0.1240	0.8034	0.0984
cg26464411	0.0179	0.6603	0.9971	0.0347	-0.0023	0.9249	0.9971	-0.0045
cg18068240	0.0246	0.2954	0.9676	0.0107	**0.0403**	**0.0050**	0.3350	0.0176
cg15645634	-0.0201	0.4232	0.9676	-0.0080	0.0132	0.3884	0.9676	0.0053
cg15910486	-0.0716	0.1195	0.8034	-0.2278	0.0023	0.9346	0.9971	0.0075
cg04111177	-0.0392	0.4624	0.9889	-0.0355	-0.0151	0.6436	0.9971	-0.0138
cg17860381	-0.0075	0.8399	0.9971	-0.0077	0.0159	0.4829	0.9889	0.0165
cg18019515	**0.0912**	**0.0004**	0.0802	0.0799	**0.0407**	**0.0095**	0.3817	0.0351
cg11152298	-0.0537	0.2322	0.9448	-0.0476	-0.0066	0.8107	0.9971	-0.0059
cg00629244	0.0077	0.7371	0.9971	0.0032	0.0240	0.0871	0.8034	0.0100
cg18146873	-0.0138	0.7223	0.9971	-0.0091	-0.0144	0.5423	0.9889	-0.0095

Supplemental Table 3.1 *(continued)*

	Moderate early life trauma: Family health issues				Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg20753294	-0.0337	0.3171	0.9676	-0.1213	0.0366	0.0751	0.8034	0.1345
cg17617527	0.0406	0.2158	0.9237	0.0399	0.0369	0.0651	0.7839	0.0363
cg06521673	-0.0028	0.9544	0.9971	-0.0017	-0.0072	0.8080	0.9971	-0.0045
cg06952416	0.0031	0.9324	0.9971	0.0110	-0.0003	0.9875	0.9971	-0.0012
cg27122725	-0.0002	0.9948	0.9971	-0.0008	0.0122	0.5775	0.9889	0.0438
cg18998365	0.0363	0.4162	0.9676	0.5625	0.0100	0.7140	0.9971	0.1551
cg07733851	0.0095	0.8421	0.9971	0.1529	-0.0233	0.4243	0.9676	-0.3728
cg08845721	-0.0197	0.6968	0.9971	-0.0447	0.0271	0.3812	0.9676	0.0604
cg17342132	-0.0402	0.3393	0.9676	-0.1493	0.0093	0.7167	0.9971	0.0341
cg06613263	-0.0255	0.5466	0.9889	-0.1023	-0.0399	0.1215	0.8034	-0.1610
cg25535999	0.0022	0.9416	0.9971	0.0053	-0.0276	0.1325	0.8034	-0.0671
cg16586394	0.0028	0.9337	0.9971	0.0049	-0.0155	0.4431	0.9846	-0.0277
cg18484679	-0.0197	0.5246	0.9889	-0.0536	0.0049	0.7945	0.9971	0.0133
cg03857453	-0.0350	0.2846	0.9676	-0.3527	-0.0178	0.3735	0.9676	-0.1783
cg19457823	-0.0064	0.8823	0.9971	-0.0224	**-0.0710**	**0.0070**	0.3500	-0.2536
cg23273257	0.0024	0.9298	0.9971	0.0029	-0.0214	0.1895	0.8870	-0.0266

	Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues				Moderate early life trauma: Overall			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg12466613	0.0272	0.1013	0.8034	0.0704	0.0304	0.1184	0.8034	0.0787
cg07589972	-0.0055	0.7437	0.9971	-0.0116	**0.0397**	**0.0439**	0.7839	0.0828
cg26720913	**-0.0370**	**0.0487**	0.7839	-0.0139	-0.0177	0.4225	0.9676	-0.0067
cg08818984	-0.0309	0.1899	0.8870	-0.0232	0.0056	0.8384	0.9971	0.0043
cg07528216	-0.0177	0.2858	0.9676	-0.0260	**-0.0417**	**0.0318**	0.7417	-0.0618
cg27345592	0.0029	0.8305	0.9971	0.0045	-0.0297	0.0639	0.7839	-0.0462
cg13648501	-0.0269	0.2456	0.9448	-0.0389	-0.0287	0.2902	0.9676	-0.0416
cg24026230	0.0037	0.7956	0.9971	0.0040	-0.0076	0.6549	0.9971	-0.0081

Supplemental Table 3.1 *(continued)*

	Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues				Moderate early life trauma: Overall			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg14558428	-0.0149	0.2616	0.9676	-0.0084	-0.0158	0.3109	0.9676	-0.0089
cg21702128	**0.0638**	**0.0156**	0.5211	0.1178	-0.0125	0.6858	0.9971	-0.0226
cg10847032	-0.0076	0.7191	0.9971	-0.0062	0.0411	0.0954	0.8034	0.0342
cg16335926	-0.0168	0.3277	0.9676	-0.0168	-0.0160	0.4257	0.9676	-0.0160
cg18849621	-0.0141	0.5338	0.9889	-0.0145	0.0035	0.8953	0.9971	0.0036
cg06968181	0.0131	0.5070	0.9889	0.0406	0.0002	0.9915	0.9971	0.0008
cg26464411	-0.0116	0.6286	0.9971	-0.0221	0.0054	0.8477	0.9971	0.0104
cg18068240	-0.0080	0.5638	0.9889	-0.0034	-0.0036	0.8223	0.9971	-0.0016
cg15645634	-0.0110	0.4531	0.9889	-0.0044	-0.0027	0.8764	0.9971	-0.0011
cg15910486	0.0155	0.5650	0.9889	0.0507	0.0095	0.7639	0.9971	0.0310
cg04111177	0.0576	0.0651	0.7839	0.0540	0.0390	0.2880	0.9676	0.0363
cg17860381	-0.0100	0.6450	0.9971	-0.0103	-0.0298	0.2437	0.9448	-0.0305
cg18019515	0.0178	0.2380	0.9448	0.0152	0.0219	0.2171	0.9237	0.0187
cg11152298	-0.0025	0.9257	0.9971	-0.0022	-0.0251	0.4171	0.9676	-0.0224
cg00629244	-0.0007	0.9584	0.9971	-0.0003	-0.0083	0.5984	0.9889	-0.0034
cg18146873	-0.0402	0.0769	0.8034	-0.0264	0.0272	0.3074	0.9676	0.0183
cg20753294	-0.0068	0.7296	0.9971	-0.0248	0.0116	0.6155	0.9928	0.0425
cg17617527	0.0016	0.9326	0.9971	0.0016	0.0098	0.6633	0.9971	0.0096
cg06521673	0.0081	0.7769	0.9971	0.0051	0.0479	0.1532	0.8284	0.0303
cg06952416	-0.0114	0.5891	0.9889	-0.0409	0.0054	0.8291	0.9971	0.0193
cg27122725	-0.0024	0.9088	0.9971	-0.0086	-0.0003	0.9910	0.9971	-0.0010
cg18998365	-0.0248	0.3427	0.9676	-0.3874	0.0129	0.6740	0.9971	0.2007
cg07733851	-0.0084	0.7649	0.9971	-0.1343	0.0136	0.6790	0.9971	0.2185
cg08845721	-0.0270	0.3631	0.9676	-0.0613	0.0378	0.2784	0.9676	0.0840
cg17342132	-0.0138	0.5768	0.9889	-0.0507	0.0413	0.1533	0.8284	0.1496
cg06613263	-0.0006	0.9813	0.9971	-0.0023	0.0210	0.4703	0.9889	0.0831
cg25535999	-0.0072	0.6835	0.9971	-0.0174	0.0124	0.5497	0.9889	0.0297

Supplemental Table 3.1 *(continued)*

	Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues				Moderate early life trauma: Overall			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg16586394	0.0295	0.1282	0.8034	0.0520	-0.0057	0.8028	0.9971	-0.0101
cg18484679	0.0003	0.9871	0.9971	0.0008	0.0390	0.0666	0.7839	0.1043
cg03857453	-0.0054	0.7788	0.9971	-0.0539	0.0001	0.9965	0.9971	0.0010
cg19457823	-0.0279	0.2709	0.9676	-0.0982	0.0118	0.6909	0.9971	0.0411
cg23273257	-0.0002	0.9916	0.9971	-0.0002	0.0358	0.0519	0.7839	0.0436

	High early life trauma			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg12466613	0.0314	0.3224	0.9676	0.0812
cg07589972	-0.0201	0.5317	0.9889	-0.0427
cg26720913	-0.0421	0.2406	0.9448	-0.0158
cg08818984	-0.0152	0.7365	0.9971	-0.0114
cg07528216	-0.0002	0.9951	0.9971	-0.0003
cg27345592	-0.0273	0.2961	0.9676	-0.0424
cg13648501	-0.0602	0.1739	0.8870	-0.0862
cg24026230	0.0038	0.8894	0.9971	0.0041
cg14558428	0.0063	0.8055	0.9971	0.0035
cg21702128	0.0851	0.0913	0.8034	0.1584
cg10847032	0.0057	0.8874	0.9971	0.0047
cg16335926	0.0285	0.3850	0.9676	0.0289
cg18849621	-0.0701	0.1049	0.8034	-0.0708
cg06968181	-0.0207	0.5816	0.9889	-0.0637
cg26464411	0.0419	0.3598	0.9676	0.0816
cg18068240	0.0139	0.5983	0.9889	0.0060
cg15645634	-0.0093	0.7408	0.9971	-0.0037
cg15910486	**0.1096**	**0.0334**	0.7417	0.3692
cg04111177	0.0514	0.3901	0.9676	0.0480
cg17860381	-0.0002	0.9971	0.9971	-0.0002

Supplemental Table 3.1 *(continued)*

	High early life trauma			
CpG site	**Estimate**	**P - value**	**q value**	**Δ % mDNA**
cg18019515	-0.0231	0.4237	0.9676	-0.0195
cg11152298	0.0153	0.7608	0.9971	0.0139
cg00629244	-0.0130	0.6138	0.9928	-0.0054
cg18146873	0.0226	0.6032	0.9889	0.0152
cg20753294	0.0006	0.9872	0.9971	0.0022
cg17617527	0.0071	0.8462	0.9971	0.0069
cg06521673	-0.0868	0.1122	0.8034	-0.0524
cg06952416	-0.0645	0.1108	0.8034	-0.2270
cg27122725	0.0329	0.4150	0.9676	0.1184
cg18998365	-0.0698	0.1628	0.8566	-1.0948
cg07733851	-0.0011	0.9834	0.9971	-0.0179
cg08845721	-0.1087	0.0555	0.7839	-0.2535
cg17342132	-0.0268	0.5699	0.9889	-0.0990
cg06613263	-0.0322	0.4967	0.9889	-0.1295
cg25535999	-0.0311	0.3558	0.9676	-0.0758
cg16586394	0.0537	0.1475	0.8284	0.0940
cg18484679	**-0.0991**	**0.0042**	0.3350	-0.2770
cg03857453	0.0173	0.6366	0.9971	0.1725
cg19457823	0.0180	0.7101	0.9971	0.0625
cg23273257	-0.0204	0.4961	0.9889	-0.0254

mDNA: Methylation; SE: Standard error

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CHAPTER 4

Early Life Trauma and Adult Leucocyte Telomere Length

4.1 Introduction

Growing evidence has linked trauma with adverse psychological and physiological health outcomes[1, 2]. Furthermore, research suggests children may be particularly vulnerable to the effects of trauma from its disruptive effect on neurological, physiological, or psychological development[3]. Childhood trauma has been associated with high risk behaviors[4, 5], cognitive impairment[6], high risk of revictimization[6], and adverse mental and physical health outcomes later in life[1, 7]. Under life course conceptual models, early life environments may lead to poor outcomes due to exposures during developmentally sensitive periods or by contributing to cumulative damage to biological systems. In a sensitive period model, trauma/adversity in early life has lasting effects on the structure or function of the body and long-term health effects, independent of subsequent adverse exposures (Figure 4.1A)[8]. Alternatively, the accumulation of risk factors over the life course may have an additive effect that results in cumulative biological damage contributing to poor health later in life or may have a trigger effect in which the final link in the chain or risk (i.e., the adult exposure) dictates the final health outcome (Figure 4.1B)[8, 9]. Evaluating the pathways of early childhood trauma on biological mechanisms of aging and disease development provides insight into the early life origins and etiology of adverse health outcomes.

Telomeres are repeating DNA protein complexes at the end of chromosomes that protect chromosomal stability and integrity[10]. Telomeres gradually shorten with each subsequent cell division, which continues until cell cycle arrest or cellular senescence; the state in which

telomeres reach a critical length and no longer have the capacity to divide, occurs[11]. Research suggests telomeres are linked to age-dependent processes[12], the development of chronic diseases[13] including cancer[14], obesity, diabetes[15, 16], cardiovascular disease[15, 17], and mortality[18]. Therefore, telomeres have been widely investigated in research as a biomarker for cellular aging and age-related diseases[19].

Due to the growing evidence linking telomere length and disease development, telomeres have been proposed as a pathway to describe how trauma and other adversities are biologically embodied "under the skin" to influence adverse health outcomes[10]. In 2010, preliminary research suggested childhood maltreatment was associated with shorter telomeres[20]. Since then, research has supported the association between childhood and adolescent trauma and shorter telomeres[21-26] and revealed mixed, reversed, and more complex associations related to age and trauma measurement[27, 28]. The type of traumatic adversity[22, 23, 29, 30], number of traumatic exposures[25, 27, 31], and timing of exposure[29, 31] have been shown to have differential or compounding effects on telomere length with increased trauma severity or chronic exposures contributing to shorter telomere length. However, few studies have looked at how the cooccurrence of specific traumas may play a role in telomere attrition.

