Mediation and Moderation of Intergenerational Epigenetic Effects of Trauma

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DEDICATION

I dedicate this work to my tirelessly patient and supportive husband who made this dream possible, and to my children who sparked my passion for change.

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I would like to thank my committee members for their support and guidance in helping me develop as a professional. Your consistent openness to my endless questions and pursuit of learning opportunities has fostered a spirit of sharing and encouragement that I will pour into my career-long teaching and research.

ABSTRACT

Trauma and early-life stress have been linked to poor mental and physical health outcomes. In fact, research has identified trauma and stress can influence epigenetic marks on genes that can alter gene activity. It is suspected that epigenetically altered gene activity is involved in behavior and mental health. This may help explain why some individuals don't experience great benefit from treatment for the effects of stress, and severe mental health symptoms can be chronic for decades or a lifetime. Moreover, some trauma-related mental health symptoms have shown generational patterns that appear linked to epigenetic marks. Therefore, this study sought to investigate the potential inter-generational influence of mother's trauma history and mental health on her offspring's DNA methylation and gene expression in umbilical cord blood.

Standardized measures were used to assess mother's trauma history and cumulative experienced fear (TLEQ), as well as mother's mental health status during pregnancy (BSI). Genome-wide and candidate gene analyses were conducted after standard quality control data cleaning procedures. Batch and chip adjustments were made using the Combat package in R software, and the False Discovery Rate was employed to control for multiple comparisons.

Results indicate mother's exposure to trauma in childhood predicts DNA methylation and gene expression in offspring. Additionally, mother's mental health status during pregnancy significantly predicts differential gene expression on 245 genes in males only. Finally, mother's fear completely mediates the influence of trauma on her mental health functioning. In conclusion, a mother's traumatic experience has potential

to influence gene regulation in her offspring. Most importantly, mother's mental health during pregnancy appears to exert a great influence on gene regulation in males compared to female offspring.

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INTRODUCTION

Childhood abuse (physical, sexual, psychological) and neglect have been identified as some of the most damaging trauma and most stressful experiences that contribute to impaired functioning and poor mental health in adulthood (Felitti et al., 1998). Psychopathology is one associated outcome (Blaze, Asok, & Roth, 2015; Yehuda, Halligan, & Grossman, 2001), with a significant role in poor quality of life and functioning in adulthood. Compelling evidence has identified associations between childhood trauma and development of affective disorders, increased comorbidity, relatively poor treatment response (Anacker, O'Donnell, & Meaney, 2014), depression, anxiety, and PTSD (Anda et al., 2006; Cicchetti & Toth, 2005; Teicher et al., 2003). Research suggests early life stress also increases risk of affective disorders such as major depression and bipolar disorder symptoms (Hammen, Brennan, Keenan-Miller, Hazel, & Najman, 2010), and animal research models of early-life stress demonstrate anxiety and symptoms similar to depression (Elliott, Ezra-Nevo, Regev, Neufeld-Cohen, & Chen, 2010; Murgatroyd, Wu, Bockmühl, & Spengler, 2010).

Childhood abuse is also associated with poor physical health. Monnat and Chandler (2015) reported that childhood abuse (physical, verbal, sexual) and other family adversities (domestic violence, divorce, depression, substance abuse, incarceration) were associated with diabetes and heart attack, functional limitations, and influenced self-perceptions of health. The overwhelming evidence of deleterious outcomes associated with trauma (an acute stress) and early life stress (acute or chronic) fuels the onslaught of investigations to identify mechanisms involved in the

observed psychological, behavioral, and health changes leading to poor quality of life. A study conducted by the CDC and Kaiser Permanente, and published in 1998, the *Adverse Childhood Experiences study,* identified adverse childhood experiences as predictors of increased risk for multiple diseases and early death (Felitti et al., 1998). The ACE study is unique because of the large sample (n = 9,508) recruited from the Kaiser Health Plan in San Diego, a measure of trauma that can capture a degree of complexity through the number of different types of adversity experienced, and detailed health information over time. Some of the childhood adverse events included physical, psychological, or sexual abuse, violence against the mother, mental illness or criminal behavior in the home, or alcohol or drug abuse in the home. The ACE score was calculated as the sum of types of childhood traumatic experiences noted by a participant. For example, a participant that experienced childhood physical abuse, domestic violence in the home, and a parent with alcoholism would be given an ACE score of three. What began as an endeavor to identify predictors of health risk became a revelation for practitioners, policy makers, and researchers alike that traumatic experiences can influence our biology. The results included associations such as individuals with ≥ 4 childhood adversities showed a greater likelihood of developing cancer, obesity, heart disease, and lung disease (Felitti et al., 1998). These findings raise more questions because of the generational patterns often associated with several of the correlated diseases.

More recently researchers have found potential for trauma- and stress-related consequences to reach subsequent generations. Initial trans-generational patterns were

thought to be the sole result of social learning (Bandura & McClelland, 1977), where children learned through observation and experience to replicate parent behavior patterns. However, more evidence has surfaced to identify genetic and epigenetic (chemical effects that change gene expression) causes that add to the social learning explanation of trans-generational psychopathology (Yehuda et al., 2001) and health risks (Bygren, Kaati, & Edvinsson, 2001). This is significant because severe or chronic trauma symptoms have been found to be resistant to treatment (Ford & Kidd, 1998; Lonergan, 2014) and do not appear to remit naturally over time (Van der Kolk, 1994). Understanding what role epigenetics play in persistent trauma-related psychopathology may help researchers and practitioners identify better intervention and prevention strategies for improved quality of life.

While genes do contribute to development, risk, and resilience, results from monozygotic twin studies show high discordance rates, suggesting that environmental factors are significant in the development of psychiatric disorders via epigenetic changes (Bagot, Labonte, Pena, & Nestler, 2014). For example, in a study of monozygotic twins, twins that experienced bullying in school showed increased DNA methylation on a gene involved in serotonin transport as well as a blunted cortisol response compared to non-bullied twins (Ouellet-Morin et al., 2013). Additionally, epigenetic patterns can influence brain maturation, altering the developmental trajectory, and creating a new one known as *reprogramming* (Bale, 2015).

These findings are significant for social work practitioners as major providers of mental health services in the United States and the largest advocate body for

underserved, marginalized, and oppressed populations. It was estimated that approximately 89% of adults will experience some form of trauma within a lifetime, although these data are based on a non-representative sample of individuals (Kilpatrick et al., 2013). However, vulnerable populations served through social work have greater risks of experiencing trauma due to the adverse environments they navigate. Consequently, epigenetics may be a logical area for social work investigation and potential intervention (Combs-Orme, 2013). This manuscript examines and summarizes research demonstrating that a mother's trauma may affect her unborn child's gene regulation influencing risk and resilience to adversity.

This document presents three manuscripts in a stream of research that synthesize the current body of epigenetic research and identify knowledge gaps, determine if trauma experiences can generate DNA methylation (methyl groups attached to DNA altering gene expression or function) in offspring, and identify altered offspring gene expression associated with mother's trauma history, fear, and current mental health. The first manuscript (Chapter II) is a systematic literature review to address the following question: What genes have been shown in research to undergo DNA methylation and expression changes in individuals who experience trauma or early-life stress and their offspring? Existing literature was used to identify genes and DNA methylation patterns associated with trauma or early-life stress. This manuscript clearly defines knowledge gaps and methodological challenges to aid improvement of future research endeavors and inform social workers in their understanding of this science.

The second manuscript (Chapter III) describes original research to answer the following question: Do mothers' trauma experiences and related outcomes, such as mental health, influence DNA methylation patterns in offspring? The third manuscript (Chapter IV) describes original research to address the following question: Do mothers' trauma and associated outcomes, such as mental health, alter fetal gene expression?

CHAPTER I

TRAUMA AND GENETICS: AN INFLUENCE ACROSS GENERATIONS

This manuscript was prepared and written by the student, Stefanie Pilkay. Feedback and editing suggestions were provided by each member of the committee, and the student completed appropriate changes and resubmitted to committee for acceptance. This article hasn't been published anywhere, nor will it be before I turn in the final version of my ETD.

ABSTRACT

Trauma is important to social work because over 60 % of trained social workers implement mental health services (i.e. substance abuse treatment, crisis intervention, and child welfare supervision and support, and in all these settings clients are likely to have experienced trauma influencing their current circumstances. Childhood maltreatment and neglect are some of the most egregious traumatic experiences known to contribute to impaired physical and mental health functioning in adulthood. Compelling evidence has identified an association between childhood trauma and development of affective disorders, increased comorbidity, poorer treatment response, depression, anxiety, and PTSD. However, extreme experiences in adulthood such as war also have generated trauma-related symptoms with potential to endure for over 20 years. To multiply the social, physical, and mental health consequences further, we now know that our experiences influence how our genes are expressed through changes in what is known as epigenetic marks. Moreover, research suggests epigenetic influence across generations. Although social work practice is rooted in the bedrock belief that environment is a crucial component for quality of life, and has long acknowledged the importance of genetic influences, the discipline has historically shied away from

targeting the influence of genes on behavior for research or intervention. As a result, social practitioners and policy advocates have been missing crucial pieces of the life quality puzzle that come from research on trauma related epigenetic influences. We conducted a systematic literature review according to the steps outlined by Cochrane. A search was completed in Google Scholar, PubMed, and PsychInfo using the following search criteria for each database: (trauma OR stress) AND (mental health OR psychopathology OR PTSD) AND (intergenerational OR transgenerational) AND (epigenetics OR DNA methylation OR expression). We included all species studied in relation to the search terms. Collected literature was reduced to 89 peer-reviewed articles after applying exclusion criteria. Despite the complexities and changes that have taken place in investigating DNA methylation, a strong pattern of relationships emerged from the review. Trauma and early-life stress associated with epigenetic marks on genes linked to stress reactivity (24 studies), emotionality (30 studies), brain development (29 studies), and inter-generational transmission (7 studies), with some studies investigating genes involved in more than one area of association. Research and practice implications are discussed.

BACKGROUND

Introduction

Trauma can generate devastating outcomes for individuals and societies. A traumatic event is subjective, but the universal ingredient is an elicited fear response that threatens the self, eliciting feelings of helplessness, and disrupting the sense of control, meaning and connection (Herman, 1997). This can also be the absence of needed experience such as neglectful environments. Trauma is important to social work because over 60 % of trained social workers implement mental health services (i.e. substance abuse treatment, crisis intervention, and child welfare supervision and support,(Gibelman, 2004), and in all these settings clients are likely to have experienced trauma influencing their current circumstances. Kilpatrick et al. (2013) assessed 25 experiences used in the DSM to diagnose Post Traumatic Stress Disorder in a non-representative sample of adults. According to their weighted (for demographic composition) estimates, approximately 89% of adults have experienced at least one traumatic event in their lifetime.