Recent life course research evaluating cumulative lifetime adversity has further suggested that exposure during early life may be particularly damaging, revealing cumulative adversity during early life predicted shorter telomere length in adulthood[24] in addition to shorter baseline telomere length and greater telomere attrition in mid-life[31]. Willis et al. proposed a social trajectory or accumulation model in which the association between childhood stressful life events and adult saliva telomere length was fully mediated through adult stressful life events[32]. To our knowledge, no study has evaluated the mediation of the association between early life trauma

during childhood and adolescence and telomere length by later life experiences in peripheral blood or evaluated how different patterns of concurrent trauma in early life may differentially affect adult telomere length. This study aims to elucidate the relationship between early life trauma and adult leukocyte telomere length, a marker of cellular aging and the potential mediating role of chronic trauma in adulthood in this relationship. Furthermore, this study uses a latent class approach to assess the role of cooccurring traumas on telomere attrition. Evaluating the effect of cooccurring traumatic experiences during specific windows of vulnerability across the life course allows for life course mechanisms to be tested, filling gaps in current knowledge about the relationships between early life stress and adult telomere length. It was hypothesized that childhood traumatic adversities will be associated with shorter telomere length independent of exposure to traumatic adversities later in life.

4.2 Methods

Study population

The Sister Study is a prospective cohort study designed to assess environmental and genetic risk factors for breast cancer and other conditions[33]. Participants are U.S. and Puerto Rican women (ages 35 to 74 years old; N = 50,884) and have a biological sister previously diagnosed with breast cancer but had not been diagnosed with breast cancer themselves at baseline (2003-2009). During the baseline visit, participants completed a telephone interview, as well as anthropometric measurements and blood sample collection; annual health updates and detailed follow-up visits are completed every 2-3 years. Requests for Sister Study data can be submitted by following the directions at http://sisterstudy.niehs.nih.gov/English/coll-data.htm.

All participants provided written informed consent. The study was approved by the Institutional Review Boards of the National Institute of Environmental Health Sciences and the

Copernicus Group. The current analysis using coded data was considered exempt from human subject research by the Institutional Review Board at the University of Wisconsin-Milwaukee.

An initial vanguard sub-sample of 740 women from the first 2086 Sister Study participants who completed the first annual health update follow-up by June 2005 was selected to conduct telomere studies. Smokers, nonwhite women, and women with high perceived stress were oversampled and the remaining women were sampled randomly[34].

We excluded 100 participants with insufficient telomere length data and an additional 73 participants who did not complete a stress and trauma questionnaire at the first follow-up visit (two to three years after enrollment). A total of 602 participants were included in the present study.

Traumatic events over the life course

Adverse experiences and traumatic events were collected during the detailed follow-up questionniare[33], which was conducted two to three years after baseline and included original items from the Brief Betrayal Trauma Survey[35] as well as items modified for The Sister Study. Participants were asked whether they had ever experienced each traumatic event in their lifetime (Possible responses: Yes, No, Don't know, Refuse to answer). Participants who answered affirmatively, were asked if they experienced the trauma before age 13 (childhood), between the ages of 13 and 17 (adolescence), after age 18 until 12 months prior to survey completion (adulthood), and during the past twelve months (recent adulthood). Traumatic events classified as occurring in recent adulthood were excluded as they would have occurred after the blood sample was collected at baseline. The 20 traumatic experiences collected in the stress and trauma questionnaire and included in this study were classified into 7 domains: 1) natural disasters, 2)

major accidents, 3) household dysfunction, 4) sexual abuse, 5) physical abuse, 6) emotional or psychological abuse, and 7) major illness before age 18.

A latent class variable was created to better capture the co-occurrence of early life traumas among Sister Study participants who completed the trauma questionnaire (n = 45,973) in order to capture the distribution of trauma profiles in the underlying Sister Study cohort. We estimated five latent class models with 2 to 6 latent clusters using the 20 types of early life trauma as indicators. Within each model, we assigned substantive classifications based on conditional response probabilities of each cluster. The latent class model with six clusters had the best model fit of the tested models while still maintaining moderate to high level certainty that participants were placed in the correct clusters, which is evaluated using entropy on a scale of 0 to 1 (entropy = 0.72). We identified six latent classes based on the proportion of women who reported each early life trauma within each cluster: 1) low early life trauma (average probability of reporting any type of early life trauma was less than 2% across all possible traumatic events; referent), 2) family health issues (highest proportions for death of a parent and major illness (not breast cancer) in someone close, 40% and 49%, respectively. Average across all other traumas = 6%), 3) sexual trauma and family drug/alcohol/mental health issues (highest proportions for unwanted sexual contact by someone close (22%) or someone not close (22%) and family drug, alcohol, and/or mental health issues (17%). Average across all other traumas = 4%), 4) high betrayal trauma and family drug/alcohol/mental health issues (highest proportions for traumas perpetrated by someone close – unwanted sexual contact (22%), hit or attached (16%), emotionally or psychologically mistreated (99%) and family drug, alcohol, and/or mental health issues (28%). Average across all other traumas = 5%), 5) moderate early life trauma: overall (average probability of reporting each early life traumatic event = 17%), and 6) high early life

trauma (average probability of reporting each early life traumatic event = 32%). Adult trauma that occurred between age 18 and 12 months prior to completing the stress and trauma questionnaire was assessed using a latent variable created from adult responses to the same 20 trauma types used to assess early life trauma (RMSEA = 0.025, CFI = 0.919).

Leukocyte telomere length

Whole blood samples were obtained during the home visit at study baseline prior to any diagnosis of breast cancer. Samples were then shipped and stored at -80°C until DNA was extracted. Relative telomere length was assessed using PCR-based assays using methods described elsewhere [34, 36, 37]. In summary, 1µL aliquots of 100 to 200 ng of template DNA was amplified for telomeric DNA and a single-copy gene. Telomere length is measured as a ratio (T/S ratio) of the relative amount of telomeric DNA (T) to the relative amount of DNA from the single-copy control gene (S). Details on how the samples were run and quality control are previously described[34].

Covariates

Covariates were age at baseline (continuous); race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, other); measures of early life socioeconomic position (SEP): highest level of education in the household at age 13 (Less than high school, High school graduate, Some college, College degree or higher), family income while growing up (Poor, Low income, Middle income, Well off), and as a child, whether there were times when the family did not have enough food to eat (Yes, No); adult educational attainment (continuous) as a measure of adult SEP; and adult health and lifestyle factors: number of pack years (continuous) and adult body mass index (BMI, continuous).

Statistical analysis

Descriptive statistics were calculated using means and standard deviations for continuous variables and frequency counts for categorical variables.

Risk ratios and 95% confidence intervals (CI) were calculated using linear regression models created to assess the association between latent classes of early life trauma and telomere length, using unadjusted, age-adjusted, and age and early life SEP-adjusted models. Model estimates represent the change in telomere length (T/S ratio) when comparing women in each latent class of early life trauma compared to women who were classified as experiencing low early life trauma. Descriptive statistics and linear regression models were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC)[38] and structural equation models.

We constructed structural equation models using Mplus version 8.3[39] to assess the relationship between early life trauma and telomere length and to evaluate potential mediation through adult trauma (Figure 4.1C). Structural equation modeling accommodates time varying variables (i.e. early life and adult SEP) and can control for confounding along multiple pathways[40]. Path coefficients were estimated using full information maximum likelihood estimation. Goodness of fit was evaluated using root mean square error of approximation (RMSEA) and the Comparative Fit Index (CFI), with RMSEA < 0.02 and CFI > 0.95 as indicators of good fit[41]. We calculated the total effect of early life trauma on telomere length including the direct effect (blue pathway) of early life trauma on telomere length, the indirect effect via adult trauma (red pathways) and indirect effects due to all other mediating pathways as seen in Figure 4.2. Model 1 adjusted for age and race/ethnicity; model 2 additionally adjusted for early life SEP and allowed adult SEP to act as a mediator; and model 3 also allowed adult health and lifestyle factors to serve as potential mediators between early life trauma and telomere length (green pathway; Figure 4.2).

4.3 Results

Sample characteristics

Characteristics of Sister Study participants included in the vanguard telomere subsample are reported in Table 4.1. Approximately 47% of women (n = 284) reported experiencing at least one early life trauma before age 18, which is slightly lower than the rate of reported early life trauma in the full Sister Study cohort (50%), but is still comparable to national surveys of childhood and adolescent trauma in which 48 to 51 percent of women report experiencing at least one trauma over the life course[42, 43]. Women who reported at least one early life trauma were slightly younger at 53.3 (standard deviation: 9.3) years compared to 55.6 (standard deviation: 9.6) years for women who reported no early life trauma. Although both groups were predominantly non-Hispanic White, a higher percentage of women who reported at least one early life trauma also reported being Hispanic or some other race/ethnicity (not non-Hispanic Black). In this subsample, the distribution of parental educational attainment was similar, however, women who reported at least one early life trauma were more likely to report growing up in poor or low-income households and to recall periods of food insecurity. Women who reported at least one early life trauma were more likely to be obese than women without experiences of early life trauma, but less likely to be past or current smokers.

Early life trauma and telomere length

When we ran individual linear regression models to assess the overall association between latent classes of early life trauma and adult telomere length, we found that women who were grouped in the latent class for high early life trauma had telomeres that were 0.112 (95% CI: -0.221, -0.004) T/S units shorter than women who were classified as having experienced low early life trauma (Table 4.2). Although we saw a trend of shorter telomere length observed in all

latent classes of moderate early life trauma, except for moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues, none of the models were statistically significant, whether they were unadjusted or adjusted for age, race/ethnicity, and early life SEP.

Life course models of trauma and telomere length

Table 4.3 presents results from life course models of the association between latent classes of early life trauma and telomere length. The initial pathway model of early life trauma and telomere length allowed for both a direct pathway (blue line) and an indirect pathway via adult trauma (red lines; Figure 4.2-A). The association between moderate early life trauma consisting primarily of family health issues on adult telomere length persisted when direct and indirect (via adult trauma (red lines) and adult health and lifestyle factors (green line)) pathways were considered together after controlling for early life SEP and adult SEP was included as a potential mediator (model 3 fit indices: RMSEA = 0.021, CFI = 0.903); experience of family health issues in early life resulted in telomeres that were 0.042 T/S units shorter than the absence of such experience after holding other covariates constant ($p < 0.05$). Furthermore, high early life trauma was associated with shorter telomere length when direct and indirect pathways were considered in the fully adjusted pathway model; high early life trauma decreased telomere length by 0.060 T/S units holding all covariates constant ($p = 0.008$). This was largely driven by the negative association attributed to the direct effect of high early life trauma on telomere length ($\beta = -0.118$, S.E. 0.057, $p = 0.038$). The indirect effect of early life trauma on telomere length, mediated by adult trauma, was not statistically significant, suggesting that high early life trauma is associated with telomere length independent of adult trauma.

Sensitivity analyses

We also conducted analyses using traditional domains of early life trauma. Using individual linear regression models, we found that women who reported experiencing a natural disaster before age 18 had longer telomeres than women who did not experience a natural disaster in early life after controlling for age, race/ethnicity, and factors of early life SEP (β = 0.124, 95% CI = 0.028, 0.220) (Supplemental Table 4.1). Conversely, women who experienced physical trauma in early life had shorter telomeres than those who did not experience physical trauma before age 18 (β = -0.083, 95% CI = -0.162, -0.004 in age-, race/ethnicity-, and early life SEP-adjusted models. We observed statistically significant inverse total and direct effects between major accidents, physical trauma, and major illness and telomere length, such that women who experienced each of these domains of early life trauma had shorter telomeres than women who did not experience that domain of early life trauma after controlling for all covariates, life course SEP, and allowing mediating pathways via adult smoking history and BMI (Supplemental Table 4.2).

4.4 Discussion

The association between childhood trauma and telomere length has been well studied, however, most studies rely on cumulative scales of childhood trauma[26] or specific exposures of child trauma (i.e., trauma domains[44, 45] or specific events[46, 47]) to represent childhood trauma and often fail to consider adolescent trauma in their measures of early life trauma. Traumas do not usually occur in isolation and summative measures may not fully capture the nuances of co-occurring traumas. We only found two studies that used latent variables to capture early life trauma[32, 48], however, it is difficult to assign substantive meaning to a unit change in these variables making them hard to interpret, so we utilized a latent class model of early life trauma to assess the effects of concomitant traumas. In our latent class model of early life trauma, only

women who were classified as having high early life trauma had significantly shorter telomeres than women classified with low early life trauma in adjusted models.

To assess whether adult trauma mediated the association between early life trauma and telomere length, we used a sensitive period model including later life risk factors assuming early life trauma affects telomere length both directly and indirectly (Figure 4.1). We found in models adjusting for late life risk factors, evidence for a sensitive period effect of early life trauma on leukocyte telomere length. In models with statistically significant direct effects between measures of early life trauma and telomere length, the direct effect of early life trauma on telomere length was in the same direction but had a larger magnitude than the total effect. This , suggests that models that adjust for potential life course mediating factors may not capture the true direct effect of early life trauma on telomere length and may instead be reporting attenuated effects.

In initial regression models, we observed a positive association between natural disasters in early life and telomere length after controlling for age and early life SEP. Although the direct effect of early life natural disasters on telomere length was also positive and statistically significant in sensitive period models that also controlled for age and early life SEP, the association was no longer statistically significant and in the opposite direction in models that controlled for SEP over the life course and allowed for additional mediating pathways through adult health and lifestyle factors. This suggests that the positive association observed with natural disasters could be attributable to other factors that occur over the life course associated with SEP or adult health and lifestyle factors, such as resilience and social support, which has been reported to moderate the association between natural disasters and other health outcomes[49, 50].