Childhood is a uniquely vulnerable time to experience trauma given the ongoing neurodevelopment into early adulthood (Perry, 2009). Childhood maltreatment and neglect are some of the most egregious traumatic experiences known to contribute to impaired physical and mental health functioning in adulthood (Felitti et al., 1998). For example, Monnat and Chandler (2015) found that childhood abuse (physical, verbal, sexual) and other family adversities are also associated with serious disease such as

diabetes and heart attack. Additional compelling evidence has identified an association between childhood trauma and development of affective disorders, increased comorbidity, poorer treatment response (Anacker et al., 2014), depression, anxiety, and PTSD (Anda et al., 2006; Cicchetti & Toth, 2005; Teicher et al., 2003). Lund, Foy, Sipprelle, and Strachan (1984) noted extreme experiences in adulthood such as war also have generated trauma-related symptoms with potential to endure for over 20 years. Furthermore, the symptoms lead to impairment in work, relationships, and physical and mental health significantly reducing quality of life (Lund et al., 1984). To multiply the social, physical, and mental health consequences further, we now know that our experiences influence how our genes are expressed through changes in what is known as epigenetic marks. Moreover, research suggests epigenetic influence across generations (Appleton et al., 2013)

Although social work practice is rooted in the bedrock belief that environment is a crucial component for quality of life, and has long acknowledged the importance of genetic influences, the discipline has historically shied away from targeting the influence of genes on behavior for research or intervention (Strohman, 2003). As a result, social practitioners and policy advocates have been missing crucial pieces of the life quality puzzle that come from research on trauma related epigenetic influences. Epigenetic research has great potential to help us understand how social injustice such as oppression, and the inherent disadvantages that accompany poverty (i.e. community violence, domestic violence, crime), can affect our biology and subsequent behavior (Combs-Orme, 2013).

Toward contributing to social workers' understanding of the importance of epigenetic research to explain the impacts of trauma on well-being, this manuscript synthesizes findings from a systematic review of existing empirical literature about how trauma influences functioning through epigenetic influences on gene regulation of individuals and their offspring. The following questions will be addressed: (1) How does trauma relate to DNA methylation in individuals? (2) How does maternal trauma relate to DNA methylation in offspring? (3) How does DNA methylation-related gene regulation alter functioning of an individual? (4) How can DNA methylation be transmitted across generations?

Understanding Trauma

Negative outcomes associated with trauma have sparked research focused on improving our understanding of etiology, symptom manifestation, and treatment outcomes for different types, combinations, and accumulations of adverse experiences in childhood and adulthood. The Adverse Childhood Experiences (ACE) study is one investigation that examined relationships between adversity in childhood, such as abuse or family deaths, and health outcomes in over 17,000 adults with Kaiser Permanente HMO insurance. The sample was largely Caucasian and not representative of those living in poverty. However, many mental and physical health outcomes, like depression and heart disease, were positively associated with four or more ACEs increasing the odds of developing most of the physical and mental health ailments investigated (Felitti et al., 1998).

Stress and Trauma

The key to relationships between traumatic events and physical and mental health outcomes is stress. Stress is also a biological response to stimuli that requires focused energy and activity often referred to as the “fight-or-flight” response. The magnitude of stress can vary according to the extent of perceived threat triggered by events, in addition to an individual’s ability to cope.

The hypothalamus pituitary adrenal axis (HPA-axis) is the stress response system that is activated by a threat to generate a spectrum of emotion from worry about basic needs to fear (Guilliams & Edwards, 2010). While fear is an adaptive response embedded in survival, excessive and uncontrolled fear is intricately woven into the development of numerous stress-related psychiatric illnesses (Mobbs et al., 2007). Whether an event elicits a stress response or not, and to what magnitude, depends upon individual perceptions (Lazarus & Folkman, 1984), and therefore identical experiences might be considered traumatic for one person while much less threatening for another. Given the close relationship between stress and trauma, this manuscript seeks to identify empirical research outlining gene-environment interaction mechanisms (DNA methylation) that may convey detrimental outcomes associated with trauma and early-life stress.

Understanding Consequences of Stress and Trauma

Research in the 1990s (often called the “decade of the brain”) discovered profound structural and functional alterations that helped illuminate many negative consequences associated with trauma and early-life stress. Multiple brain regions can

be affected, and there are differences based on type (Agorastos et al., 2014), timing (Andersen et al., 2008), chronicity (De Bellis et al., 1999; Kaufman & Charney, 2001), and complexity of trauma (Perry, 2009). These differences suggest variation in magnitude of elicited stress.

Repeated exposure to stress can impair the HPA-axis (Perry, 2009), and the cascade of neurochemicals and repeated triggering of other parts of the brain's limbic system can lead to alterations in other brain regions. One such example occurs in the hippocampus, the part of the brain associated with learning and memory, where Kaufman and Charney (2001) noted from a review of the research literature that stress reduces neurogenesis in the dentate gyrus of the hippocampus. Other researchers have identified associations between repeated stress and reduced hippocampal volume in adulthood (Graham, Heim, Goodman, Miller, & Nemeroff, 1999).

Cortisol is a steroid hormone released during HPA-axis activation that has been implicated in dysfunctional stress response. It serves a survival purpose during threat of danger through multiple biological processes that result in increased blood glucose for energy use during the fight-or-flight response (Huether & McCance, 2013). Cortisol released during stress can cause a build-up of excess extracellular glutamate (overall excitatory neurochemical) in the brain. In manageable amounts, and for average durations, this is part of an adaptive response. However, during chronic stress activation, such as what occurs with a sensitized HPA-axis, the brain gets bathed in this excitatory neurochemical. This creates a toxic environment (excitotoxicity) that kills neurons and breaks down neuron connections (synapses) (Goodman, Bruce, Cheng, &

Mattson, 1996). It is believed that an altered HPA-axis and subsequent excitotoxicity is at the root of some structural and functional brain changes associated with trauma and early-life stress (Goodman et al., 1996; Weiss, 2007).

The potential for trauma and early-life stress to alter brain structure and function is moderated by two basic neurodevelopmental principles. First, the brain develops and organizes in a bottom-up fashion where fundamental processes for survival develop first and higher thinking cortical brain regions are developed last (Perry, 2009). For example, the hindbrain (responsible for breathing and heart rate) is completely developed at full-term birth, but the prefrontal cortex (responsible for executive functions and involved in top-down processing to inhibit the emotion-focused amygdala) is still in development until approximately 25 years of age (Miller & Cummings, 2013). Second, a growing brain organizes itself to accommodate environmental experiences (Perry, 2009). Combination of these two developmental principles makes childhood a uniquely vulnerable time to experience trauma or stress because so much of the brain is still being developed and organized.

Epigenetics and Trauma: The Environment and Gene Regulation

Deoxyribonucleic acid (DNA) is made up of 145 to 150 base pairs attached to a backbone of sugar-phosphate (Bagot & Meaney, 2010). Epigenetic modifications, which occur in response to experience and environment, alter chromatin and change gene expression but not the actual DNA sequence (Bale, 2015). Three primary epigenetic modifications include: DNA methylation, non-coding RNAs, and histone modifications (Jaenisch & Bird; Ptak & Petronis, 2008).

DNA methylation (wherein a methyl group attaches to DNA) has been studied most and is considered the most stable epigenetic mark with potential to continue throughout life (Bagot, Labonte, Pena, & Nestler, 2014; Blaze et al., 2015). These methyl groups can cause a reaction leading to the detachment of acetyl groups (Moore, Le, & Fan, 2013) thereby typically reducing gene expression (although it is possible for methylation to also increase gene expression in some cases) (Bagot et al., 2014). Epigenetic modifications from early-life stress influence genes involved in signaling between cells (Suderman et al., 2014) and neurobiological substrates into adulthood (McGowan et al., 2009; Murgatroyd, Wu, et al., 2010; Weaver et al., 2004). These foundational biological processes can influence behavior such as later life psychopathology that has been linked to correlations between DNA methylation and early-life events (Blaze et al., 2015). These epigenetic mechanisms appear to be involved in adaptation to trauma; in other words, they seem to mediate the relationship between childhood traumatic experiences and outcomes of well-being. For example, DNA methylation appears to play a role in individual differences for PTSD risk and resilience (Zovkic, Meadows, Kaas, & Sweatt, 2013), and may be responsible for salient prenatal stress effects on behavior and the brain (Bock, Wainstock, Braun, & Segal, 2015). In the current study, we conducted a systematic review of the research of epigenetic modifications in relation to trauma and early-life stress to promote further understanding of the mechanisms involved in trauma-related detrimental outcomes.

METHODS

We conducted a systematic literature review according to the steps outlined by Cochrane (Higgins & Green, 2011). A search was completed in Google Scholar, PubMed, and PsychInfo using the following search criteria for each database: (trauma OR stress) AND (mental health OR psychopathology OR PTSD) AND (intergenerational OR transgenerational) AND (epigenetics OR DNA methylation OR expression). We included all species studied in relation to the search terms. Google Scholar produced 9,690 results. PubMed identified 27 and PsychInfo located 19 results. There was significant replication of articles. We identified an initial 592 articles for further examination. Exclusion criteria included: non-peer reviewed literature, research investigating specific health conditions not related to stress activation or trauma, epigenetic marks other than DNA methylation, and studies that focused on predispositions without epigenetic changes (SNPs). A single nucleotide polymorphism is a type of variation in a gene. Collected literature was reduced to 89 peer-reviewed articles based on these exclusion criteria.