Previous studies of the association between early life trauma and telomere length have reported mixed results with just over half of studies reporting statistically significant negative associations between recalled early stressful life events measured in adulthood and telomere length[26]. In a review by Willis et al., the authors note that the heterogeneity in findings appear to be unrelated to the type of measure used to assess early life stress and whether the studies also adjusted for adult health and lifestyle factors or experience of trauma over the life course[26]. However, studies that adjust for potential mediators as confounders may result in estimates that do not fully capture the effect of early life trauma on adult telomere length. We found only one study that used SEM to evaluate different life course models of childhood stressful life events before 18 years in saliva telomere length[32]. Using financial and social/traumatic adversity to create an adversity score first constructed in Puterman et al.[24], Wallis et al. reported that there was no direct effect of childhood stressful life events on adult telomere length, with all effects mediated through adult stressful life events. Observed differences between that study and the current study could be due to 1) the fact that we capture a wider spectrum of early life traumas that may be internalized differently, 2) the differences in study populations, and 3) the limited ability to directly compare qPCR measures of telomere length across populations, let alone measures from different cell types (i.e., saliva vs. leukocytes)[51].

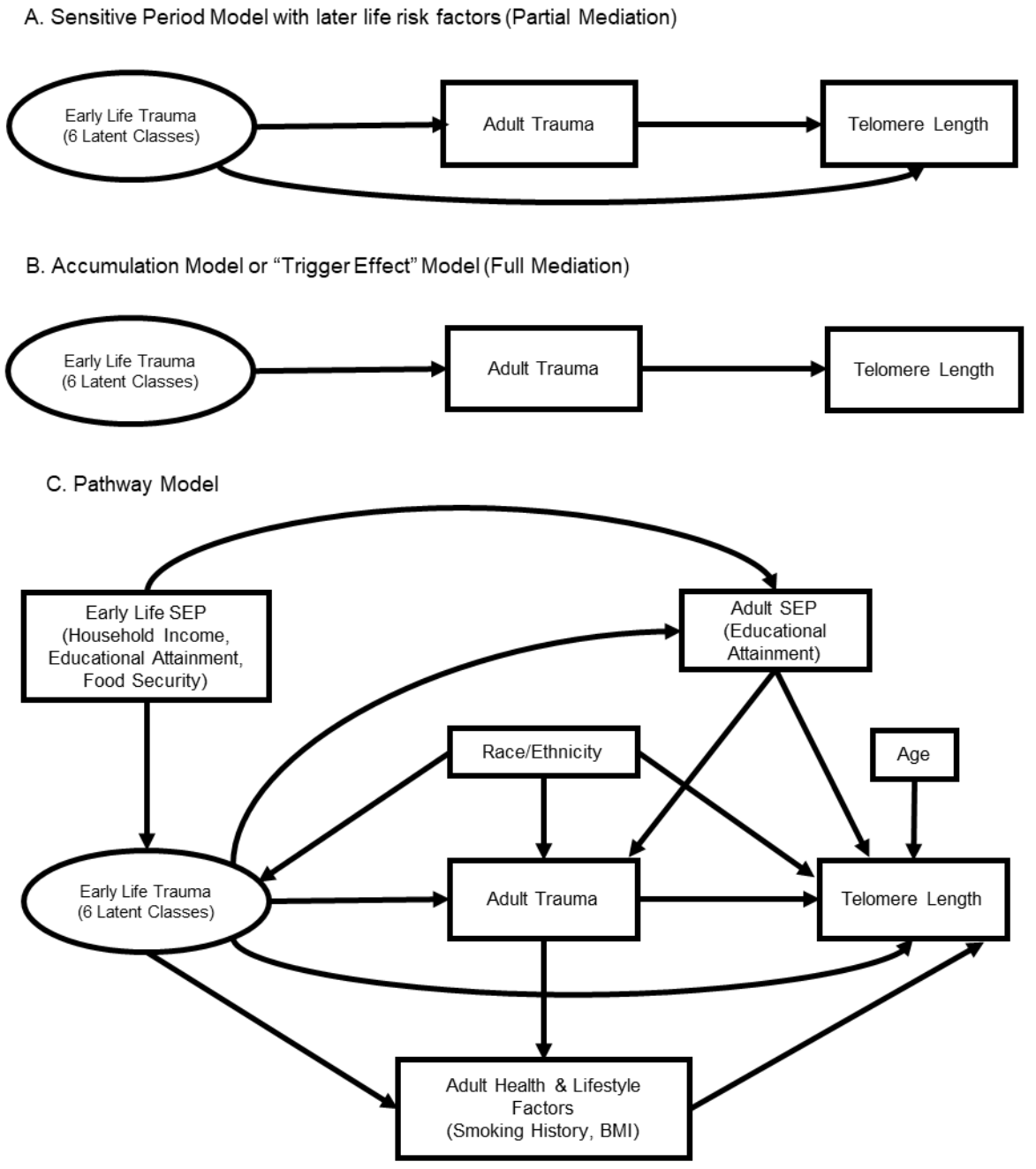
This study has several limitations. First, this study presents a cross-sectional measure of telomere length. Although we know that experience of early life trauma and recalled adult trauma all occurred prior to measure of telomere length, we do not know how telomere length is changing over time in response to experiences of trauma over the life course. Longitudinal studies that capture serial measures of telomere length over the life course would be better suited to elucidate the timing associated with trauma experience, embodiment, and telomere

modification. Second, although we captured a diverse spectrum of early life traumatic experience in this sample, the duration and magnitude of these experiences during childhood and adolescence are unknown. Persistent exposure to traumatic experiences may differentially affect cellular division and subsequent telomere shortening. Finally, this study utilizes a small subsample of the total Sister Study cohort, and purposefully oversampled women with higher perceived stress, were current smokers, and were non-Hispanic White, which may limit its generalizability to other samples. Additional samples that are both larger and more diverse are needed to confirm the results observed in this study.

In summary, this paper builds upon previous literature by utilizing interpretable latent measures of early life trauma that extends into adolescence and exploring potential pathways through which early life trauma may affect leukocyte telomere length in adulthood. Unlike a previous study that first explored structural life course models of childhood trauma and salivary telomere length and found that a social trajectory model in which the effect of child stressful life events was fully mediated by adult stressful life events[32], we found that high early life trauma was directly associated with leukocyte telomere length independent of mediating effects of adult trauma and other adult health and lifestyle factors. This suggests that early life is a sensitive period in which trauma is embodied and affects telomere length independent of adult pathways. As a result, prevention efforts that address early life trauma would be most successful in mitigating the effect of early life trauma on telomere weathering and associated poor health outcomes.

4.5 Tables and Figures

Figure 4.1. Life course models of the relationship between early life trauma and adult telomere length (A, B) and pathway model (C).



SEP: Socioeconomic position; BMI: Body mass index

Table 4.1. Characteristics of a sub-sample of 602 Sister Study participants based on experience of early life traumas before age 18

	No Early Life Trauma (n = 318)		Any Early Life Trauma (n = 284)		p-value
	n	%	n	%	
Age at baseline, mean (SD)	55.63	(9.57)	53.28	(9.29)	0.002
Race/Ethnicity					0.211
Non-Hispanic White	274	86.16	237	83.45	
Non-Hispanic Black	19	5.97	15	5.28	
Hispanic	4	1.26	11	3.87	
Other	21	6.6	21	7.39	
Highest level of education in the household at age 13, %					0.301
Missing	1	0.31	6	2.11	
Less than high school	63	19.81	52	18.31	
High school graduate	123	38.68	114	40.14	
Some college	63	19.81	58	20.42	
College degree or higher	68	21.38	54	19.01	
Family income while growing up, %					0.005
Missing	1	0.31	0	0	
Poor	10	3.14	28	9.86	
Low income	71	22.33	74	26.06	
Middle income	216	67.92	166	58.45	
Well off	20	6.29	16	5.63	
As a child, were there times when your family did not have enough food to eat?, %					0.062
Yes	22	6.92	32	11.27	
No	296	93.08	252	88.73	
Body Mass Index					0.025
Missing	1	0.31	0	0	
Underweight	5	1.57	5	1.76	
Normal weight	139	43.71	99	34.86	
Overweight	95	29.87	77	27.11	
Obese	78	24.53	103	36.27	
Smoking Status					0.125
Never smoked	149	46.86	143	50.35	
Past smoker	104	32.7	72	25.35	
Current smoker	65	20.44	69	24.3	

Table 4.2. Telomere length by latent class of early life trauma

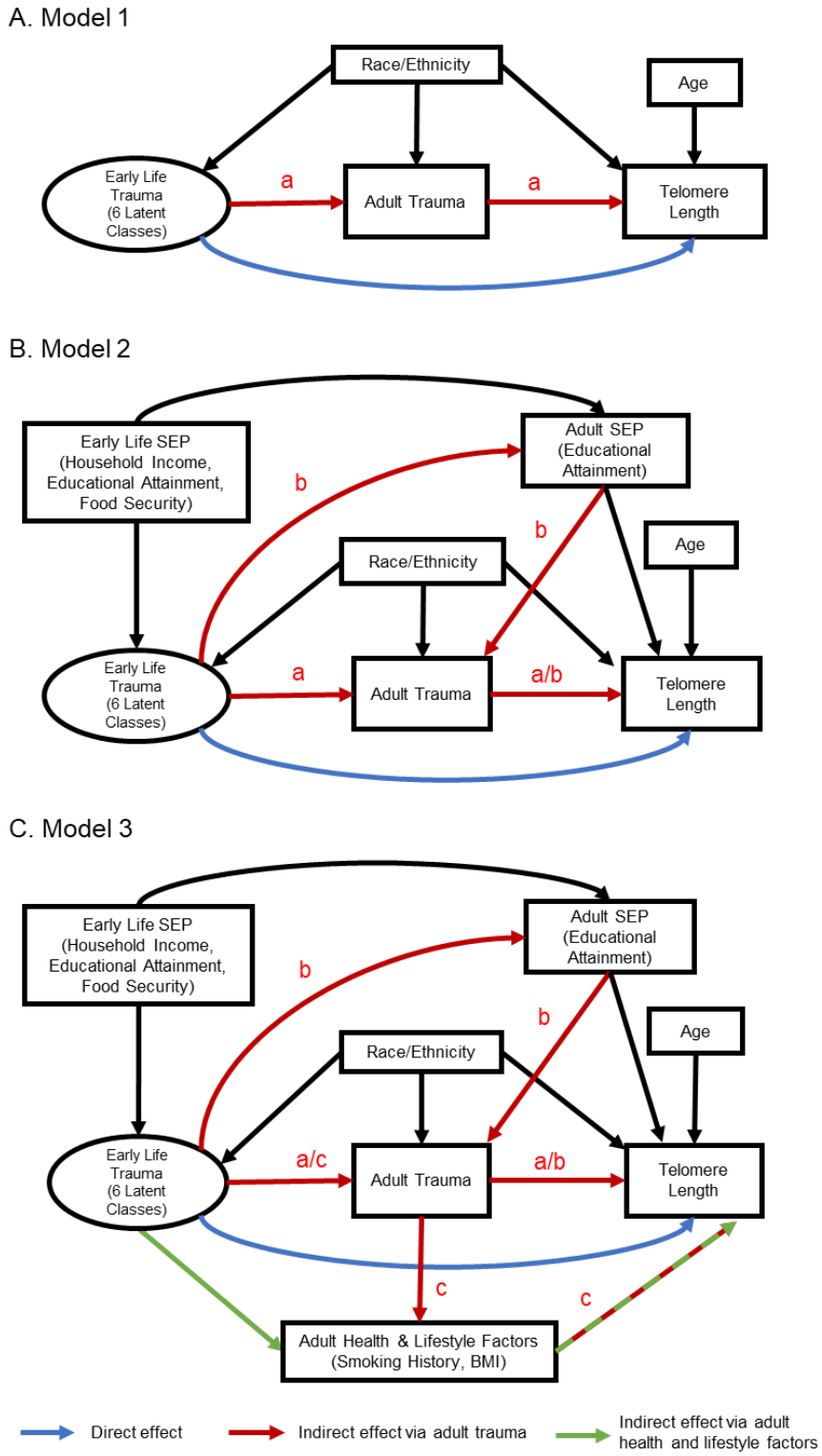
Trauma Latent Class	N	Model 1[a] β (95% CI)	Model 2[b] β (95% CI)	Model 3[c] β (95% CI)
Low early life trauma	468	Ref	Ref	Ref
Moderate early life trauma: Family health issues	13	-0.043 (-0.179, 0.094)	-0.078 (-0.210, 0.055)	-0.068 (-0.206, 0.070)
Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues	40	0.056 (-0.024, 0.136)	0.028 (-0.049, 0.106)	0.027 (-0.052, 0.105)
Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues	36	-0.032 (-0.116, 0.052)	-0.046 (-0.128, 0.035)	-0.040 (-0.122, 0.042)
Moderate early life trauma: Overall	25	-0.043 (-0.143, 0.057)	-0.072 (-0.169, 0.025)	-0.058 (-0.157, 0.040)
High early life trauma	20	**-0.117 (-0.227, -0.006)**	**-0.139 (-0.247, -0.031)**	**-0.112 (-0.221, -0.004)**

[a] unadjusted

[b] Adjusted for age

[c] Additionally adjusted for early life socioeconomic factors (early life household income, household educational attainment, and food security)

Figure 4.2 Structural equation model diagrams of the relationship between early life trauma and adult leukocyte telomere length



SEP: Socioeconomic position; BMI: Body mass index

Table 4.3. Estimates for the association between latent classes of early life trauma (compared to low early life trauma) and adult leukocyte telomere length.