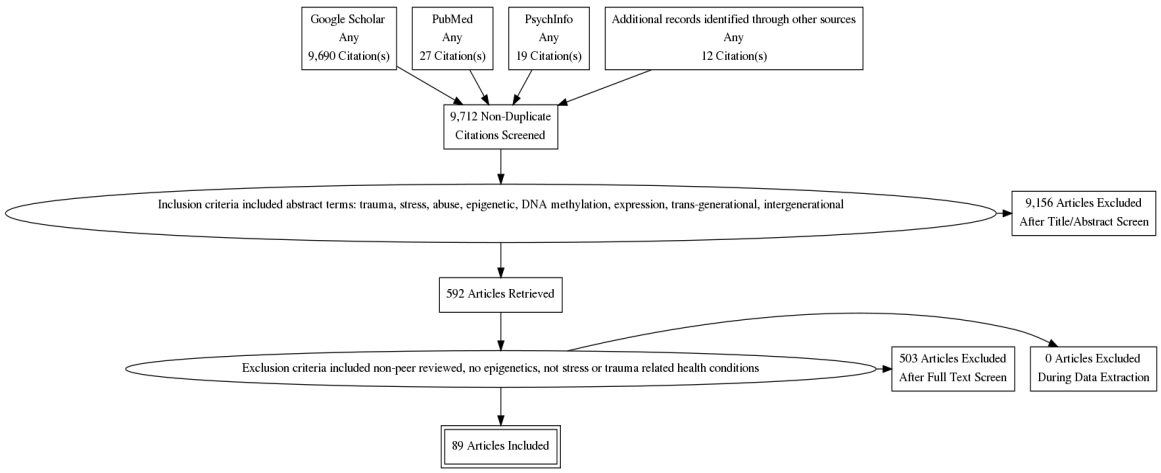


Figure 1. Overall results of the systematic review

RESULTS

Effect size reporting is considered prudent to include in systematic reviews. However, investigations of epigenetic relationships still predominantly do not include effect sizes. This could be due to a slower transition to a new format of reporting or the rapid advancements in measurement technology. Furthermore, it is presently unknown if effect size will carry the same weights for epigenetic marks. In social science literature a small effect size may not be clinically significant, whereas a “small” effect size in comparison of epigenetic marks across groups might carry large biological consequences. In summation, this relatively new science is still undergoing rapid growth and change across tools and analytic approaches that may be hindering effect size reporting. Consequently, effect sizes are not included in this review, and results must be considered within this context.

Despite the complexities and changes that have taken place in investigating DNA methylation, a strong pattern of relationships emerged from the review. Trauma and early-life stress associated with epigenetic marks on genes linked to stress reactivity (24 studies), emotionality (30 studies), brain development (29 studies), and inter-generational transmission (7 studies), with some studies investigating genes involved in more than one area of association. Most importantly, many relationships also linked to neurobiological alterations and mental health functioning. The following section reports on these findings and provides an explanation of the processes involved in those findings. Studies that examined relationships in more than one category outlined in this review will be noted in each appropriate section with the accompanying results to

eliminate repetition. Results tables have been constructed to organize the genes related to glucocorticoids, serotonin, brain development, and studies that investigated the whole-genome. This organization streamlines reference for the reader according to how the results are discussed within the text.

Genes That Influence Stress Reactivity

Of the 89 articles included in this review, 24 studies identified 42 relationships between trauma, stress activity genes, and behavior/symptoms. The overall findings outline how traumatic experiences can alter activity of glucocorticoid genes resulting in altered stress reactivity that can associate with adverse mental health symptoms. The stress response is a cascade of hormones throughout the brain and body with potential for alteration in multiple areas. Genes that instruct any part of stimulation, production, or reception of these hormones can generate changes in stress reactivity ranging from increased sensitivity to impaired negative feedback that shuts down the stress response.

Cortisol is the primary hormone that activates glucocorticoid receptors and two genes that regulate the expression of glucocorticoid receptors (***NR3C1, FKBP5***) were replicated in studies in this review (see Table 1). The activation of glucocorticoid receptors in the brain sends a signal back to the HPA-axis that stress activation was successful and needs to shut down now. However, when alteration occurs in genes regulating glucocorticoid receptors, the stress response can become dysregulated in different ways with potential to influence behavior and adversely affect mental health. This section will outline dysfunction in the stress response and the links between DNA

methylation, expression of the glucocorticoid receptor genes, and differential activation of the stress response.

Stress response dysfunction

Stress reactivity and the HPA-axis are prominent in trauma-related literature across disciplines. Brain imaging, cortisol, heart-rate, and skin conductivity measures inform understanding of what is happening during stress and potential dysfunction of the stress response and subsequent consequences. However, research examining DNA methylation and gene expression provides deeper insight into the underlying mechanisms controlling stress response dysfunction. Investigation revealed changes in DNA methylation in childhood immediately after a traumatic event (Naumova et al., 2012), more changes during adolescence that were not present in the first measurement (Essex et al., 2013), and additional methylation changes in adulthood (Klengel et al., 2013). These changes over time were on genes involved in regulating stress response hormones (Klengel et al., 2013) that have been found to be related to the development of psychopathology when stress activation is dysregulated (Perroud et al., 2011; Tyrka, Price, Marsit, Walters, & Carpenter, 2012).

Animal research has found early-life stress to be associated with salient methylation patterns on genes involved in the HPA-axis. The methylation resulted in increased gene activity that increased ACTH protein expression (adrenocorticotropic hormone) (Wu, Patchev, Daniel, Almeida, & Spengler, 2014) and corticosterone levels (the rodent equivalent of cortisol) (Chen et al., 2012; Murgatroyd, Patchev, et al., 2010). These gene changes are consistent with a greater hormonal stress response

(Murgatroyd, Patchev, et al., 2010), but could possibly lead to a breakdown of the negative feedback loop through compensatory responses in receptors or a depletion of available corticosterone to send the shutdown signal.

Stress response dysfunction symptoms

Results from the review also revealed that epigenetic marks associated with trauma or stress were correlated with unfavorable mental health symptoms and behavior. For example, 6 studies in this review reported on epigenetic changes found in people diagnosed with borderline personality disorder (BPD). This disorder is marked by severe dysregulation of mood and behavior along with heightened stress reactivity. In a female sample, DNA methylation increased with childhood maltreatment and BPD symptoms, suggesting methylation as a mechanism for child abuse to influence development of BPD (Prados et al., 2015). In addition, examination of rat pups repeatedly separated from their mothers during the first 10 days of life suggest that these epigenetic changes are long-lasting (Murgatroyd, Patchev, et al., 2010); methylation changes in genes involved in regulating stress hormone levels were still present one year after separation. These epigenetic patterns correlated with increased gene expression, corticosterone hypersecretion, and stress response dysfunction symptoms such as memory discrepancies and passive stress management behaviors such as floating with minimal movement in a forced swim test (Murgatroyd, Patchev, et al., 2010).

Stress response dysfunction predictors

Identifying what predicts dysfunction in the stress response increases our ability to find new avenues for trauma-related prevention and intervention. DNA methylation research is developing this understanding, but gene expression is beginning to reveal new insight as well. Gene expression is essentially the transcription (copy) of a gene, with more transcriptions representing greater gene activity. Research measures gene expression using mRNA, the step in gene transcription that acts as intermediary between a gene and the protein it encodes. Findings from the current review suggest correlational relationships between trauma and methylation and gene expression can occur without presence of mental health symptoms. In a sample of mixed-sex, mostly African American individuals with PTSD and different childhood adversities, gene expression was differently and more greatly influenced by DNA methylation in those with a history of childhood abuse compared to those without an abuse history (Mehta et al., 2013). This relationship was later supported in a study by Gola et al. (2014) examining PTSD and trauma in a mixed-sex and ancestry (race) sample. Reduced glucocorticoid receptor (*GRa*) expression was found in individuals with chronic or severe PTSD using whole blood. In addition, *GRa* expression correlated negatively with the number of trauma events for both with PTSD and the control groups (Gola et al., 2014) supporting the previous findings that trauma experience exerts influence on gene regulation, and supports the proposition that epigenetic influence on gene regulation may be a mechanism for trauma-related mental health symptoms.

Glucocorticoid receptors

One suspected mechanism of stress-related disorder development is the alteration of genes involved with glucocorticoid receptors (GR) that are essential for proper functioning of the stress response negative feedback (Meaney et al., 1996). When genes involved in functioning of these receptors are altered by DNA methylation, the stress response suffers dysregulation. For example, in a post-mortem brain sample from male suicide victims, history of childhood maltreatment associated with differential global (overall) methylation (Labonté et al., 2012), and increased methylation on one particular GR gene (*NR3C1*) associated with reduced GR expression in the hippocampus (McGowan et al., 2009). Reduced expression of glucocorticoid receptors would impair negative feedback of the HPA-axis and increase likelihood of chronically elevated stress hormones. These findings were replicated in abused adolescents (11-14 years) with increased methylation on the *NR3C1* gene compared to non-abused children (Romens, McDonald, Svaren, & Pollak, 2015). Lower stress reactivity was found to be associated with reduced methylation on GR genes, but education level and lifestyle differences accounted for much of the relationship (de Rooij et al., 2012).

In fact, a number of studies reveal that methylation on gene loci (CpG sites) on the glucocorticoid receptor gene also appears to be related to environmental factors such as parental caregiving (Hao, Huang, Nielsen, & Kosten, 2011) and sibling environment (Kosten, Huang, & Nielsen, 2014). Rats exposed to minimal-to-low maternal care during infancy showed increased methylation of the glucocorticoid receptor in the hippocampus compared to high-maternal-care pups. Notably, these

effects could be reversed when pups were removed from low maternal care mothers and raised by high-maternal-care mothers (Weaver et al., 2004). Female adolescent rats from same-sex litters also showed hyper-methylation of the GR gene (*NR3C1*) in the nucleus accumbens (part of the reward system of the brain) compared to females from mixed-sex litters and males (Kosten et al., 2014), which would be expected to reduce expression of the GR gene.

Human studies have begun to report the same methylation pattern of *NR3C1* in individuals with altered stress reactivity. For instance, early-life parental loss or maltreatment is associated with increased methylation on a *NR3C1*, and the methylated gene is associated with diminished cortisol response (Tyrka et al., 2012). Support for these findings was demonstrated in a group of Caucasian male veterans with PTSD who showed reduced expression of GR genes in whole blood compared to controls (M. W. Logue et al., 2015). Ancestry wasn't considered, so patterns of reduced GR gene expression may manifest differently among alternate ethnicities.

Linking GR and mental health

Findings from the review also included numerous studies providing evidence consistent with the assertion that alterations to glucocorticoid receptor genes also have potential downstream effects on behavior. Many of these studies implicated NR3C1. For example, childhood abuse predicted increased methylation of *NR3C1* in post-mortem suicide victims' brains and mood disorder patients' blood. The increased methylation corresponded with reduced gene expression, which is expected to disturb suppression of the HPA-axis (McGowan et al., 2009; Perroud et al., 2011). Furthermore, Heinrich et

al. (2015) recently discovered that individuals with externalizing disorders have reduced methylation on *NR3C1*, compared to those with depressive disorder and controls.