	Model 1[a]			Model 2[b]			Model 3[c]		
	β (T/S units)	**SE**	**p-value**	**β (T/S units)**	**SE**	**p-value**	**β (T/S units)**	**SE**	**p-value**
Moderate early life trauma: Family health issues									
Direct	-0.046	0.036	0.200	-0.065	0.039	0.096	-0.099	0.058	0.087
Total	-0.029	0.025	0.253	-0.043	0.022	0.051	**-0.042**	**0.021**	**0.046**
Total Indirect	0.017	0.017	0.313	0.023	0.023	0.327	0.057	0.044	0.193
Indirect - Adult Trauma[d]	0.017	0.017	0.313	0.023	0.021	0.281	0.033	0.029	0.255
Moderate early life trauma: Sexual trauma and family drug/alcohol/mental health issues									
Direct	0.000	0.030	0.997	-0.007	0.034	0.846	-0.024	0.044	0.582
Total	0.021	0.021	0.316	0.024	0.018	0.181	0.025	0.018	0.165
Total Indirect	0.021	0.018	0.247	0.031	0.025	0.215	0.050	0.037	0.179
Indirect - Adult Trauma[d]	0.021	0.018	0.247	0.031	0.024	0.199	0.042	0.032	0.187
Moderate early life trauma: High betrayal trauma and family drug/alcohol/mental health issues									
Direct	-0.036	0.025	0.150	-0.037	0.024	0.120	-0.040	0.029	0.163
Total	-0.019	0.019	0.305	-0.017	0.017	0.322	-0.018	0.017	0.307
Total Indirect	0.017	0.015	0.266	0.020	0.016	0.212	0.022	0.023	0.324
Indirect - Adult Trauma[d]	0.017	0.015	0.266	0.020	0.016	0.223	0.027	0.022	0.207
Moderate early life trauma: Overall									
Direct	-0.040	0.024	0.096	-0.035	0.024	0.132	-0.046	0.027	0.096
Total	-0.028	0.019	0.147	-0.018	0.016	0.264	-0.019	0.017	0.249
Total Indirect	0.012	0.012	0.306	0.017	0.015	0.258	0.027	0.021	0.203
Indirect - Adult Trauma[d]	0.012	0.012	0.306	0.017	0.015	0.251	0.023	0.019	0.243
High early life trauma									
Direct	**-0.086**	**0.040**	**0.029**	**-0.093**	**0.042**	**0.026**	**-0.118**	**0.057**	**0.038**
Total	**-0.061**	**0.024**	**0.012**	**-0.060**	**0.023**	**0.008**	**-0.060**	**0.023**	**0.008**
Total Indirect	0.025	0.024	0.285	0.033	0.028	0.238	0.058	0.045	0.200
Indirect - Adult Trauma[d]	0.025	0.024	0.285	0.033	0.028	0.242	0.046	0.038	0.223
	RMSEA = 0.024; CFI = 0.907			RMSEA = 0.022; CFI = 0.905			RMSEA = 0.021; CFI = 0.903		

[a]Adjusted for race/ethnicity and age

[b]Additionally adjusted for early life socioeconomic factors (early life household income, household educational attainment, and food security) and adult socioeconomic position (educational attainment)

[c]Additionally adjusted for adult lifestyle factors (smoking history and body mass index)

[d] Pathways from Figure 4.2 - Model 1: a; Model 2: Additionally b; Model 3: Additionally c

CFI: Comparative fit index; RMSEA: Root mean square error of approximation; SE: Standard error

4.6 Supplemental materials

Supplemental Table 4.1 Telomere length (T/S ratio) by early life trauma domains

Trauma Domain	N	Model 1[a] β (95% CI)	Model 2[b] β (95% CI)	Model 3[c] β (95% CI)
Natural Disasters	25	**0.101 (0.001, 0.200)**	**0.097 (0.000, 0.194)**	**0.124 (0.028, 0.220)**
Major Accidents	15	-0.013 (-0.152, 0.127)	-0.049 (-0.187, 0.088)	-0.052 (-0.187, 0.083)
Household Dysfunction	102	0.008 (-0.045, 0.062)	-0.010 (-0.062, 0.043)	-0.011 (-0.063, 0.042)
Sexual Trauma	101	0.007 (-0.047, 0.060)	-0.008 (-0.061, 0.044)	-0.005 (-0.058, 0.047)
Physical Trauma	44	**-0.079 (-0.158, 0.000)**	**-0.091 (-0.169, -0.014)**	**-0.083 (-0.162, -0.004)**
Psychological Trauma	183	-0.017 (-0.060, 0.027)	-0.027 (-0.070, 0.015)	-0.019 (-0.062, 0.024)
Major Illness	9	-0.074 (-0.256, 0.108)	-0.092 (-0.270, 0.086)	-0.068 (-0.243, 0.108)

[a]Unadjusted

[b]Adjusted for age

[c]Additionally adjusted for early life socioeconomic factors (early life household income, household educational attainment, and food security)

Supplemental Table 4.2 Standardized estimates for the association between domains of early life trauma and adult leukocyte telomere length (Telomere length/Single copy gene (T/S) ratio)

Trauma Domain		Model 1[a]			Model 2[b]			Model 3[c]		
		β (T/S units)	**SE**	**p-value**	**β (T/S units)**	**SE**	**p-value**	**β (T/S units)**	**SE**	**p-value**
Natural Disasters	Direct Effect	**0.040**	**0.020**	**0.045**	-0.004	0.079	0.957	-0.017	0.085	0.838
	Total Effect	0.037	0.020	0.056	-0.015	0.074	0.845	-0.042	0.073	0.571
	Total Indirect Effect	-0.003	0.002	0.212	-0.010	0.011	0.353	-0.024	0.022	0.275
	Indirect Effect via Adult Trauma	-0.003	0.002	0.212	-0.006	0.010	0.559	-0.004	0.009	0.666
		RMSEA = 0.000; CFI = 1.00			RMSEA = 0.063; CFI = 0.692			RMSEA = 0.064; CFI = 0.619		
Major Accidents	Direct Effect	-0.168	0.087	0.054	**-0.251**	**0.075**	**0.001**	**-0.318**	**0.101**	**0.002**
	Total Effect	**-0.169**	**0.082**	**0.040**	**-0.239**	**0.064**	**<0.001**	**-0.247**	**0.065**	**<0.001**
	Total Indirect Effect	-0.001	0.011	0.962	0.012	0.018	0.492	0.071	0.048	0.143
	Indirect Effect via Adult Trauma	-0.001	0.011	0.962	0.013	0.017	0.427	0.015	0.019	0.429
		RMSEA = 0.039; CFI = 0.959			RMSEA = 0.050; CFI = 0.809			RMSEA = 0.050; CFI = 0.760		
Household Dysfunction	Direct Effect	-0.009	0.062	0.884	-0.024	0.063	0.699	-0.028	0.066	0.667
	Total Effect	-0.022	0.058	0.706	-0.027	0.058	0.641	-0.001	0.021	0.956
	Total Indirect Effect	-0.013	0.013	0.331	-0.003	0.015	0.855	-0.029	0.058	0.609
	Indirect Effect via Adult Trauma	-0.013	0.013	0.331	-0.006	0.014	0.673	-0.002	0.018	0.913
		RMSEA = 0.086; CFI = 0.875			RMSEA = 0.071; CFI = 0.754			RMSEA = 0.070; CFI = 0.595		
Sexual Trauma	Direct Effect	-0.014	0.070	0.839	-0.056	0.071	0.434	-0.053	0.072	0.463
	Total Effect	-0.027	0.063	0.667	-0.055	0.063	0.381	-0.063	0.063	0.319
	Total Indirect Effect	-0.013	0.015	0.385	0.001	0.017	0.968	-0.010	0.018	0.570
	Indirect Effect via Adult Trauma	-0.013	0.015	0.385	-0.002	0.016	0.881	-0.001	0.016	0.970
		RMSEA = 0.070; CFI = 0.920			RMSEA = 0.063; CFI = 0.810			RMSEA = 0.067; CFI = 0.644		

Supplemental Table 1 (*Continued*)

Trauma Domain		Model 1[a] β (T/S units)	Model 1[a] SE	Model 1[a] p-value	Model 2[b] β (T/S units)	Model 2[b] SE	Model 2[b] p-value	Model 3[c] β (T/S units)	Model 3[c] SE	Model 3[c] p-value
Physical Trauma	Direct Effect	**0.192**	**0.071**	**0.007**	**-0.244**	**0.070**	**0.001**	**-0.261**	**0.073**	**<0.001**
	Total Effect	**-0.190**	**0.065**	**0.004**	**-0.231**	**0.062**	**0.000**	**-0.244**	**0.063**	**<0.001**
	Total Indirect Effect	0.002	0.012	0.857	0.013	0.015	0.415	0.017	0.021	0.418
	Indirect Effect via Adult Trauma	0.002	0.012	0.857	0.013	0.015	0.387	0.012	0.014	0.411
		RMSEA = 0.017; CFI = 0.994			RMSEA = 0.056; CFI = 0.827			RMSEA = 0.064; CFI = 0.629		
Psychological or Emotional Trauma	Direct Effect	-0.076	0.053	0.154	-0.089	0.054	0.102	-0.089	0.054	0.103
	Total Effect	-0.082	0.051	0.108	-0.088	0.052	0.087	-0.091	0.052	0.079
	Total Indirect Effect	-0.006	0.010	0.536	0.001	0.011	0.954	-0.002	0.013	0.878
	Indirect Effect via Adult Trauma	-0.006	0.010	0.536	-0.001	0.011	0.940	0.002	0.012	0.884
		RMSEA = 0.047; CFI = 0.954			RMSEA = 0.063; CFI = 0.789			RMSEA = 0.067; CFI = 0.609		
Major Illness	Direct Effect	-0.099	0.089	0.266	**-0.250**	**0.084**	**0.003**	**-0.317**	**0.117**	**0.007**
	Total Effect	-0.103	0.081	0.204	**-0.238**	**0.076**	**0.002**	**-0.250**	**0.073**	**0.001**
	Total Indirect Effect	-0.004	0.014	0.760	0.012	0.017	0.507	0.067	0.055	0.217
	Indirect Effect via Adult Trauma	-0.004	0.014	0.760	0.013	0.016	0.429	0.014	0.019	0.437
		RMSEA = 0.000; CFI = 1.000			RMSEA = 0.039; CFI = 0.872			RMSEA = 0.045; CFI = 0.788		

[a]Adjusted for race/ethnicity and age

[b]Additionally adjusted for early life socioeconomic factors (early life household income, household educational attainment, and food security) and adult socioeconomic position (educational attainment)

[c]Additionally adjusted for adult lifestyle factors (smoking history and body mass index)

[d] Pathways from Figure 4.2 - Model 1: a; Model 2: Additionally b; Model 3: Additionally c

CFI: Comparative fit index; RMSEA: Root mean square error of approximation; SE: Standard Error

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CHAPTER 5

Conclusion

5.1 Conclusion

This dissertation examined the potential effects of biological embedding of early life trauma on adult health outcomes, specifically breast cancer incidence, DNA methylation of the hypothalamus-pituitary-adrenal (HPA) axis gene – *NR3C1*, and leukocyte telomere length, within a cohort of women residing in the U.S. or Puerto Rico with a first degree family history of breast cancer. As early life traumatic events do not commonly occur in isolation, this dissertation uses a latent class approach to evaluate the effect of different early life trauma profiles on the three target measures of adult health and biological embedding of trauma. A latent class variable was created to capture the potential clustering of 20 different early life traumatic experiences. I observed patterns that suggest women who reported moderate early life trauma consisting jointly of sexual trauma and household dysfunction (i.e., family drug, alcohol, and/or mental health issues) were at higher risk for developing pre- and post-menopausal breast cancer, as well as a moderate decrease in DNA methylation among cytosine-phosphate-guanine (CpG) sites within the *NR3C1* gene body compared to women classified as low early life trauma. There was no association between moderate early life trauma consisting jointly of sexual trauma and household dysfunction and leukocyte telomere shortening compared to low early life trauma. However, women who were classified as having high early life trauma had significantly shorter telomeres than women classified with low early life trauma but did not show any statistically significant differences in incident breast cancer risk nor *NR3C1* methylation. Although there was a trend in the positive direction between most latent classes of early life trauma and elevated risk for post-menopausal breast cancer as well as shorter telomere length, I did not observe any additional statistically significant associations between the latent classes of early life trauma not previously

mentioned and differential *NR3C1* methylation overall, among promoter-associated CpG sites, or within the gene body.

The experience of early life trauma is highly predictive of future revictimization in adulthood[1-3], contributing to the accumulation of trauma over the life course and subsequent negative impacts on health. However, this dissertation attempted to elucidate whether early life trauma may impact adult health independent of subsequent trauma by serving as a sensitive period during which the biological embedding of trauma may have a stronger effect on developing systems. Moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues was the only latent class of early life trauma to exhibit a statistically significant overall effect on *NR3C1* methylation, which was observed as hypomethylation of CpG sites located within the gene body, compared to low early life trauma. This association was driven by the direct effect of moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues as no statistically significant indirect effects were observed via pathways mediated by adult trauma. A similar pattern emerged for the relationship between early life trauma and leukocyte telomere shortening, featuring the high early life trauma latent class. Early life trauma was associated with significantly shorter leukocyte telomeres among women classified as high early life trauma compare to women classified as experiencing low early life trauma. Again, this association was driven by the direct effect between high early life trauma and telomere length, with no statistically significant indirect effects or indirect effects specifically mediated by adult trauma. These results suggest that early life may serve as a sensitive period for during which the effects of specific clusters of traumatic experiences may differentially impact future health outcomes independent of subsequent trauma trajectories.