Suspected traumatic/stress event(s) and subsequent mental health outcomes that have been linked to DNA methylation strongly suggest these epigenetic changes constitute a mechanism that delivers the negative effects of trauma. For instance, trauma and stress-related mental health symptoms show the same epigenetic patterns even though mental health can be heritable or the result of experience other than trauma. In a large sample of peripheral blood from mostly women with borderline personality disorder, a positive association was found between childhood physical abuse and methylation of *NR3C**1*** that positively correlated with clinical severity of mental health symptoms (Martín-Blanco et al., 2014). In a study investigating male and female children (n = 184) with diverse ancestry, child maltreatment positively associated with methylation on *NR3C1* that was also positively associated with individual reports of perceived stress (Tyrka et al., 2015).

In addition to *NR3C1*, DNA methylation is also considered a mechanism of gene-environment interactions related to polymorphisms (common gene variations) of other GR genes. A few years ago Binder et al. (2008) suggested DNA methylation is the mechanism for the relationship between the GR gene *FKBP5* risk allele and child maltreatment in mostly African American samples. *FKBP5* is linked to development of glucocorticoid resistance, and studies found reduced methylation associated with childhood maltreatment (Binder et al., 2008). This correlated with greater risk for PTSD (Binder et al., 2008) and increased expression and dysregulation of the stress

response, which is consistent with impaired HPA-axis negative feedback (Klengel et al., 2013). The study did include some different ancestries; however, the samples were mostly African American (>95%) and may not have had sufficient sample sizes of other ethnic groups to identify ancestry-associated variance in methylation.

Serotonin and Emotionality

This section will evaluate links among trauma, early-life stress, serotonin, and emotionality found in the reviewed research articles. A total of 30 studies, identifying 28 relationships are included in this section because they identified predictors of altered emotionality, genes involved in serotonin activity (see Table 2) and subsequently modified emotion regulation, gene-environment interactions, and more recently discovered alternative contributors.

Anxiety is a unique chronic stress that can be heritable, or due to adversity. Since anxiety is considered both a chronic stressor and an emotion it requires careful consideration. Research has identified biological markers unique to people suffering from anxiety, such as men and women who showed overall increased global DNA methylation (average of methylation across the genome). More importantly, as anxiety scores increased so did expression of genes involved in regulating the epigenetic process itself (*DNMT1/3A, EZH2,* and *IL-6*) (Murphy et al., 2015).

Emotionality predictors

Serotonin-related genes, like some GR genes, were included in many candidate gene analyses based upon understanding of the involvement of serotonin in anxiety and depression-related disorders. Serotonin is a neurotransmitter found primarily in the

central nervous system and gastrointestinal tract. It is thought to affect mood, sleep, memory, social behavior, appetite, and sexual interest and function. Childhood trauma is associated with increased methylation on serotonin-related genes in females (Beach, Brody, Todorov, Gunter, & Philibert, 2011; Vijayendran, Beach, Plume, Brody, & Philibert, 2012) and samples of both sexes (Booij et al., 2015; Kang, Kim, Stewart, et al., 2013; Ouellet-Morin et al., 2013; Provenzi et al., 2015).

In research on monkeys, serotonin transporter gene *5HTT (also known as SLC6A4)* DNA methylation was elevated and associated with increased stress reactivity in those exposed to early-life stress such as variable foraging demand compared to controls (Kinnally et al., 2011). On the opposite end of the stress reactivity spectrum, a group of women who suffered child sex abuse showed increased methylation in the promoter region (close to where transcription starts)of *5HTT* that positively correlated with antisocial personality disorder symptoms (Beach et al., 2011), which is typically associated with reduced stress reactivity (Lovallo, 2013). A sample of night shift nurses showed reduced methylation on the serotonin transporter gene (*SLC6A4*) for which work stress and burnout equally contributed (Alasaari et al., 2012). These findings suggest a complex dysregulation of serotonin signaling following both childhood maltreatment and stress in adulthood.

Serotonin receptor genes and emotion

The review findings suggest one possible mechanism leading to mental health symptoms involves the gene *SLC6A4* identified in regulation of available serotonin in the synaptic cleft (a microscopic gap between neurons), and known to moderate facets

of emotional behavior (Kang, Kim, Stewart, et al., 2013). Differential methylation on the SLC6A4 gene predicted poorer quality of life and greater disability (Kang, Kim, Stewart, et al., 2013). Alasaasri et al., (2012) suggested that decreased methylation on this serotonin gene has potential to cause increased gene expression, leading to greater serotonin reuptake in the synaptic clefts, thereby terminating serotonin activity, with potential over time to result in depressed mood (Alasaari et al., 2012). It is important to note that one study examining the link between SLC6A4 expression and symptoms of depression did not find differential methylation that reached statistical significance and they reported a conflicting trend for increased methylation (Philibert et al., 2008). However, the review findings suggest a balance of gene activity is necessary to stave off mental health symptoms because too little activity is also linked to poor mental health.

Reduction of *SLC6A4* activity has been associated with heightened stress reactivity (Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013). Increased methylation on the promoter region was associated with greater perceived stress and difficulties in social and occupational functioning in a mixed-sex sample (n = 108) with major depressive disorders (Kang, Kim, Stewart, et al., 2013). As noted previously, chronic stress activation can lead to compensatory responses in the brain to counter the over-production and imbalance of cortisol. Monozygotic twin studies revealed findings suggestive of this phenomenon. Twins showed increased methylation on the serotonin transporter gene that was associated with a blunted cortisol response under stress (Babenko, Kovalchuk, & Metz, 2015; Ouellet-Morin et al., 2013) that is consistent with

previous findings linking this methylation pattern to antisocial personality disorder traits (which is known to be indicative of reduced stress reactivity) (Beach et al., 2011).

Risk and resilience to the development of PTSD have been associated with activity of the serotonin transporter gene *SLC6A4*. In a mixed-sex and ancestry sample, degree of methylation on *SLC6A4* moderated the effect of the number of traumatic events on PTSD development. Specifically, the greater the methylation on this gene the more resilient the individual was to developing PTSD (Koenen et al., 2011). One could infer that protection from anxiety/fear related disorders might be a risk for the development of dissociative type responses given potential to blunt the cortisol response and diminish stress reactivity. As an example, methylation on SLC6A4 could result in protection from developing PTSD, but could increase the risk of developing antisocial personality disorder.

The activity of genes for specific serotonin receptors has been linked to other mental health symptoms and disorders. Clinical severity of mental health symptoms as it relates to DNA methylation on a serotonin receptor gene (HTR3A) was assessed in 346 adult European men and women with bipolar disorder, ADHD, and borderline personality disorder. Childhood abuse was associated with greater methylation on the serotonin *5-HT3a* gene as well as greater symptom severity for individuals, but only for those with a specific gene variant (Perroud et al., 2015).

Serotonin gene-environment mechanisms

A variant of the serotonin gene *SLC6A4* was identified as a contributing factor to environmental influence on methylation and expression changes. In a sample of

Caucasian males only, methylation was the mechanism for a gene-environment interaction of a specific genotype (*5-HTTLPR*) with early and recent life stress showing increased global methylation, and cortisol responses reflected some of the changes (Duman & Canli, 2015). This gene-environment interaction was replicated in an animal study of adult male rats. Early-life stress interacted with the *SLC6A4* genotype (a sequence variation in a serotonin transporter gene) altering DNA methylation of the corticotropin-releasing factor (CRF) gene in the central amygdala, which regulates fear, anxiety, and other stress related behavior. Furthermore, *CRF* methylation correlated with *CRF* expression and subsequent stress coping behavior (van der Doelen et al., 2015). The gene-environment interaction also appears to act as a moderator on already existing environmental influences. One such example in whole blood from a mixed-sex sample of Caucasians in Germany reported that mother's stress during pregnancy or childhood abuse exposure was associated with reduced expression of the serotonin transport gene (*SLC6A4*). Moreover, individuals who also had the serotonin gene variant (*5-HTTLPR S*) had 32% less expression related to prenatal stress and 56% less expression related to childhood maltreatment (Wankerl et al., 2014).

Other contributors

Not all genes associated with depression are involved with serotonin. Some relationships link depression symptoms with differential DNA methylation on genes involved in neural circuitry development (*TPPP*), neural plasticity (*GRIN1*), and the stress response system (*ID3*) among mixed sexes and ancestries (Weder et al., 2014). Other findings linked early-life stress to differential DNA methylation across species,

such as a gene associated with major depressive disorder (*MORC1*), suggesting an influence on the magnitude of symptoms (Nieratschker et al., 2014). Greater expression of *FKBP5* was related to a two-fold increase in depression episodes (Binder et al., 2004). These gene expression changes have also been linked to behavior. Glucocorticoid receptor expression was synthetically decreased in rodents that then showed depression-like behaviors linked to poor HPA-axis inhibition (Boyle et al., 2005; Ridder et al., 2005). More recent research suggests DNA methylation may be a mechanism for more complex behaviors related to depression and anxiety such as eating disorders. A group of women with bulimia-spectrum disorder (BSD) and a history of childhood sexual abuse showed increased methylation on a gene that affects dopamine receptors (*DRD2*) compared to those with no eating disorder (Groleau et al., 2014). Additionally, those comorbid with BSD and borderline personality disorder showed an increase in methylation on *DRD2* (Groleau et al., 2014).

Brain Development

This section will discuss the 29 studies (25 relationships) in the review that examined the epigenetic influence on brain development related to trauma or stress. We will first explore genes (*RELN, BDNF*) involved in neurodevelopment and how the findings in the review demonstrate that DNA methylation may be one mechanism for structural and functional changes in the brain (see Table 3). Second, we will synthesize the literature to explain the complexity and challenges in linking DNA methylation in human peripheral tissue to altered activity in the brain. Finally, we will outline differential *BDNF* methylation and the links to mental health.

Genes and Brain Development

Environmental influences on brain development are of special interest to researchers and practitioners alike. It is not surprising, therefore, that early-life stress and trauma have been investigated to identify potential effects on brain development. In a recent genome-wide analysis on men with histories of depression (5-10 years after a temporary separation from parents in childhood), DNA methylation was significantly different on multiple genes spanning different areas of growth and function especially involved in brain development. However, it was depression, not early-life stress, that related to differential methylation in this group of males (Khulan et al., 2014).