Prior studies investigating the relationship between early life trauma and incident breast cancer, *NR3C1* methylation, or leukocyte telomere length has been highly mixed due to heterogeneity in the definitions used to classify early life adversity (namely trauma, stress or stressful life events, and adverse childhood experiences – ACEs), methods used to quantify early life trauma, and the timing of exposure (i.e., childhood, adolescence, or both). The findings from this dissertation improve upon the current literature by utilizing comprehensive models of early life trauma and its impact on adult health to build a more robust picture of these associations. Future research will need to work towards addressing these discrepancies before a unified picture of the association between early life trauma and these health outcomes can be elucidated.

This dissertation found that early life represents a sensitive period during which trauma can be biologically embedded and affect telomere length independent of adult pathways. As a result, prevention efforts that address early life trauma would be most successful in mitigating the effect of early life trauma on telomere weathering and associated poor health outcomes. Furthermore, this work supports the use of comprehensive measures, rather than summative scores, individual events, or trauma domains that have been used traditionally to elucidate the effects of early life trauma on measures of stress embodiment and adult health. Our work also supports the use of life course models to evaluate various pathways that may ultimately influence the observed associations between measures of early life trauma and adult health.

5.2 Aim 1 – Early Life Trauma and Incident Breast Cancer

The first aim of this dissertation investigated the association between early life trauma and incident breast cancer risk, using traditional measures, as well as a latent class model, of early life trauma. There were no statistically significant associations between early life trauma and incident breast cancer risk using traditional measures of early life trauma. A latent class

model identified six unique early life trauma profiles that reflected not only overall severity (i.e., low and high overall early life trauma), but also combinations of traumatic events (e.g., sexual trauma and family drug, alcohol, and/or mental health issues). Model results suggested that women who reported moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues had potentially higher risk of developing pre- and post-menopausal breast cancer compared to women who reported low early life trauma (pre-menopausal breast cancer HR 1.19, 95% CI 0.89, 1.59; post-menopausal breast cancer HR 1.09; 95% CI: 0.93, 1.29) after adjusting for race/ethnicity and childhood socioeconomic position (SEP), which was captured as highest level of education in the household at age 13, family income while growing up, and food security as a child. Estimated effects were also in the positive direction for other early life trauma profiles, except for high early life trauma, and post-menopausal breast cancer when compared to the low early life trauma profile. Therefore, the role of early life trauma on breast cancer risk may be more nuanced than what may be captured by a cumulative trauma score, a single traumatic event, or traditional trauma domains.

Aim 1 builds upon previous literature on the relationship between early life trauma and breast cancer incidence, which continues to be inconsistent. The observed variation in results from previous studies and the current dissertation may in part be due to the heterogeneous nature of breast cancer (i.e., differences in class, stage, and molecular subtypes), as well as methodological differences in the operationalization of stress, adversity, and trauma (e.g., summative scores, singular traumatic events, and trauma domains) and exposure periods (i.e., childhood, adolescence, or both) in these studies. Similar to prior research that has utilized summative trauma scores and/or trauma domains, there was no statistically significant association between early life trauma and incident breast cancer. Prior studies have not assessed

the relationship between early life trauma and breast cancer molecular subtype. To my knowledge, only one study has looked at the association between chronic psychological stress and breast cancer tumor subtype, which reported that women with breast cancer who reported a history of chronic stress also had a high percentage of human epidermal growth factor receptor 2 (HER2) amplified tumors—a form of aggressive breast cancer—compared to women with breast cancer and no history of chronic distress[4]. In this dissertation, the direction of effects varied slightly between risk for estrogen receptor (ER) positive and negative breast cancer, with slightly higher risk observed for ER positive breast cancer for most early life trauma domains and latent classes of early life trauma. The hazard ratios for ER negative breast cancer were higher than those for ER positive breast cancer for women reporting early life sexual trauma (domain) or classified as experiencing moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues when compared to women who did not experience of sexual trauma or women classified as low early life trauma, respectively; however, these associations did not reach statistical significance.

The mechanisms through which traumatic experiences that occur during sensitive periods of breast development and may subsequently affect breast cancer risk are complex and poorly understood. One potential biologic pathway though which early life trauma could affect breast cancer risk is modification of stress response mechanisms. The HPA axis is one of the primary stress response mechanisms and functions via a series of biochemical feedback pathways. Persistent activation of the HPA axis resulting from exposure to chronic stress can decrease the its functionality, contributing to greater allostatic load and disease susceptibility[5, 6]. Experiences of early life trauma may predispose women to a chronic dysregulation of stress management mechanisms including immune function and inflammatory processes[7-9]. Early life trauma could

also affect breast cancer risk through the alteration of circulating hormones that affect pubertal changes and early breast development and subsequent reproductive behaviors. Younger age at menarche[10], an indicator of accelerated breast development, has been associated with increased breast cancer risk. Previous studies have reported an association between childhood sexual abuse and lower age at menarche[11], but less is known about the potential relationship between early life trauma and other known breast cancer risk factors, including delayed age at first birth, parity, and time between pregnancies[12-14]. Other life course factors that may be affected by early life trauma and subsequently modify breast cancer risk include adult health and lifestyle factors (e.g., alcohol use[15-17] and body mass[18-21]) or adult stress and coping mechanisms. In these analyses of early life trauma and incident breast cancer risk, I did not adjust for these factors due to their role as potential mediators. Future studies should assess these pathways to discover if environmental hazards, social factors, or health and behaviors in adulthood may differentially affect the association between early life trauma and incident breast cancer risk.

5.3 Aim 2 – Early life trauma and circulating *NR3C1* methylation

The second aim of this dissertation examined the relationship between early life trauma and patterns of DNA methylation of the HPA axis gene, nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) in peripheral blood, using the latent class model of early life stress developed in Aim 1 (Chapter 2). Only the latent class consisting of early life sexual trauma and family drug, alcohol, and/or mental health issues was associated with moderate decrease in methylation of CpG sites within the *NR3C1* gene body ($\beta = -0.021$, $p = 0.036$, % methylation change = -0.08) after controlling for age, breast cancer status, body mass index (BMI), smoking history, and proportions of white blood cell types. In similar models, there were no statistically significant associations between latent classes of early life trauma and NR3C1 methylation

overall, among promoter-associated CpG sites, or within the gene body. Various CpG sites were associated with differential methylation at several CpG sites, however, these associations did not survive correction for multiple testing. In life course structural equation models, potential mediating effects via adult trauma and other adult SEP and health and lifestyle factors were assessed and no direct nor indirect effects between latent classes of early life trauma and adult *NR3C1* methylation were identified.

Aim 2 builds upon previous literature investigating the association between early life trauma and DNA methylation of HPA axis genes, specifically the glucocorticoid receptor gene – *NR3C1*. Whereas previous studies have relied on summative trauma scores and traditional trauma domains, to our knowledge, this is one of the few studies to utilize a latent class approach to assess the co-occurrence of traumatic events in early life and the first to evaluate the relationship between early life trauma and adult *NR3C1* methylation using structural equation models and a life course approach to observe potential mediating effects through adult SEP, trauma, and health and behavioral factors. Other studies that control for SEP measures over the life course, adult trauma, and/or adult health and lifestyle factors may mask complex mediating pathways of the observed relationship between early life trauma and *NR3C1* methylation in adulthood. Using traditional measures of early life adversity and trauma, findings from previous studies have been mixed with some studies reporting hypermethylation[22-25], hypomethylation[25-27], and no changes[28-30] in *NR3C1* methylation overall, in promotor-specific regions, and at individual CpG sites. The variation in these results may in part be due to the heterogeneity in the measurement of early life trauma—both the measures used and the exposure period assessed, the targeted *NR3C1* CpG sites, the method used to capture *NR3C1* methylation, whether the studies corrected for multiple testing, as well as the study populations represented, which differed by

age, sex, and proportion of participants selected for known psychiatric and mental health disorders.

The latent class of moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues was associated with hypomethylation of CpG sites located in the *NR3C1* gene body. This association was replicated, albeit attenuated, in life course SEM models when both direct and indirect effects were considered and was largely driven by the direct effect of moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues. Although most research in this field has focused on the methylation of CpG sites within the proximal promotor region of *NR3C1* due to its prominent role in *NR3C1* transcription, methylation changes in the bodies of genes have been reported to play a role in differential promotor usage, transcription elongation, or alternative splicing[31, 32]. Structural equation models did not identify direct or indirect effects via adult SEP, trauma, smoking, or BMI in the relationship between latent classes of early life trauma and *NR3C1* methylation overall. Compared to other toxic exposures, such as smoking and tobacco use, methylation associated with the embedding of early life psychological stress and trauma tend to be less pervasive and associated with smaller changes in methylation, which may make them hard to disentangle from subsequent behavioral and environmental exposures associated with a history of trauma[28].

The lack of associations between early life trauma and *NR3C1* methylation overall and at individual CpG sites that have been observed in previous studies, as well as this dissertation, could be attributed in part to the long period between the experience of early life trauma and the measurement of adult *NR3C1* methylation. Although DNA methylation is thought to be a durable epigenetic modification, methylation levels can be modified over time and may not persist over

the life course[33, 34]. As such, different risk or protective factors (e.g., coping and support) experienced over the life course that were not explored in the current models may further mediate the association between the reported trauma and the observed methylation patterns. A comprehensive study by Marzi et al. looked at the relationship between childhood trauma and *NR3C1* methylation at age 18, thus eliminating the long period between exposure and measurement, and found no associations[28]. This suggests that biological embedding of early life trauma may not occur immediately but may result from the chronic psychological stress associated with reliving the trauma over time or may contribute to behaviors later in life that have a stronger impact on DNA methylation. Future research should assess why the combination of early life sexual trauma and household dysfunction may differentially affect methylation of *NR3C1*, and potentially other HPA axis genes, compared to other clusters of early life trauma and whether alternative pathways through adult behaviors may exist that may mediate or modify the association between early life trauma and *NR3C1* methylation as no direct association between early life trauma and *NR3C1* methylation was observed.

5.4 Aim 3 – Early life trauma and leukocyte telomere length

The third aim of this dissertation assessed the relationship between early life trauma and leukocyte telomere length, a biomarker of cellular aging, using the latent six class model of early life trauma developed in Aim 1 (Chapter 2). Women who were classified as experiencing high early life trauma had significantly shorter telomeres than women who were classified as experiencing low early life trauma after controlling for age and early life SEP (β = -0.112; 95% CI = -0.221, -0.004). This association was maintained in life course structural equation models when direct and indirect pathways were considered after adjusting for age, race/ethnicity, and early life and adult SEP, and allowing for mediating pathways through adult smoking history and

BMI; high early life trauma decreased telomere length by 0.060 T/S units holding all covariates constant ($p = 0.008$). This was largely driven by the negative direct effect of high early life trauma on telomere length ($\beta = -0.118$, S.E. 0.057, $p = 0.038$). The indirect effect of early life trauma on telomere length that was mediated by adult trauma was not statistically significant, suggesting that high early life trauma was associated with telomere length independent of adult trauma.

Aim 3 fills several gaps in the previous literature on early life trauma and adult leukocyte telomere length. To our knowledge, this is the first analysis to use structural equation modeling and a life course approach to assess the relationship between early life trauma and leukocyte telomere length. A previous study used SEM to evaluate different life course models of childhood stressful life events before age 18 years in saliva telomere length and reported an association between childhood stressful life events and shorter saliva telomere length, which was fully mediated by the experience of stressful life events in adulthood[35]. Experience of high early life trauma was associated with shorter telomere length, albeit in leukocytes, the observed association was due to the significant direct effect of early life trauma on telomere length and was not mediated by adult trauma, SEP, nor indicators of adult health or health behavior. Observed differences between that study and the current dissertation could be due to how a wider spectrum of early life traumas were captured in the Sister Study and the degree to which they may be internalized differently, variations in study populations, and the limited ability to directly compare qPCR measures of telomere length across populations, let alone measures from different cell types (i.e., saliva vs. leukocytes)[36]. Our findings suggest that childhood and adolescence represent a sensitive period in which trauma is embodied and affects telomere length independent of adult pathways. As a result, prevention efforts that address early life trauma

would be most successful in mitigating the effect of early life trauma on telomere weathering and associated poor health outcomes (e.g., cancer[37], obesity, diabetes[38, 39], and cardiovascular disease[38, 40], and mortality[41]).

Previous studies of the association between early life trauma and telomere length have reported mixed results with just over half of studies reporting statistically significant negative associations between recalled early stressful life events measured in adulthood and telomere length[42]. In a review by Willis et al., the authors note that the heterogeneity in findings appear to be unrelated to the type of measure used to assess early life stress and whether the studies also adjusted for adult health and lifestyle factors or experience of trauma over the life course[42]. However, studies that inappropriately adjust for potential mediators may result in estimates that do not fully capture the effect of early life trauma on adult telomere length. Furthermore, in our models with statistically significant direct effects between measures of early life trauma and telomere length, the direct effects suggested a stronger negative association with telomere length than measured total effects, suggesting that models that do not address life course mediating factors may not capture the true direct effect of early life trauma on telomere length and may instead report attenuated effects. Our results suggested that clusters of traumatic experiences in early life may have differential effects on adult leukocyte telomere length, but the greatest effect was observed when comparing the extreme latent classes of early life trauma (i.e., high early life trauma vs. low early life trauma).

Future research on the relationship between early life trauma and leukocyte telomere length should consider how duration of clustered early life traumatic experiences may affect leukocyte telomere length and how positive and negative coping behaviors over the life course may contribute to the observed effects. As such, due to the myriad of pathways that early life

trauma can alter telomere length (e.g., directly as well as via socioeconomic factors, health behaviors, and coping mechanisms), life course models will be necessary in teasing out potential points of intervention to lessen the impact of early life trauma on telomere weathering.

5.5 Strengths and Limitations

This is the first study to comprehensively assess the relationship between early life trauma and risk of incident breast cancer overall and by breast cancer tumor subtype and to utilize this latent measure of early life trauma in life course structural equation models to evaluate the association between early life trauma and adult circulating *NR3C1* methylation and leukocyte telomere length. I used data from participants of the Sister Study, a large prospective cohort of U.S. and Puerto Rican women with a sister diagnosed with breast cancer. Due to the prospective design of the Sister Study and the nature of the exposure, I can ensure temporality between the experience of early life trauma and breast cancer diagnosis, adult *NR3C1* methylation, and adult leukocyte telomere length. Our outcomes of interest had high validity, with breast cancer diagnosis verified with medical records for approximately 86% of women with breast cancer and a 99% agreement between medical records and self-report for breast cancer; *NR3C1* methylation and leukocyte telomere length were measured and checked for quality using standard practices. Experiences of early trauma were combined for childhood (before age 12) and adolescence (ages 13-17). Prior studies that have assessed the association between early life adversity in breast cancer have focused primarily on childhood adversity, however, studies that limit their scope to childhood may overlook the impact of trauma on pubertal development and subsequent reproductive pathways associated future breast cancer risk. Furthermore, although other studies have evaluated different methods of assessing adverse

childhood experiences[43], this is the first study to utilize and compare different methods of evaluating breast cancer risk in a single cohort.

Whereas previous studies have utilized summary measures, domains, or distinct events to assess early life trauma, this dissertation utilized a latent class approach for early life trauma and a latent variable approach for adult trauma to capture potential nuanced roles of concomitant traumas on incident breast cancer risk, adult *NR3C1* methylation, and adult telomere length. In addition, this is the first study to look at the relationship between early life trauma and *NR3C1* methylation and adult leukocyte telomere length using a life course framework and structural equation model approach. These models were able to control for covariates of interest including age, race/ethnicity (except for *NR3C1* methylation analyses, which were limited to non-Hispanic White women), early life and adult SEP, and adult health and lifestyle factors—smoking status and BMI—based on their potential relationships to our exposure and outcomes. Other studies that directly control for life course SEP measures, adult trauma, and/or adult health and lifestyle factors may hide complex mediating pathways that affect the overall relationship between early life trauma and our outcomes of interest.

This dissertation has several limitations. First, the Sister Study consists of an enriched sample of women who did not have breast cancer but had at least one biological sister diagnosed with breast cancer at baseline. In addition, the Sister Study population are generally older, more educated, and more likely to be healthy and economically sound, which may mask the effects of early life trauma in more vulnerable populations. However, the distribution of breast cancer risk factors in the Sister Study cohort resembles that of the general population, which suggests that the results observed in this study may still be generalizable and remain internally valid[44]. Furthermore, the participants included in the *NR3C1* methylation and leukocyte telomere

analyses were small samples of the total Sister Study cohort. The *NR3C1* methylation sample were strictly non-Hispanic White women who were sampled in a case-cohort fashion while the leukocyte telomere sample purposefully oversampled women with high perceived stress, were current smokers, and were not non-Hispanic White, which may limit the generalizability of these samples to larger populations. Additional samples that are both larger and more diverse are needed to confirm the results observed in Aims 2 and 3. Furthermore, previous studies have demonstrated sex and gender differences in the associations between early life adversity/trauma and adult health outcomes (e.g., cancer[45] and pulmonary disease[46]). As such, the results reported in this dissertation may not be generalizable to men. Future studies in more diverse populations could help to strengthen the findings observed in this dissertation.

Second, although the number and type of early life traumatic experiences that occurred in childhood or adolescence were captured, the duration nor the magnitude of these exposures over these two periods remain unknown. Some trauma types tend to have shorter exposure periods (e.g., natural disasters) whereas other trauma types may be more persistent (e.g., emotional or psychological mistreatment). The duration and repetition of trauma over potentially sensitive time periods over the life course may play an important role in the association between early life trauma and adult indicators of biological embedding of early life trauma. The Sister Study collected information on a comprehensive list of traumas, however, the possibility that there may be other types of stress and adversity with higher likelihood to be biologically embedded and subsequently play a larger role in the risk of breast cancer, DNA methylation, and leukocyte telomere length remains unknown. In addition, although temporality between reported early life trauma and breast cancer diagnosis can be assured, non-differential exposure misclassification may exist as a result of the long period between experienced trauma and accurate reporting.

Previous studies have reported between 60 and 81 percent of study participants accurately disclosing previous experiences of sexual and physical trauma[47-49]. This underreporting has been attributed to the inability to recall the traumatic experience, as well as avoidance of potential stigmas associated with disclosing early life trauma. Concurrent mental health issues (e.g., psychological distress) can contribute to higher recall and reporting of childhood traumatic experiences[50]. Furthermore, the Sister Study does not capture adverse childhood experiences as presented by the ACE study, instead uses a trauma framework; as a result, the observed results cannot be directly compared with other studies that have used the traditional categories for ACEs. However, Bethell et al. demonstrated that although variability exists among measures of adverse childhood experiences, most measures remain similar and cumulative scores from these measures reflect consistent association with poor health outcomes[43].

Finally, this study presents single measures of DNA methylation of a single stress response gene, *NR3C1*, and of leukocyte telomere length in later adulthood. Although *NR3C1* is pivotal in the stress response, attributable to the role of glucocorticoid receptors in HPA axis signaling, it is a single gene in a complex network of mechanisms that characterize the stress management process. The study of DNA methylation of additional HPA axis genes would provide a more global picture of the role of early life trauma on methylation of these genes. Furthermore, it is unknown when epigenetic modification becomes stabilized following experiences of psychological stress and trauma and the degree to which these changes can be modified post exposure. Persistent exposure to traumatic experiences may differentially affect DNA methylation or cellular division and subsequent telomere shortening. Longitudinal studies that capture serial measures of DNA methylation and telomere length over the life course would

be better suited to elucidate timing associated with trauma experience, embodiment, and epigenetic modification.

5.6 Future Directions

This dissertation builds upon the current literature on the relationship between early life trauma and three indicators of adult health, incident breast cancer, *NR3C1* methylation, and leukocyte telomere length. Future studies should focus on refining measures to assess early life trauma and their impact on health and future elucidate additional pathways through which early life trauma may affect adult health.

Prior studies in this field have depended on summative trauma scores, individual traumatic events, and trauma domains, and fail to consider the effects that may result from the cooccurrence of different traumatic experiences in early life, resulting in mixed findings on the relationship between early life trauma and the outcome studied in this dissertation. I utilized a latent class approach to identify substantive classes of early life trauma in order to assess how the cooccurrence of different early life traumatic experiences may affect the embodiment of psychosocial stress and subsequent health in adulthood. The latent class variable created in this dissertation is likely not the final measure to be used to evaluate early life trauma since latent classes are specific to the study population and may differ from study to study depending on the underlying distribution of reported early life trauma. However, one of the primary goals of the latent class measure was to capture the cooccurrence of different early life traumas. Results from future studies that utilize similar approaches can be used to elucidate patterns of cooccurring early life trauma that impact adult health. Identifying clusters of early life traumatic experiences can inform effective public health policies and programs to prevent trauma profiles that have the

largest impact on health or to help mitigate the effect of high-risk trauma profiles in vulnerable populations.

The life course models used in this dissertation include potential mediating roles of adult trauma, socioeconomic position, and health and lifestyle factors. However, measures of stress management, such as coping, social support, and depression, may also serve as mediating factors between early life trauma and the indicators of adult health featured in this dissertation. Future studies should consider these factors to gain a greater understanding of the relationship between early life trauma and adult health.

Finally, early life trauma and SEP are highly correlated with higher rates of reported early life trauma and child maltreatment associated with low childhood SEP[51, 52], including receipt of public assistance[53], poverty[54], homelessness[55], and neighborhood disadvantage[56]. Studies have evaluated the association between neighborhood and socio-structural barriers and breast cancer[57, 58], DNA methylation of stress management-related genes[59], and telomere length[60, 61], however, there is a paucity of research on the effect of early neighborhood on these indicators of adult health. Future studies that utilize similar life course models as used in this dissertation can start to disentangle the effects of early life trauma, SEP, and neighborhood effects on incident breast cancer, DNA methylation of stress management genes, and telomere shortening.

5.7 Dissertation Summary

Using data from participants in the Sister Study, a large prospective cohort of women, this dissertation attempted to capture a nuanced measure of early life trauma by assessing how clusters of different types of trauma may work together to affect three indicators of adult health that are vulnerable to the biological embedding of psychosocial stress: 1) breast cancer

tumorigenesis, 2) epigenetic modification of the stress management gene, *NR3C1*, and 3) leukocyte telomere length, a measure cellular aging. Despite representing different physiological mechanisms, these measures represent various aspects of the stress response. Methylation of HPA axis genes, such as *NR3C1*, contributes to modified expression of genes related to immune response, inflammatory pathways, and cell apoptosis, contributing to disease risk and negative health impacts[62]. Furthermore, leukocyte migration is a key feature of the stress response and inflammatory processes, which is partially regulated by glucocorticoid signaling[63] and characterized by telomere shortening[64]. In addition, breast cancer tumorigenesis, DNA methylation of HPA axis genes, and telomere weathering are all ongoing biological processes that start in early life and proceed over the life course, potentially making them more vulnerable to the effects of chronic stress. Childhood and adolescence represent potentially vulnerable periods for breast cancer development due to the rapid proliferation of undifferentiated mammary cells and heightened susceptibility to tumorigenic changes[65, 66]. Since this dissertation was unable to evaluate the process of breast cancer tumorigenesis, incident breast cancer risk was used to assess whether the effects of early life trauma are observable not only in relatively durable biological mechanisms of stress embodiment but also remain detectable in downstream health outcomes, such as breast cancer incidence.

In order to build upon previous literature that have relied heavily on summative scores of trauma and adversity, traditional trauma domains, and individual events to represent early life trauma, a latent class model of early life trauma was used to assess the potentially nuanced effects of concomitant traumatic experiences. This dissertation also used a life course approach using structural equation modeling to evaluate how adult trauma, SEP, and health behaviors may

mediate the relationship between early life trauma and adult measures of biological embedding of psychosocial stress and overall health.

The relationship between early life trauma and these indicators of adult health are complex. Results from this dissertation suggest higher incident pre- and post-menopausal breast cancer risk and hypomethylation of CpG sites located in the *NR3C1* gene body among women classified as experiencing moderate early life trauma consisting of sexual trauma and family drug, alcohol, and/or mental health issues in early life compared to women classified as experiencing low early life trauma. However, there was no association between the latent class for high early life trauma and incident breast cancer risk nor differential *NR3C1* methylation, compared to low early life trauma. This would suggest that early life trauma consisting primarily of sexual trauma and family drug, alcohol, and/or mental health issues may contribute to different social, behavioral, and/or biological trajectories that can subsequently affect incident breast cancer risk and adult *NR3C1* methylation, unique from other clusters of varying traumatic experiences.

Conversely, the strongest association between early life trauma and shorter telomere length was observed for women who experienced high early life trauma compared to women who experienced low early life trauma. In life course structural equation models, the latent class for high early life trauma was associated with shorter leukocyte telomere length compared to the latent class for low early life trauma when direct and indirect pathways were considered after adjusting for age, race/ethnicity, and early life and adult SEP, and allowing for mediating pathways through adult smoking history and BMI. This association was largely driven by the direct effect between high early life trauma and adult leukocyte telomere length, which hinted at a stronger inverse association than measured total effects, suggesting that early life trauma

models that do not address life course mediating factors may not capture the true direct effect of early life trauma on specific health outcomes and may instead be reporting attenuated effects.. These associations were not observed between latent classes of early life trauma and differential adult *NR3C1* methylation in similar life course structural equation models. These findings suggest that the process of biological embedding of early life trauma may differ for various indicators of adult health depending on how proximal they are to key stress response mechanisms. Experience of clustered traumatic experiences or overall high levels of early life trauma may have differential effects on health by directly impacting biological mechanisms, exposing individuals to negative socioeconomic or environmental hazards in adulthood, or contributing to adaptive or maladaptive behaviors that subsequently influence of adult health.

Breast cancer, DNA methylation of *NR3C1*, and leukocyte telomere length have all previously been associated with traditional measures of psychosocial stress and early life trauma. However, they represent fundamentally different biologic mechanisms and there were no apparent sweeping trends across these three outcomes associated with specific patterns of early life trauma. These differences may be due to the long period between exposure of early life trauma and our outcomes of interest and the durability and magnitude of the effect of the biological embedding of early life trauma on these outcomes compared to more proximal risk (e.g., hormonal and reproductive factors for breast cancer or health behaviors such as smoking that may contribute to increased DNA methylation or telomere shortening) and protective (e.g., coping mechanisms and social support) factors.

In conclusion, these findings suggest that the role of early life trauma on breast cancer risk, *NR3C1* methylation, and leukocyte telomere length may be more nuanced than a cumulative trauma score, a single traumatic event, or a trauma domain can capture. This dissertation benefits

public health by setting a precedence of using comprehensive measures of early life trauma and utilizing life course models to fully understand the relationship between the experience of early life trauma and adult manifestations of the biological embedding of stress and trauma. Our findings contribute to the current understanding of the relationship between latent measures of early life trauma and incident breast cancer risk, adult circulating *NR3C1* methylation, and adult leukocyte telomere length and support the need to identify potential windows for interventions targeted at minimizing these negative health outcomes.

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Jennifer M.P. Woo, M.P.H.