Reelin is a multifunctional protein, encoded by *RELN*, involved in multiple aspects of brain development. During pregnancy, it aids neuron migration in the embryo. However, in a review of the literature on reelin, D'Arcangelo (2014) noted it also aids neuron growth and maturation, as well as synaptic activity in the mature brain. Qin et al. (2011) found reduced *RELN* expression in the hippocampus of maternally-deprived rat pups that negatively associated with DNA methylation. Blaze, Scheuing, and Roth (2013) also found early-life maltreatment to be linked to differences in methylation on *RELN* in male rat pups. Research has continued to show how adversities, such as maltreatment, in early-life have potential to alter DNA methylation and gene expression involved in the central nervous system (Blaze et al., 2015; Weder et al., 2014). Additionally, those epigenetic changes in the brain can emerge immediately (Naumova et al., 2012), later during adolescence (Essex et al., 2013), and even in adulthood on genes associated with psychopathology (Perroud et al., 2011;

Tyrka et al., 2012). These changes could affect the role of *BDNF* in neurogenesis and synaptogenesis (Perroud et al., 2013) and be predictive of suicide attempts (Kang, Kim, Lee, et al., 2013; Keller et al., 2010). *BDNF,* a brain development gene that has been heavily investigated, has been shown to be sensitive to early-life maltreatment (Blaze et al., 2013; Roth, Matt, Chen, & Blaze, 2014) and can manifest with sex-specific differential DNA methylation (Blaze et al., 2013).

Altered Brain Structure and Function

Learning and memory difficulties have long been associated with childhood trauma and early-life stress (Vogel & Schwabe, 2016). DNA methylation may be a mechanism for differential volume and functioning in the hippocampus (a brain region essential for learning and memory). Childhood maltreatment has been linked to greater methylation and decreased expression of glucocorticoid receptors in the hippocampus (Labonte et al., 2012; McGowan et al., 2009).

Rodent research suggests DNA methylation is a major mechanism for fear memory creation and maintenance (Zovkic & Sweatt, 2013). Trauma has been linked to differential methylation in the hippocampus, with reduced methylation on a gene involved in neuronal cell signaling (*Dlgap2*) that negatively correlated with expression of stress-related behavior in rats (Chertkow-Deutsher, Cohen, Klein, & Ben-Shachar, 2010). Trauma is often considered the presence of an unwanted event, but it can also be the absence of needed experience as is the case with neglectful environments. Absence of a parent-child relationship, as in institutional care, generates increased

methylation, possibly altering activity on genes involved in cellular signaling, immune system response, and development of functional brain networks (Naumova et al., 2012).

It is common in human studies to use peripheral tissues such as blood, saliva, and buccal cells for examining gene regulation changes. However, as noted previously this makes it difficult to infer what effect DNA methylation has on the brain and subsequently behavior. This is where multiple rodent studies help to inform what epigenetic alterations might be happening in specific brain regions. Early-life adversity has been found to influence the prefrontal cortex (executive functions) and limbic system (emotional center). The prefrontal cortex, for example, has shown influence from early-life caregiving. Parental care has been linked to epigenetic changes in the medial prefrontal cortex on genes involved in cognition and the development of psychopathology (Blaze et al., 2013). Moreover, maltreated rats displayed greater methylation on the brain development gene *BDNF* resulting in altered *BDNF* expression in the prefrontal cortex (Roth, Lubin, Funk, & Sweatt, 2009).

Areas in the limbic system found to be affected are the hippocampus and amygdala. The hippocampus was found to be sensitive to gestational factors manifesting in depression-like behaviors related to methylation on a specific gene responsible for generating dilation of coronary and cerebral vessels (*CALCA*) (Jiao, Opal, & Dulawa, 2013). The amygdala is functionally connected to the hippocampus and showed greater methylation in the promoter region of the neurotensin receptor gene (*NTSR1*) following maternal deprivation in rodents. Activity in the neurotensin

receptor1 in the amygdala was found to reduce conditioned fear freezing behavior, whereas blocking these receptors increased freezing behaviors (Toda et al., 2014).

BDNF and Mental Health

DNA methylation has potential to differentially influence mental health and associated behavior. Two studies identified that Major Depressive Disorder and bipolar disorder type II are associated with increased DNA methylation on different locations of the BDNF gene, suggesting differential methylation patterns on these loci might affect mood and thereby behavior (D'Addario et al., 2012; Fuchikami et al., 2011). These findings were later supported in women who showed increased methylation on specific *BDNF* gene sites according to group status (bulimia nervosa, borderline personality disorder, childhood maltreatment) (Thaler et al., 2014).

It can be alarming to think about the potential for childhood experience to alter foundational processes for brain development. Fortunately, epigenetic changes have also proven sensitive to positive environmental stimuli and those changes have been reflected in behavior alterations. Dialectical behavior therapy was employed as intervention for a group of adult female patients diagnosed with borderline personality disorder and showing increased methylation on the *BDNF* gene. After only four weeks of treatment, responsive patients experienced decreased methylation with a decrease in symptoms, whereas unresponsive patients showed higher methylation (Perroud et al., 2013). Potential to reverse epigenetic changes through intervention has been replicated in men and mixed ancestry groups (Yehuda et al., 2013). In a sample of veterans diagnosed with PTSD, DNA methylation on *FKBP5* reversed in subjects who also

exhibited a reduction in symptoms following treatment, whereas methylation on *NR3C1* did not change but predicted treatment outcome in responders and non-responders (Yehuda et al., 2013).

Individual behavior patterns have also shown influence on epigenetic changes leading to variability in risk or resilience. For example, animal research found differences between sedentary and physically active rats when exposed to acute restraint stress. Acute restraint stress in sedentary rats, compared to active rats, linked to lower global DNA methylation in brain regions responsible for learning and memory (hippocampus), the multifunctional cortex as well as greater *BDNF* expression in the periaqueductal gray region of the brain (Rodrigues et al., 2015). The periaqueductal gray brain region is linked to fear, anxiety-related, and defensive behaviors. Active rats, however, did not show restraint-induced epigenetic changes, suggesting physical activity has potential to moderate stress influence on DNA methylation (Rodrigues et al., 2015). A study investigating gene expression replicated this relationship in a sample of stressed mice that showed exercise was a protective factor against stress and increased expression of the brain development gene *BDNF* (Ieraci, Mallei, Musazzi, & Popoli, 2015).

Inter-generational Effects

Inter-generational influence (parent to offspring) is a relatively recent area of investigation, and research suggests multiple pathways by which parental experience might influence gene expression in their offspring. One path is through the mother's altered state during pregnancy such as stress or mental health issues. Another path is

through behavioral transmission such as an abused mother who abuses her offspring, then generating similar epigenetic marks. Some research has suggested a heritable component to epigenetic patterns in the germ line and imprinted genes, but these possibilities are still under investigation. In this section, we will identify parent-offspring epigenetic relationships outlined in 7 studies investigating inter-generational transmission.

Prenatal Exposure

Results indicate the mother's environment can induce stress influencing gene regulation in the fetus. Placental tissue from healthy newborns showed differential DNA methylation on a gene responsible for encoding an enzyme that protects the fetus from increased levels of maternal cortisol (*HSD11B2*). Infants whose mothers suffered socioeconomic adversity, especially in male offspring, had decreased methylation (Appleton et al., 2013). These, and other methylation changes on the GR gene *NR3C1*, were associated with different neurobehavioral phenotypes regarding habituation, excitability, and asymmetrical reflexes (Appleton, Lester, Armstrong, Lesseur, & Marsit, 2015). For example, infants with high methylation across *NR3C1* and *HSD11B2* showed higher habituation scores, which may lead to greater neurobehavioral difficulties for these children in processing and adapting to environmental stimuli (Appleton et al., 2015).

Prenatal stress-related methylation changes on *HSD11B2* and the serotonin-related gene *SLC6A4* correlated with heightened stress-related behavioral outcomes in another study (Bagot et al., 2014). During pregnancy, the mother's stress response

exposes the fetus to stress which alters the newborn's subsequent stress reactivity. For example, maternal stress positively associated with offspring stress sensitivity as observed in increased glucocorticoid production during stress activation, amplified stress behaviors and cognitive deficits (Kapoor, Kostaki, Janus, & Matthews, 2009; Lemaire, Koehl, Le Moal, & Abrous, 2000; Mueller & Bale, 2008). Prenatal stress research suggests potential to program stress neuro-circuitry with salient epigenetic alterations (Bale, 2015). Male mice offspring showed increased production of corticosterone to a stressor that was correlated with greater corticotropin releasing factor levels in the amygdala as well as reduced expression of glucocorticoid receptors in the hippocampus (Mueller & Bale, 2008).

Sex differences found in inter-generational models examining maternal stress during pregnancy may be resultant of sex-specific timing of natural epigenetic processes. For example, primordial germ cells experience a re-methylation of imprinted genes after birth for females and before birth in males (Bale, 2015). An insult occurring during these sensitive periods may have more influence on epigenetic processes. However, these early-life epigenetic patterns can be altered by maternal care (Bale, 2015).

LIMITATIONS

There are two types of genetic studies: candidate gene studies, which examine specific genes of interest based on previous research, and epigenome-wide studies, which investigate every available gene. Initially, I found that candidate gene studies dominated research because a tool to measure every gene simultaneously wasn't

available early on in this science. This resulted in research on genes according to what literature suggested about brain functioning, perhaps missing vital genes that were not obvious.

Examination of brain-related genes initially required examination of the brain so researchers either used rodents (rats and mice) or post-mortem brains. Eventually scientists could use peripheral tissues such as blood, saliva, and buccal cells (cheek swabs) to measure DNA methylation and gene expression. However, it was difficult to draw inferences about how epigenetic changes found in peripheral tissue could be manifested in the brain to influence behavior. Recently investigators have identified DNA methylation correlations between peripheral tissues and the brain (Smith et al., 2015). However, heterogeneity in brain tissue complicates conclusions from peripheral tissue. For example, rat models of prenatal stress have shown differential BDNF levels across brain regions and sex (Luoni et al., 2015; Roth et al., 2014). Furthermore, rats chronically exposed to cats had increased methylation and reduced expression of *BDNF* in two hippocampus regions (dorsal dentate gyrus and dorsal CA1) but not in two other hippocampus regions (ventral dentate gyrus and ventral CA1) (Roth, Zoladz, Sweatt, & Diamond, 2011). Tissue specificity has been discovered in the dorsal and ventral hippocampus, and central and basolateral complex of the amygdala in male and female rats (Roth et al., 2014), and the nucleus accumbens (NAc), which is related to reward processing, in a sample of adult male rats exposed to early-life maternal separation (Anier et al., 2014).