EDUCATION

2014 – Current	**Ph.D.** in Epidemiology, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI (Expected May 2020)
2014 – 2017	**Graduate Certificate** in Geographic Information Systems (GIS), University of Wisconsin-Milwaukee, Milwaukee, WI
2010 – 2012	**M.P.H.** in Public Health Practice, Loma Linda University School of Public Health, Loma Linda, CA
2004 – 2008	**B.S.** in Physiological Science with a minor in Chinese Language, University of California, Los Angeles, Los Angeles, CA

RESEARCH INTERESTS

My research interests involve the mechanisms through which stress related to early life trauma/adversity and social environment become biologically embedded that can contribute to epigenetic changes along stress management and immune/inflammatory pathways (i.e., epigenetic modification of stress management genes and markers of cellular aging) and ultimately impact health. In my dissertation, I am investigating how early life trauma may become biologically embedded to affect incident breast cancer risk, DNA methylation of the glucocorticoid receptor gene (*NR3C1*), and leukocyte telomere length. Following my dissertation, my long-term goals are to obtain a rigorous postdoctoral fellowship with a dynamic research team and ultimately transition to becoming an independent investigator and to continue to study the epigenetic implications of stress embodiment, as well as its interaction with early life neighborhood environment, on the biologic manifestation of breast cancer and other chronic diseases.

EXPERIENCE – Academic and Professional

2019 – current	**Graduate Research Assistant**, Amanda Simanek Research Group, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI
2019 – current	**Special Volunteer**, Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC
Summer 2019	**Summer Research Intern,** NIH Summer Internship Program (Advisor: Dale P. Sandler, Ph.D.), Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC

2017 – 2018 **Project Director**, Young Women's Health History Study, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI

2013 – 2018 **Research Intern/Graduate Research Assistant**, Young Women's Health History Study, Joseph J. Zilber School of Public Health, University of Wisconsin-Milwaukee, Milwaukee, WI

2008 – 2014 **Research Coordinator/Staff Research Associate**, Department of Pediatrics, Division of Allergy/Immunology/Rheumatology, University of California, Los Angeles, Los Angeles, CA

2007 – 2010 **Researcher**, UCLA Lupus Genetics, Laboratory of Betty P. Tsao, Ph.D., Department of Medicine, Division of Rheumatology, University of California, Los Angeles, Los Angeles, CA

ADDITIONAL TRAINING

2019 *Epigenetics Bootcamp*. Columbia Mailman School of Public Health, New York, NY

2017 *Structural Equation Modeling*, Statistical Horizons, LLC, Chicago, IL

FELLOWSHIPS, HONORS, AND AWARDS

2020 **UWM Student Excellence Award for Outstanding Graduating Student**, University of Wisconsin-Milwaukee, Milwaukee, WI

2020 **Finalist, Three Minute Thesis**, University of Wisconsin-Milwaukee, Milwaukee, WI

2019 **Third Place, Graduate Category**, 2019 Summer Internship Program Poster Session, National Institute of Environmental Health Sciences, Research Triangle Park, NC

2019 **Finalist, Three Minute Thesis**, University of Wisconsin-Milwaukee, Milwaukee, WI

2018 – 2019 **UWM Distinguished Dissertation R1 Fellowship**, University of Wisconsin-Milwaukee, Milwaukee, WI

2018 **Excellence in Public Health Research Award**, Wisconsin Public Health Association, Green Bay, WI

2016 – 2017 **UWM Distinguished Graduate Student Fellowship**, University of Wisconsin-Milwaukee, Milwaukee, WI

2014	**ACR RRF/Abbott Medical and Graduate Student Achievement Award**, American College of Rheumatology/Research Education Foundation, Boston, MA
2014	**Finalist**, **2014 Mobile App Challenge**, Medical College of Wisconsin, Milwaukee, WI
Spring 2012	**ACR REF/Abbot Health Professional Graduate Student Research Preceptorship**, American College of Rheumatology/Research Education Foundation, Department of Pediatrics, Division of Allergy/Immunology/ Rheumatology, University of California, Los Angeles, Los Angeles, CA
2012	**Delta Omega Honor Society Award**, Loma Linda University School of Public Health, Loma Linda, CA
2011	**ARHP Graduate Student Recognition Award**, Association of Rheumatology Health Professionals, Chicago, IL
Summer 2011	**ACR REF/Abbot Health Professional Graduate Student Research Preceptorship**, American College of Rheumatology/Research Education Foundation, Department of Pediatrics, Division of Allergy/Immunology/ Rheumatology, University of California, Los Angeles, Los Angeles, CA
2010	**ACR REF/Abbott Medical and Graduate Student Achievement Award**, American College of Rheumatology/Research Education Foundation, Atlanta, GA
2008	**Harold Paulus Prize for Best Paper**, Anand Malaviya Symposium on Inflammation and Immune Therapies, Department of Medicine, Division of Rheumatology, University of California, Los Angeles, Los Angeles, CA
Summer 2008	**Gina M. Finzi Memorial Student Summer Fellowship**, Lupus Foundation of America, Inc., Department of Medicine, Division of Rheumatology, University of California, Los Angeles, Los Angeles, CA

PEER REVIEWED PUBLICATIONS

PubMed Bibliography**:**
https://www.ncbi.nlm.nih.gov/myncbi/1b3hjbm37CUQ7/bibliography/public/

1. Rubinstein TB, Ogbu EA, Rodriguez M, Waqar L, **Woo JMP** et al. Prioritized Agenda for Mental Health Research in Pediatric Rheumatology from the Childhood Arthritis and Rheumatology Research Alliance Mental Health Workgroup [published online ahead of print, 2020 Jan 15]. *J Rheumatol*. 2020;jrheum.190361. doi:10.3899/jrheum.190361

2. Woo KMT, Barron GH, Daugherty AL, **Woo JMP**, Kehoe SP, Aguilar LAB, Cavanaugh SM. Measurements of the radiographic cardiac silhouette of osprey (*Pandion haliaetus*). *AJVR*. 2019 Sep; 80(9).

3. Knight A, Vickery M, Faust L, Muscal E, Davis A, Harris J, Hersh AO, Rodriguez M, Onel K, Rubinstein T, Washington N, Weitzman ER, Conlon H, **Woo JMP**, Gerstbacher D, and von Scheven E, for the CARRA Investigators. Gaps in Mental Health Care for Youth with Rheumatologic Conditions: A Mixed Methods Study of Perspectives from Behavioral Health Providers. *Arthritis Care Res (Hoboken).* 2019 May;71(5):591-601. (Epub 2019 Apr 8). PMID: 29953741.

4. Yen EY, Shaheen M, **Woo JMP**, Mercer N, Duan L, Li N, Karlamangla A, McCurdy DK, Singh RR. 46-Year Trends in Systemic Lupus Erythematosus Mortality According to Sex, Race, Ethnicity, and Geographic Region in the United States. *Ann Intern Med.* 2017 Dec 5;167(11):777-785. (Epub 2017 Oct 31). PMID: 29086801.

5. Phillippi K, Hoeltzel M, Byun Robinson A, Kim S, **Childhood Arthritis and Rheumatology Research Alliance (CARRA) Legacy Registry Investigators.** Race, Income, and Disease Outcomes in Juvenile Dermatomyositis. *J Pediatr*. 2017 May;184:38-44.e1. (Epub 2017 Mar 3). PMID: 28410093.

6. Beukelman T, Kimura Y, Ilowite NT, Mieszkalski K, Natter MD, Burrell G, Best B, Jones J, Schanberg LE, **CARRA Registry Investigators.** The new Childhood Arthritis and Rheumatology Research Alliance (CARRA) registry: design, rationale, and characteristics of patients enrolled in the first 12 months. *Pediatr Rheumatol Online J*. 2017 Apr 17;15(1):30. PMID: 28416023.

7. Amarilyo G, Tarp S, Foeldvari I, Cohen N, Pope TD, **Woo JMP**, Christensen R, Furst DE. Biological agents in polyarticular juvenile idiopathic arthritis: A meta-analysis of randomized withdrawal trials. *Semin Arthritis Rheum*. 2016 Dec;46(3):312-318. PMID: 27989499.

8. Hong KM, Kim HK, Park SY, Poojan S, Kim MK, Sung J, Tsao BP, Grossman JM, Rullo OJ, **Woo JMP**, McCurdy DK, Rider LG, Miller FW, Song YW. CD3Z hypermethylation is associated with severe clinical manifestations in systemic lupus erythematosus and reduces CD3ζ chain expression in T cells. *Rheumatology*. 2016 Dec 10. PMID: 27940592.

9. Tarp S, Amarilyo G, Foeldvari I, Christensen R, **Woo JMP**, Cohen N, Pope TD, Furst D. Efficacy and safety of biological agents for systemic juvenile idiopathic arthritis: A systematic review and meta-analysis of randomised trials to Rheumatology. *Rheumatology (Oxford)*. 2016 Apr. 55(4):669-79. PMID: 26628580.

10. Amarilyo G, Furst DE, **Woo JM**, Li W, Bliddal H, Christensen R, Tarp S. Agreements and Discrepancies between FDA Reports and Journal Papers on Biologic Agents Approved for Rheumatoid Arthritis: A Meta-Research Project. *Plos One*. 2016 Jan 25;11(1):e0147556. PMID: 2680830.

11. Martin N, Nakamura K, Paila U, **Woo J**, Brown C, Wright J, et al. Homozygous mutation of MTPAP causes cellular radiosensitivity and persistent DNA double strand breaks. *Cell Death and Disease.* Mar 2014; 5(3): e1130. PMID: 24651433.

12. Amarlilyo G, Rullo OJ, McCurdy DK, **Woo JMP**, Furst DE. Folate usage in MTX-treated juvenile idiopathic arthritis (JIA) patients is inconsistent and highly variable. *Rheumatol Int*. 2013 Feb 12. PMID: 23400770.

13. Rullo OJ, **Woo JMP**, Parsa MF, Hoftman ADC, Maranian P, Elashoff DA, Niewold TB, Grossman JM, Hahn BH, McMahon MA, McCurdy DK, and Tsao BP. Plasma levels of osteopontin identify patients at risk for organ damage in systemic lupus erythematosus. *Arthritis Res Ther*. 2013 Jan 23;15(1):R18. PMID: 23343383.

14. Amarilyo G, **Woo JMP**, et al. Publication outcomes of abstracts presented at the ACR/ARHP Annual Scientific Meeting. *Arthritis Care Res*. 2012 Oct 8. doi: 10.1002/acr.21864

15. **Woo J**, et al. Treatment with apolipoprotein A-1 mimetic peptide reduces lupus-like manifestations in a murine lupus model of atherosclerosis. *Arthritis Research and Therapy*. 2010 May 18; 12(3):R93. PMID: 2048278.

16. Rullo OJ, **Woo JMP**, Wu H, Maranian P, Hoftman, ADC, Brahn B, McCurdy D, Cantor RM, Tsao BP. Association of IRF5 polymorphisms with activation of the Interferon-alpha pathway. *Annals of Rheumatic Disease*. 2009 Oct 23; 69(3):611-7. PMID: 19854706.

In Progress

1. **Woo JMP**, Liu P, Parsa MF. Hoftman ADC, Amarilyo G, Yen E, McCurdy DK, Rullo OJ, For the CARRA Registry Investigators. Racial differences in juvenile systemic lupus erythematosus within 18 months of diagnosis: An analysis of the Childhood Arthritis and Rheumatism Research Alliance Registry and a tertiary care center cohort.

2. **Woo JMP**, Sandler DP, Simanek A, Gaston S, O'Brien KM, Auer PL, Headley Konkel R, Meier HCS. Early life trauma and incident breast cancer risk. [*tentative author list and title*]

3. **Woo JMP**, Auer PL, Simanek A, Headley Konkel R, Sandler DP, Meier HCS. Early life trauma and methylation of the glucocorticoid gene (*NR3C1*). [*tentative author list and title*]

4. **Woo JMP**, Lynch E, Simanek A, Auer PL, Headley Konkel R, Parks C, Sandler DP, Meier HCS. Early life trauma and adult leukocyte telomere length. [*tentative author list and title*]

PRESENTATIONS

Oral Presentations

Woo JMP. (2019). CARRA Coordinator Pre-Meeting Sessions. Workshop at the 2019 CARRA Annual Scientific Meeting, Louisville, KY.

Woo JMP. (2018). CARRA Coordinator Pre-Meeting Session. Workshop at the 2018 CARRA Annual Scientific Meeting, Denver, CO.

Woo JMP. (2017). CARRA Coordinator Pre-Meeting Session. Workshop at the 2017 CARRA Annual Scientific Meeting, Houston, TX.

Murray J, **Woo JMP**, Xie H. (2016). Gotta Go! A Case Study in Mapping Inclusive Facilities for A Diverse Community. Oral presentation at the 2016 GIS and Health Symposium, Washington, D.C.

Woo JMP. (2015). Geographic Trends in the Distribution and Treatment Practices of Juvenile Systemic Lupus Erythematosus in the U.S.: An Analysis of the Childhood Arthritis and Rheumatology Research Alliance Registry. Oral presentation at the 2015 American Public Health Association Annual Meeting, Chicago, IL.

Woo JMP. (2015). U.S. Geographic Trends in the Distribution and Treatment Practices of Juvenile Systemic Lupus Erythematosus: An Analysis of the Childhood Arthritis and Rheumatology Research Alliance Registry. Oral presentation at the 2015 Zilber School of Public Health Graduate Student Research Symposium, Milwaukee, WI.

Woo J. (2011). Improving Efficiency through Electronic Notes in Pediatric Rheumatology Clinic. Oral presentation at the UCLA Quality Forum: Case Studies from the Frontlines of the Quality Revolution, Los Angeles, CA.

Poster Presentations

Woo JMP, Meier HCS, Sandler DP. (2019). Early Life Traumatic Experiences and Incident Breast Cancer Risk in Adult Women. Poster presented at the National Institute of Environmental Health Sciences 2019 Summer Internship Poster Session, Research Triangle Park, NC.