The introduction of genome-wide analysis raised questions about previous candidate gene findings. Genes previously identified in isolated analyses were not the most statistically significant, if they reached significance at all, when included in a genome-wide investigation. Furthermore, demographic traits such as sex (Roth et al., 2014) and race (Zhang et al., 2011) have been found to be associated with methylation patterns aside from predictor variables. For example, rat models looking at early-life maltreatment discovered DNA methylation patterns differed between sexes (Doherty, Forster, & Roth, 2016; Roth et al., 2014). Therefore, sex should always be considered a covariate, at a minimum, in a mixed-sex human sample, and included whenever possible to investigate sex-specific interactions. Findings from human studies are also more generalizable if a variety of races is represented and controlled as a covariate to minimize spurious findings. Finally, epigenetic studies often suffer small sample sizes (less than 100) due to financial expense and statistical multiple-comparison control that can diminish ability to detect even large relationships.

Publication bias, also known as dissemination bias (Bax & Moons, 2011), refers to investigators' decisions to submit findings for publication or not, reviewers' and editors' determinations to accept for publication, and how these three decision points can each determine what information is shared. This process is critical to note because reporting only significant findings can skew perception of the actual variable relationships and alter intervention decisions inappropriately (Strüver, 2016). In clinical research for medicine this is addressed with a mandate by the World Health Organization and the Declaration of Helsinki that all human trial results must be

published within 12 months of study completion which is expected to be honored by researchers, reviewers, and editors alike (Strüver, 2016). However, other research is less protected from the potential occurrence of dissemination bias. During this review process, it became clear that null findings are either not occurring as frequently or not published as often as statistically significant results. Although we took special care to note research designs such as demographic covariates that could influence results, replications to support evidence, and the absence of significance where available, there are bound to be unpublished null findings that would influence perspectives of the cumulative body of knowledge.

DISCUSSION

Trauma and early-life stress have potential to generate changes on a molecular level that influence brain development and alter vulnerability to stress in adulthood. In this review, we have outlined research identifying trauma and early-life stress linked to epigenetic patterns on genes found to influence stress reactivity, emotionality, and brain development. Research has revealed how experience can alter DNA methylation on genes involved in glucocorticoid receptors that associate with stress reactivity, can predict treatment outcome (*NR3C1*), and respond to treatment interventions (*FKBP5*) to improve quality of life (Yehuda et al., 2013). Furthermore, findings suggest alterations to a serotonin transporter gene (*SCL6A4*) can show similar effects on stress reactivity, in addition to the more often identified changes in emotionality (Miller et al., 2013). More specifically, DNA methylation on SCL6A4 could represent resilience in the form of protection from developing PTSD, or risk by increasing potential to develop antisocial

personality disorder. However, it could be argued that the discovered experiential influence on the *BDNF* gene may have the most profound effect on human development and the varying degrees of risk and resilience among us. As noted, the brain derived neurotrophic factor gene is mostly involved in regulating neurogenesis and synaptogenesis for brain development and regeneration. Influence on the *BDNF* gene to increase activity could result in resilience to chronic stress activation that has potential to degrade neurons and synapses. However, epigenetic marks that decrease *BDNF* gene activity could increase potential for noticeable structural changes due to excitotoxicity from chronic stress and reduced neurogenesis. The combination of these factors could result in someone being at greater risk for detrimental outcomes due to trauma, early-life stress, and even chronic stress in adulthood. Moreover, the growing evidence that experience can exert influence across generations compounds the potential for generating risk and resilience. However, inter-generational transmission is still under significant scrutiny as more evidence is necessary to replicate findings and clarify mechanisms in human models.

The mounting evidence supporting DNA methylation as a mechanism of trauma and early-life stress consequences is difficult to ignore for social work scientists, educators, and practitioners. We have only just begun to examine these changes and can expect further technological advances to provide more avenues for research that will elucidate other results of trauma, indicate interactions that will change our interpretations of these findings, and even call attention to further limitations in our knowledge. For now, we must remember that epigenetic changes are not uniformly

negative and we have potential to alter epigenetic patterns through environmental and behavioral interventions to improve quality of life for our clients. Our current body of knowledge provides enough insight to inform our practical understanding of immediate and delayed trauma responses and psychopathology, vulnerability resulting from early-life adversity, and the resilience embedded within us all given the right environment exposures.

Perhaps, one day soon, the research of epigenetics will become a tool for social workers to apply to practice, advocacy, and policy for the betterment of populations suffering social disparities. We hope the research landscape will begin to change with more treatment efficacy investigations including DNA methylation and expression variables. The potential information gained could better inform our understanding about individuals who experience fewer or no treatment benefits at all. For now, the call for social workers to consider epigenetic research is imperative (Combs-Orme, 2013). As this review has shown, epigenetics is clearly relevant to trauma and stress-related research with tremendous potential for learning about how the environment can influence our biology and behavior throughout the lifespan.

CHAPTER II

MOTHER'S HISTORY OF TRAUMA AND FEAR PREDICTS DIFFERENTIAL DNA METHYLATION AND GENE EXPRESSION IN NEWBORN

This manuscript was written independently by the student, Stefanie Pilkay. The complete draft was submitted to each committee member for feedback and the student completed appropriate changes. The final draft was re-submitted to the committee for approval. This article hasn't been published anywhere, nor will it be before I turn in the final version of my ETD.

ABSTRACT

Trauma has been found to exert significant influence on mental and physical health throughout the lifespan. Furthermore, we now know that our experience can influence our genes' activity with potential to generate effects on development, health, and behavior. This study sought to investigate the potential for trauma experiences to influence gene regulation across generations. Specifically, does the mothers' trauma history predict DNA methylation or gene expression in her newborn. The results indicate that the mothers' trauma history can predict DNA methylation and expression in her newborn. Moreover, the type of trauma experienced by the mother generates different influence on gene regulation in her offspring on genes involved in androgen receptors (*HOXD4*), monitoring protein transport through cells (*HSPA5*), and neurogenesis and synaptogenesis (*BDNF*). Further research should consider the potential for historical trauma experiences to influence DNA methylation and gene expression in offspring that could predispose risk or resilience to adversity throughout life.

INTRODUCTION

An unprecedented study on over 17,000 adults, conducted by the CDC and Kaiser Permanente, assessed early-life adverse experiences in relation to potential health outcomes and discovered almost all of the investigated outcomes, were related to the number of adversities experienced in childhood (Felitti et al., 1998). This adverse childhood events study (ACEs) revealed that trauma and early-life stress have been linked to disturbing outcomes such as increased likelihood of obesity, depression, sleep disturbances, alcohol and drug abuse, lung disease, liver disease, cancer, and attempted suicide (Felitti et al., 1998).

Estimates suggest the majority of adults will experience at least one form of trauma in their lifetime (Kilpatrick et al., 2013), and childhood maltreatment is one such type notoriously linked to poor physical and mental health throughout the lifespan (Felitti et al., 1998). Trauma and stress that occurs during childhood have greater potential to influence the quality of life because neurodevelopment proceeds rapidly in childhood (Perry, 2009). These potential experiential insults during rapid neurodevelopment are thought to explain many links between childhood trauma and outcomes such as affective disorders, less benefit from treatment in adulthood (Anacker et al., 2014), PTSD (Anda et al., 2006; Cicchetti & Toth, 2005; Teicher et al., 2003), and chronic illnesses like diabetes and heart disease (Monnat & Chandler, 2015). Some types of adult trauma, such as war, are also associated with mental health impairment that can continue for more than 20 years (Lund et al., 1984).

While some intervention and prevention strategies provide benefit, negative consequences from trauma experiences are still prevalent, and some individuals remain in need of something more or different for recovery (Lonergan, 2014). In the past social work has not been involved in **epigenetic** research (Strohman, 2003). However, some social work investigators are making strong arguments for a need to acknowledge, educate about, and participate when possible in **epigenetic** inquiry given the potential influence on brain development (Combs-Orme, 2013) and other behavioral outcomes. This is paramount for social work practice because more than half of these practitioners provide mental health treatment (Gibelman, 2004) in areas such as child welfare, crisis intervention, or substance abuse to populations with greater likelihood of having experienced trauma. Moreover, social workers are in pursuit of 12 grand challenges that include ensuring healthy development for all youth and advancing long and productive lives. Early-life trauma is not an event with temporary consequences. We've outlined how trauma in early-life can have lasting effects into adulthood. Additionally, we still aren't certain if, and how, these early-life trauma experiences can generate lasting consequences that pass from mother to child. It is essential that we develop a clearer understanding of the pathways underlying trauma-related outcomes to maximize potential to provide healing.

Insight from Epigenetics

Experience can influence how our genes behave via **epigenetic** marks (Bale, 2015). (See Table 1 for terms in **bold** in text.) Recently, advances in **epigenetics** have provided insight into the molecular mechanisms that may contribute to the development

of disease and psychiatric disorders following trauma exposure. **Epigenetic** marks, such as **DNA methylation**, are sensitive to the environment with potential to appear immediately (Naumova et al., 2012) or later in development (Essex et al., 2013). These changes can last a lifetime (Bagot et al., 2014), and may be indicative of chronic impairment, but have also been observed to change with intervention in conjunction with symptom improvement (Yehuda et al., 2013). Depending upon where **methyl groups** attach on a gene, they can increase or decrease activity regulated by that gene (Bagot et al., 2014). **DNA methylation** can alter gene activity, which, in turn, is associated with psychosocial stress, childhood trauma, PTSD (Klengel, Pape, Binder, & Mehta, 2014; McGowan et al., 2009; Mehta et al., 2013; Oberlander et al., 2008; Ressler et al., 2011; Smith et al., 2011) and clinical severity of mental health symptoms (Martín-Blanco et al., 2014; Yehuda et al., 2013).

Animal models of PTSD have found that **DNA methylation** can result from fear experiences, reinforcing fear cues and contextual triggers in the **amygdala** and **hippocampus** that are resistant to change (Zovkic & Sweatt, 2012). Fear is an important variable to consider as it is embedded in standardized trauma measures such as the Traumatic Life Events Questionnaire, and it has been found influential in relationships between trauma events, anxiety, and posttraumatic stress (Daugherty, 1998). This path of investigation has potential to help us understand more about how experience can influence our biology and behavior, suggesting new opportunities for intervention.

The link between **DNA methylation** and trauma has emerged on specific genes of interest that are involved in brain development, emotionality, and stress reactivity. For example, **DNA methylation** differences in the serotonin transporter (*SLC6A4*) and brain-derived neurotrophic factor (*BDNF*) genes have been reported in individuals with histories of abuse or traumatic stress, particularly early in life (Almli, Fani, Smith, & Ressler, 2014; Logue et al., 2013; Roth, Lubin, Funk, & Sweatt, 2009; Sipahi et al., 2014). Genes involved in the HPA-axis have also been differentially methylated in association with childhood maltreatment and early-life stress such as glucocorticoid receptor genes (*NR3C1* [McGowan et al., 2009], *FKBP5* [Binder et al., 2008], and a transcription factor gene *STAT5B* [White et al., 2015]). Methylation of these and other genes that regulate the stress-response have potential to modify an individual's ability to cope with stress or risk for PTSD (Mill & Heijmans, 2013).

We can now show that **epigenetic** marks can generate downstream effects on the brain and behavior, which adds to the convincing argument for the importance of **epigenetics** in social work practice. Some of these implications include the previously noted altered stress reactivity, brain development, and serotonin activity with potential to influence emotional regulation, cognition, and aggression. Furthermore, altered state of the mother during pregnancy has been linked to **epigenetic** changes in offspring with potential to influence development.

Epigenetic Influence Parent to Child

The potential prenatal influence emphasizes the importance of viewing trauma through an inter-generational lens to identify potential risk and resilience within the

individual. Improved knowledge of **epigenetic** associations could inform social work practice to help hinder unhealthy generational cycles such as sexual and physical abuse, and possibly trauma-related mental illness. For example, prenatal exposure to stress can alter glucocorticoid genes with **DNA methylation** resulting in a sensitized stress response upon birth (Kapoor et al., 2009). However, sensitive and responsive caregiving appropriate to develop a healthy attachment can reverse these negative effects and "reset" stress activation to proper functioning (Bale, 2015). An assessment during pregnancy to identify the mother's stress level may provide opportunity for prevention and intervention. Stress reduction methods can be shared with mothers to prevent potential consequences due to prenatal exposure to stress, and nurturing caregiving behaviors can be discussed to ensure proper development of a newborn's stress response.

DNA methylation and gene expression research on humans often relies on proximal tissues such as blood, saliva, or buccal cells (cheek cells). These proxy tissues do not reveal the exact patterns as in brain tissue, and therefore it complicates the process of making conclusions about findings in relation to potential brain function and behavior. However, researchers have compared the proxy tissues and identified some patterns of similarity that make the proximal tissues appropriate for research within the context of the natural limitations of tissue specificity (Smith et al., 2015).

Since **DNA methylation** is highly sensitive to negative and positive experiences, current social work interventions can exert effects on a molecular level with potential to alter unhealthy developmental trajectories and outcomes. The more we understand

about the mechanisms by which an individual's experience affects their subsequent behavior, such as epigenetic patterns and associated genes and behaviors, the more opportunities we can identify to intervene for improved quality of life. Therefore, this study aimed to address the question: Does mother's trauma history and cumulative experienced fear influence **DNA methylation** and expression in offspring umbilical cord blood?

Initial **epigenetic** studies were **candidate gene** analyses based upon our understanding of brain function, but more recent technical developments have allowed **genome-wide** analysis. However, **genome-wide** studies often reveal that the **candidate gene** relationships are not replicated within the genome-wide analysis, or have smaller effect sizes compared to other genes. It is possible that we may not understand as much about the brain as we thought, that other genes involved in more primary functions may be playing unexpected roles in behavior, or simply may be due to differences between proximal and brain tissue. Therefore, it is important to include **genome-wide** analyses and **candidate gene** investigations within one study to support meaningful conclusions. Therefore, this study examined how maternal self-reported trauma history and related cumulative experienced fear affect **DNA methylation** and expression in the offspring's umbilical cord blood via **genome-wide** and **candidate gene** investigations.

METHODS

Sample and Procedures

This study approach combines social work and **epigenetic** research, and was approved by the University of Tennessee Institutional Review Board. This cross-discipline collaboration provides a new path for social work inquiry relevant to our constituent populations suffering diverse adversities. Data for this study were obtained from a larger longitudinal investigation (*N* = 1503) of the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) study. Participants in the CANDLE study were healthy women, aged 18 - 40 years, recruited from local prenatal clinics in Shelby County, Tennessee. Participants were followed through pregnancy and delivery; and the mother-child pairs are being followed until eight to ten years after birth. Selection criteria included singleton pregnancy and absence of several complications. Numerous measures were used to assess a broad range of variables including trauma experiences, and **DNA methylation** and gene expression (on a sub-sample).

The current study uses a subsample of the CANDLE cohort that participated in cord blood collection after birth to allow for investigating inter-generational transmission of trauma experience and related fear on **epigenetic** patterns and gene expression. The subsample is a volunteer group that agreed to participate in cord blood collection after birth. The cord blood has been used in previous studies to investigate **DNA methylation** and gene expression (Adkins, Krushkal, Tylavsky, & Thomas, 2011; Adkins, Thomas, Tylavsky, & Krushkal, 2011; Adkins, Tylavsky, & Krushkal, 2012;

Krushkal et al., 2014). This subsample provides a large enough mix of sex and racial groups to control for these covariates, and cell variation will be identified and controlled as covariates.

Measures

Detailed variable information can be seen in Table 2. Traumatic experiences were using responses from the Traumatic Life Events Questionnaire (TLEQ; Kubany, 2000). This 23-item self-report measure covers 22 types of traumatic events that including potential traumas such as childhood abuse, warfare, domestic violence, and natural disasters. For each item respondents are asked to indicate if the event occurred (0 = no, 1 = yes) and if the event resulted in "intense fear, helplessness, or horror" in response to the event (0 = no, 1 = yes). In one item respondents are asked at what age did the trauma occur. All trauma events noted prior to age eighteen (n = 45) were considered childhood exposure, and all others as adulthood exposure (n = 134). Type of trauma was computed with all trauma events involving another person deemed "interpersonal" (n = 127) and all other traumas considered "non-interpersonal" (n = 70). The presence or absence of fear was identified for each event (0 = no, 1 = yes), and summed to create the cumulative fear score with higher values indicating more fear. The fear summary score was evaluated within the TLEQ for psychometric properties and determined to be an adequate fear measure (Johnson, Heffner, Blom, & Anthenelli, 2010; Kubany et al., 2000). Complexity of trauma was constructed to assess the exposure to multiple types of trauma, such as physical or sexual abuse, during childhood by summing the total number of different types of childhood abuse exposure

similar to an ACE (Felitti et al., 1998) score (no childhood abuse = 0, one type of childhood abuse = 1, two types of childhood abuse = 2). Childhood trauma was also dichotomized (no child abuse = 0, yes child abuse = 1).

The TLEQ has been found to have adequate overall reliability with multiple populations, as well as good content validity as assessed by an expert panel, and good discriminant validity (Kubany et al., 2000). Furthermore, the TLEQ is considered an accepted trauma measure and has been used for comparison to assess newly established brief trauma measures (Gray, Litz, Hsu, & Lombardo, 2004)

Umbilical cord blood is widely accepted as representative of the fetus/newborn, is used to assess health of the newborn, and has been used in multiple studies investigating **DNA methylation** in offspring (Boeke et al., 2012; Broberg et al., 2014; Herbstman et al. 2012; Hoyo et al., 2012; Kile et al., 2012; Koestler et al., 2013; Nomura et al., 2013; Relton et al., 2012). The Infinium HumanMethylation27 **Bead chip** by Illumina, which has been widely used in **epigenetics** research, is a **DNA methylation** analysis tool that measures approximately 27,000 of available gene locations that could be methylated (image seen in Figure 1). CANDLE investigators followed manufacturer's instructions to process the cord blood and beadchip as follows: 1) used EZ **DNA methylation** reagents (Zymo Research, Orange CA, USA) to perform **bisulfite conversion** of DNA from the umbilical cord blood; 2) Samples were then processed and hybridized. The Illumina Human WG-6 expression array measures **mRNA** expression with probes to assess 14,495 of the genes expressed in cord blood.

Analyses

Measurement error was addressed with COMBAT, an R package, that removed most of the noise to maximize signal by adjusting for **chip** and **batch effects**. It is necessary to control for chip and batch effects because differences among the bead chips and among the batches of processed bead chips could relate to the outcome variable and confound the results. The new adjusted data set created by COMBAT was used for all methylation analyses. Methylation beta values, one of the outcome variables, are the estimation of methylation based on the intensity ratio of methylated to unmethylated on each measured gene location. Gene expression, the other outcome variable, is estimated by measuring the **mRNA** transcription (copy) of a gene with more transcription representing greater expression (Marioni, Mason, Mane, Stephens, & Gilad, 2008). Methylation beta values and expression values were analyzed in regression tests in the R package MethLAB (Kilaru, Barfield, Schroeder, Smith, & Conneely, 2012) to control for the covariates child sex, child race, and cell type, as well as applying the recommended multiple comparison control False Discovery Rate *p*-value correction (Storey, 2010).

This investigation focused on main effects of mother's history of type, timing, and complexity of trauma, and her cumulative experienced fear on **DNA methylation** and gene expression in the newborn. Although **DNA methylation** can alter gene expression, methylation does not always correlate with changes in expression. Therefore, expression was included in this study to develop a more comprehensive picture of gene regulation patterns in relation to mother's trauma and fear experiences.

Genome-wide and **candidate gene** analyses were completed for genes repeatedly identified in trauma- or stress-related research with theorized potential to significantly influence brain and behavior development. We focused on three genes that can influence stress reactivity and are involved in glucocorticoid (cortisol) receptor production and function (*NR3C1*, *FKBP5*), and transcription factor activity (*STAT5B*). In addition, we targeted one gene that is important for brain development and is involved in neurogenesis and synaptogenesis (BDNF); and one gene that has potential to influence emotions and behavior through serotonin transport activity (SLC6A4).