Rubinstein T, Waqar L, **Woo J**, Ogbu E, Lapin WB, Ng L, Treemarcki E, Knight A, for the SLE CARRA Mental Health Workgroup. (2018). Research Priorities for Addressing Mental Health Needs of Pediatric Patients with Rheumatologic Disease. Poster presented at ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Goh YI, Henderson LA, **Woo JMP**, Malloy M, Riordan ME, CARRA Research Coordinator Advisory Committee, CARRA Translational Research and Technology Committee Biobanking Workgroup. (2018). Approaching Pediatric Patients to Donate Biological Specimens for Research: Best Practice Guidelines to Support Healthcare Providers and Research Coordinators. Poster presented at CARRA Annual Meeting, Denver, CO.

Woo JMP, Thayer BP, Steinmetz CN, Pathak DR, Hamilton AS, Beebe-Dimmer JL, Velie EM. (2017). Accuracy of recall for geocoding residential addresses throughout the lifecourse among U.S. born women under 50 years of age. Poster presented at SER Annual Meeting, Seattle, WA.

Velie EM, **Woo JMP**, Pathak DR, Hamilton AS, Beebe-Dimmer JL, Carnegie NB, Thayer BP, Schwartz KL. (2017). Racial and socioeconomic disparities in breast cancer subtype among young non-Hispanic Black and White women in the Young Women's Health History Study. Poster presented at SER Annual Meeting, Seattle, WA.

Knight A, Vickery M, Faust L, Muscal E, Davis A, Harris J, Hersh A, Rodriguez M, Onel K, Schanberg LE, Rubinstein T, Gottlieb BS, Washington N, Weitzman E, Conlon H, **Woo J**, Dana Gerstbacher D, von Scheven E, and for the CARRA Investigators. (2017). Social Worker Perspectives on Improving Mental Health Care for Adolescents with Rheumatologic Conditions: A Mixed Methods Study. Poster presentation at ACR Pediatric Rheumatology Symposium, Houston, TX.

Woo JMP, Malloy MM, Jegers JA, et al. (2016). The Steroid Taper App: Making of a Mobile App. Poster presentation at CARRA Annual Meeting, Toronto, Canada.

Deng Y, Grossman JM, Fu Q, Martin WJ, James JA, Merrill JT, Kamen DL, Gilkeson GS, Boackle SA, Putterman C, Salmon JE, Kyttaris VC, Tsokos GC, Quirk MC, Kamble S, Barcelona M, Magdangal E, Sahakian L, Lee SY, Lin TY, Chen W, **Woo JMP**, Rullo OJ, McCurdy DK, Hahn BH, McMahon MA, Bae SC and Tsao BP. (2015). Functional Androgen Receptor Variants Associated With Increased Damage in Systemic Lupus Erythematosus. Poster presentation at ACR/ARHP Annual Scientific Meeting, San Francisco, CA.

Woo JMP, Rullo OJ, McCurdy DK. (2015). U.S. Geographic Trends in the Distribution and Treatment Practices of Juvenile Systemic Lupus Erythematosus: An Analysis of the Childhood Arthritis and Rheumatology Research Alliance Registry. Poster presentation at ACR Rheumatology Research Workshop, San Diego, CA.

Woo JM and Farrell J. (2015). Endoscopic Ultrasound practice variations based on US Medicare Data. Poster presentation at Digestive Disease Week, Washington, D.C.

Yen EY, **Woo JMP**, McCurdy DK, Singh RP. (2014). Racial, Gender and Geographic Differences in Systemic Lupus Erythematous and Lupus Nephritis Mortality Rates in the Unites States, 1968-2010. Oral presentation at ACR/ARHP 2014 Annual Scientific Meeting, Boston, MA.

Woo JMP, Rullo OJ, McCurdy DK. (2014). U.S. Geographic Trends in the Distribution and Treatment Practices of Juvenile Systemic Lupus Erythematosus: An Analysis of the Childhood Arthritis and Rheumatology Research Alliance Registry. Poster presentation at ACR/ARHP 2014 Annual Scientific Meeting, Boston, MA.

Tarp S, Amarilyo G, Foeldvari I, Cohen N, Pope T, **Woo JMP**, Christensen R, and Furst D. (2013). Short Term Efficacy Of Biologic Agents In Patients With Systemic Juvenile Idiopathic Arthritis: Network Meta-Analysis Of Randomized Trials. Poster presentation at ACR/ARHP 2013 Annual Scientific Meeting, San Diego, CA.

Amarilyo G, Tarp S, Foeldvari I, Cohen N, Pope T, **Woo JMP**, Christensen R, and Furst D. (2013). Efficacy and Safety Of Biologic Agents In Patients With Poly-Articular Juvenile Idiopathic Arthritis: Network Meta-Analysis Of Randomized Controlled Withdrawal Trials. Poster presentation at ACR/ARHP 2013 Annual Scientific Meeting, San Diego, CA.

Yen E, **Woo JMP**, McCurdy D. (2013) Sex-Related Differences and Trends In Mortality Of Juvenile-Onset Systemic Lupus Erythematosus (SLE) In The United States Over The Last Forty Years, 1971–2010. Oral presentation at ACR/ARHP 2013 Annual Scientific Meeting, San Diego, CA.

Woo JMP, et al. (2013). Ancestral group differences in pediatric SLE early disease severity: an analysis of the CARRAnet Registry. Poster presented at ACR Rheumatology Research Workshop, Dallas, TX.

Tarp S, Amarilyo G, **Woo JM**, Li W, Bliddal H, Christensen R, Furst DE. (2013). Agreements and discrepancies between the food and drug administration (FDA) reports and journal papers on biologic agents approved for rheumatoid arthritis: A meta-epidemiological study. Poster presented at the 2013 Annual EULAR Congress, Madrid, Spain.

Amarlilyo G, Rullo OJ, McCurdy DK, **Woo JMP**, Furst DE. (2012). Folate Usage in Methotrexate-treated Juvenile Idiopathic Arthritis Patients is Inconsistent and Highly Variable. Poster presented at ACR/ARHP 2012 Annual Scientific Meeting, Washington, D.C.

Woo JMP, et al. (2012). Ancestral group differences in pediatric SLE early disease severity: an analysis of the CARRAnet Registry. Poster presented at ACR/ARHP 2012 Annual Scientific Meeting, Washington, D.C.

Woo JMP, et al. (2012). Factors contributing to non-publication of abstracts presented at the ACR/ARHP Annual Scientific Meeting. Poster presented at ACR/ARHP 2012 Annual Scientific Meeting, Washington, D.C.

Woo JMP, et al. (2012). Ethnic differences of early disease severity in pediatric SLE at an urban tertiary care center. Poster presented at 2012 ACR/ARHP Rheumatology Research Workshop, Denver, CO.

Woo JMP, et al. (2011). Ethnic differences of early disease severity in pediatric SLE at an urban tertiary care center. Poster presented at 2011 ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Woo JMP, et al. (2011). Implementation of an electronic interface for pediatric rheumatology medical record documentation in an academic pediatric rheumatology outpatient clinic: an 18-month update. Poster presented at 2011 ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Amarilyo G, **Woo JMP**, et al. (2011). Publication outcomes of abstracts presented at the ACR/ARHP Annual Scientific Meeting. Poster presented at 2011 ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Parsa MF, McCurdy DK, Rullo OJ, **Woo JMP**, et al. (2011). Ultrasound and Plasma Osteopontin Levels Improve Assessment of Remission in Oligo-Arthritis. Poster presented at 2011 ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Rullo OJ, **Woo JMP**, et al. (2011). Plasma levels of osteopontin identify patients at risk for organ damage in systemic lupus erythematosus. Poster presented at 2011 ACR/ARHP Annual Scientific Meeting, Chicago, IL.

Woo J, et al. (2011). Implementation of an electronic interface for pediatric rheumatology medical record documentation improves physician and patient utilization of time in clinic. Poster presented at 2011 ACR/ARHP Pediatric Rheumatology Symposium, Miami, FL.

Rullo OJ, **Woo JMP**, et al. (2011). Plasma Osteopontin as a Marker for Future Organ Damage in Pediatric Systemic Lupus Erythematosus. Poster presented at 2011 ACR/ARHP Pediatric Rheumatology Symposium, Miami, FL.

Liu P, **Woo J**, et al. (2011). Ethnic Differences in Pediatric SLE Early Disease Severity: A Comparison between Hispanic-Americans and European-Americans. Poster presented at 2011 ACR/ARHP Pediatric Rheumatology Symposium, Miami, FL.

Woo J., et al. (2010). Implementation of an electronic interface for medical record documentation in an academic pediatric rheumatology outpatient clinic. Poster presented at 2010 American College of Rheumatology Annual Scientific Meeting, Atlanta, GA.

Rullo OJ, **Woo JMP**, Hoftman ADC, Niewold TB, et al. (2010). Plasma Osteopontin as a Marker for Damage in Pediatric Systemic Erythematosus. Poster presented at 2010 American College of Rheumatology Annual Scientific Meeting, Atlanta, GA.

Woo J., et al. (2009). Treatment with Apolipoprotein A-1 Mimetic Peptide Prevents Lupus-like Manifestations in a Murine Lupus Model of Accelerated Atherosclerosis. Poster presented at 2009 American College of Rheumatology Annual Scientific Meeting, Philadelphia, PA.

Rullo OJ, **Woo JMP**, Hoftman ADC, McCurdy DK, Tsao BP. (2009). Circulating Osteopontin Levels are Elevated in Male Pediatric SLE and Their Unaffected Siblings. Poster presented at 2009 American College of Rheumatology Annual Scientific Meeting, Philadelphia, PA.

Woo J., et al. (2008). Effects of Statin and Apolipoprotein A-1 Mimetic Peptide in a Mouse Model of Atherosclerosis in Lupus. Poster presented at the Anand Malaviya Symposium on Inflammation and Immune Therapies, Los Angeles, CA.

Woo J., et al. (2008). Effects of Statin and Apolipoprotein A-1 Mimetic Peptide in a Mouse Model of Atherosclerosis in Lupus. Poster presented at the 2008 UCLA Department of Medicine Research Day, Los Angeles, CA.

TEACHING

University of Wisconsin-Milwaukee

Spring 2019, 2020	*Guest Lecturer*, Joseph J. Zilber School of Public Health, PH763: Field Epidemiology, Topic: Working with SAS
Spring/Fall 2017 – 2019	*Guest Lecturer*, Joseph J. Zilber School of Public Health, PH101: Introduction to Public Health, Topic: Disease Detectives: Public Health in Action (Field Epidemiology)
Fall 2018	*Student Instructor*, PhD Statistics Workshop, Joseph J. Zilber School of Public Health (Enrollment: 5)
Fall 2017	*Guest Lecturer*, Joseph J. Zilber School of Public Health, PH768: Cancer Epidemiology, Topic: Cancer Epidemiology Statistics
Summer 2017	*Guest Lecturer*, SOCWORK794: Evaluation of Programs, Topic: Show me the money: How to make a budget
Spring 2017	*Guest Lecturer*, Joseph J. Zilber School of Public Health, PH759: Applied Quantitative Methods for Studying Population Health and Health Disparities, Topic: Data Analysis as a Grad Student
Spring 2016	*Guest Lecturer*, Joseph J. Zilber School of Public Health, PH759: Applied Quantitative Methods for Studying Population Health and Health Disparities, Topic: Qualitative Response Regression Models (Parts 1-3)

COMMITTEE MEMBERSHIP

University of Wisconsin-Milwaukee Joseph J. Zilber School of Public Health, Milwaukee, WI

2019 – current	**Co-president**, Public Health Graduate Student Association
2018 – current	**Member**, Health Research Symposium Planning Committee
2017 – current	**Member**, Evaluation Workgroup
2015 – current	**Member**, Seminars Committee
2015 – 2018	**PhD Student Representative**, Graduate Program Committee
2015	**Member**, Values Accreditation Workgroup
2015	**Member**, Practice and Community Engagement Ad Hoc Committee
2015	**Member**, Mission and Goals Accreditation Workgroup
2014 – 2017	**Blood Drive Organizer**, Public Health Graduate Student Association

University Wisconsin-Milwaukee, Milwaukee, WI

2018 – current	**Member**, Subcommittee on Graduate Courses & Curriculum
2018 – current	**Member**, Scholastic Affairs Committee

Society for Epidemiologic Research

2019 – current	**Lead**, Twitter Initiative Group
2018 – current	**Member**, Twitter Initiative Group

Childhood Arthritis & Rheumatology Research Alliance

2014 – 2019 **Chair**, Research Coordinator Advisory Committee
2012 – 2014 **Member**, Research Coordinator Advisory Committee

Pediatric Arthritis and Lupus (PAL) Foundation

2012 – 2014 **Founding Board Member**

PROFESSIONAL SERVICE

2019, 2020 Mock Interviewer, Zilber School of Public Health MPH Mock Interview Day
2018 – 2019 Abstract and film reviewer, American Public Health Association

COMMUNITY SERVICE

2014 – current **Volunteer**, Milwaukee Habitat for Humanity, Milwaukee, WI
2014 – 2020 **Volunteer**, UCLA Mattel Children's Hospital, Department of Rheumatology, Los Angeles, CA
2014 – 2020 **Volunteer**, Children's Hospital of Wisconsin, Milwaukee, WI
2013 – 2020 **Volunteer**, Down Syndrome Association of Wisconsin, Milwaukee, WI
2016 **Volunteer**, Southeast Asian Literacy Program, Milwaukee, WI

PROFESSIONAL MEMBERSHIPS

2017 – current Wisconsin Public Health Association (WPHA)
2016 – current Society for Epidemiologic Research (SER)
2015 – current American Public Health Association (APHA)
2012 – current Childhood Arthritis & Rheumatology Research Alliance (CARRA)
2009 – current Association of Rheumatology Health Professionals (ARHP)
2012 – 2013 Delta Omega Honor Society (Kappa Chapter)

SKILLS

Analytic Programs: ArcGIS, MPlus, SAS, STATA