The gene expression data analysis included standard quality control (QC) procedures to identify low quality data for removal from analyses. First, data were screened to determine what genes could be considered "expressed". Detected p-values greater than .01 were set to NAs as standard in expression data ("Not Available"; equivalent to missing data) and considered not detected. Samples with less than 10% of the **gene probes** detected were eliminated, and 134 samples remained. Probes with less than 10% of the samples detected within each dataset were eliminated (12,388 probes remained). Second, we normalized the data for analysis by completing quantile normalization, scaled the data, and completed log2 transformation.

RESULTS

The subsample is slightly over half African American mothers (African American 55%, Caucasian 45%) with similar mean ages (M = 25.04, SD = 5.15 vs. M = 28.56, SD = 4.61). Of the 216 newborn umbilical cord samples (male = 106, female = 110), **DNA**

methylation data were available for the majority (*n* = 196). Expression data (*n* = 100) showed a range of 12.73 (*Min* = 2.71, *Max* = 15.44).

The trauma variables were assessed for degree of correlation given their natural relatedness. Trauma complexity did not significantly correlate with trauma type (r = .129, p = .087, bootstrap CI [-.006, .238]. However, complexity of trauma did exhibit a moderate negative correlation with timing of trauma (r = -.589, p < .001, bootstrap CI [-.713, -.438], and an expected high positive correlation with the dichotomized childhood abuse variable (r = .931, p < .001, bootstrap CI [.925, .949].

Genome-wide analyses revealed that the timing of mother's trauma (*childhood vs. adulthood*) and the *complexity* of trauma were not statistically significantly related to expression or methylation. The type of trauma, however, did predict umbilical cord blood **DNA methylation** and expression. After examining males and females together while controlling for sex, we examined males and females separately to determine if and how relationships might vary between the sexes. Mother's exposure to interpersonal trauma was associated with increased **DNA methylation**, in males only, of CpG sites in the gene (*HOXD4*) involved in stimulating transcription of androgen receptors as seen in Figure 2 boxplot (*M* = 1.66, *SD* = .48, FDR *p* < .05) *B* = .027, bootstrap 95% CI (.017,.037). Methylation did not correlate with expression of *HOXD4*. **Candidate gene** analyses identified mothers who experienced childhood abuse associated with increased expression, in males only, of the gene (*BDNF*) involved in neurogenesis and synaptogenesis as seen in Figure 3 boxplot (*M* = 1.16, *SD* = .37, FDR *p* = .001) *B* = .471, bootstrap 95% CI (.056, .865).

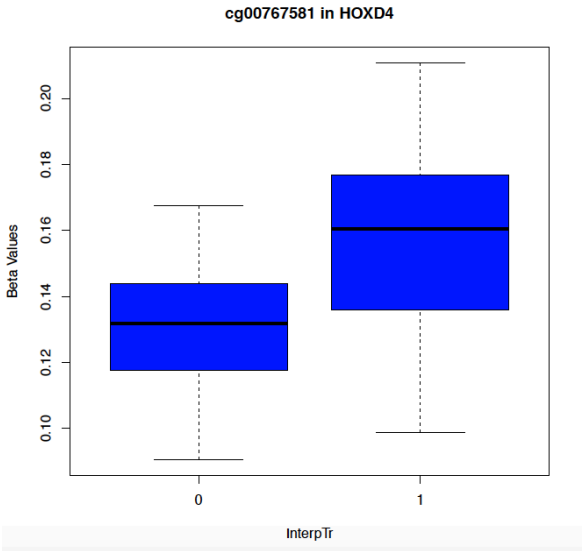


Figure 2. Mothers' Interpersonal Trauma (0 = no, 1 = yes) and HOXD4 DNA methylation.

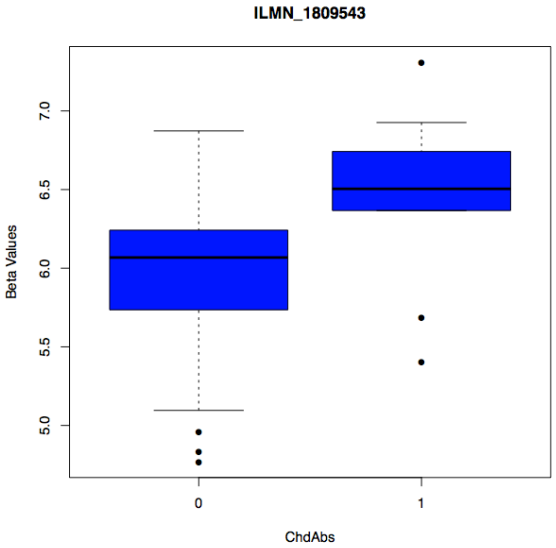


Figure 3. Relationship between Mothers' child abuse exposure (0 = no, 1 = yes) and BDNF expression in males

Mother's experience of child abuse in genome-wide analysis was associated with reduced expression, in females only, of CpG sites in the gene (*HSPA5*) suspected to be involved in supporting protein transport through cells as seen in Figure 4 boxplot (*M* = 1.2, *SD* = .4, FDR *p* = .02) *B* = -.626, bootstrap 95% CI (-.897, -.296).

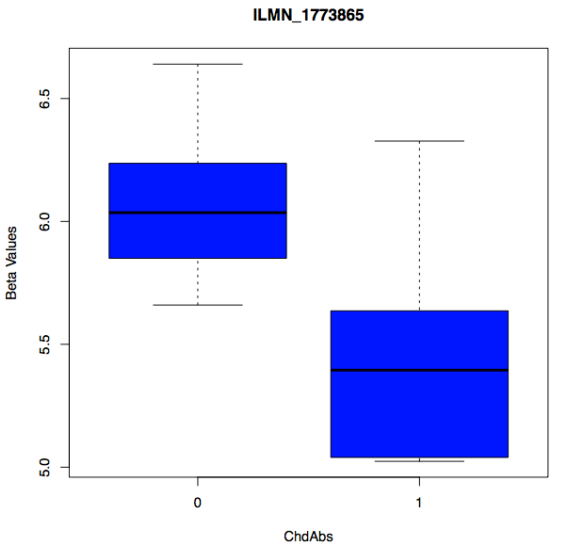


Figure 4. Relationship between Mothers' child abuse and HSPA5 expression in females

Genome-wide analysis examining mother's cumulative experienced fear did not reveal a statistically significant relationship. **Candidate gene** analysis, however, did identify differential **DNA methylation** and expression for the gene (*BDNF*) involved in brain development. Specifically, as mother's perception of experienced fear increased so did **DNA methylation**, in males only, on *BDNF* as seen in Figure 5 scatterplot (*M* = 1.62, *SD* = 1.52, *p* = .001) *B* = .004, bootstrap 95% CI (.001, .006).

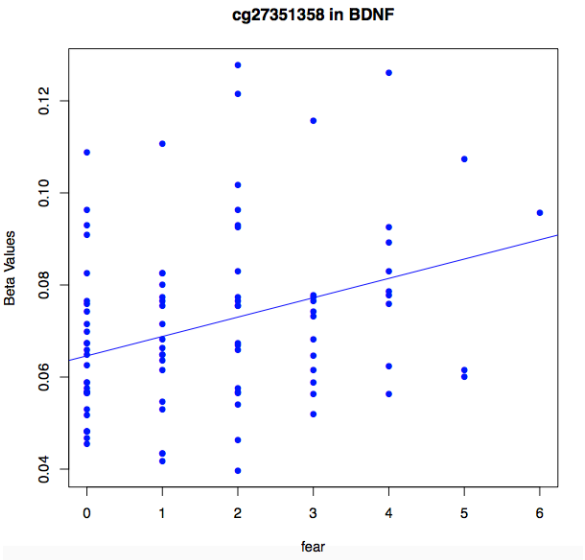


Figure 5. Relationship between Mothers' fear and BDNF expression in males.

However, methylation did not correlate with *BDNF* expression in males. However, mother's fear associated with reduced *BDNF* expression in females (as would have been expected for males with increased methylation) as seen in Figure 6 scatterplot (*M* = 2.71, *SD* = 2.47, *p* = .004) *B* = -.123, bootstrap 95% CI (-.222, -.038).

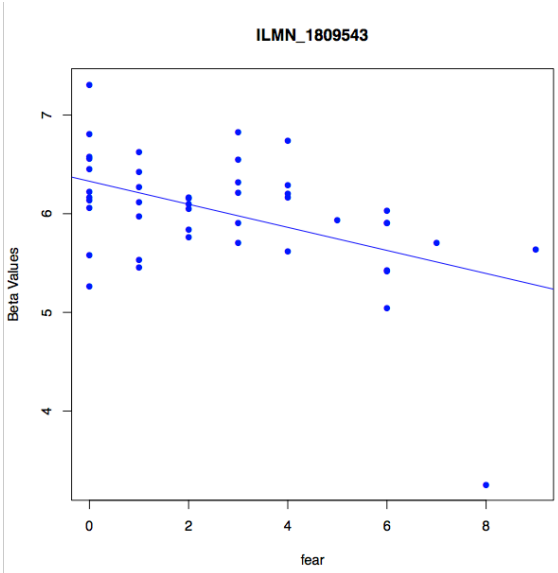


Figure 6. Relationship between Mothers' fear and BDNF expression in females.

LIMITATIONS

This study was conducted on one population sample in an urban community of west Tennessee. Furthermore, trauma measures are only as good as the honesty and accuracy with which they are completed. Emotional stimulation is often triggered with remembering traumatic events which can lead to deliberate suppression or secrecy (Perrott, Morris, Martin, & Romans, 1998; Summit, 1983). It is also not uncommon for adults to be incapable of recalling traumatic events from childhood (Widom, Weiler, & Cottler, 1999). As a result, an accurate measure of trauma can never be guaranteed, although all self-report measures potentially have these limitations. Furthermore, retrospective self-reports are the most ethical approach to assessing trauma exposure in humans. Even health records must predominantly rely on self-reports of events.

Tissue specificity is a consideration for this study. Although many studies have used cord blood to investigate DNA methylation in newborns, it is still uncertain how these gene regulation patterns might be reflected in the brain. However, proxy tissue is still the least invasive and most ethical approach to investigations with living humans. Therefore, all findings must be considered within the context of this naturally occurring limitation.

Genome-wide analysis provides opportunity to discover unknown mechanisms involved in trauma-related consequences. However, a **genome-wide** approach is conducted at a cost consisting of a larger number of statistical tests required to complete the investigation. Multiple comparison adjustment was applied to the *p*-values, and this is currently the most widely used method to conduct **genome-wide DNA**

methylation analysis. This issue is also common for fMRI studies examining voxels, which can run over 100,000. The greatest concern is increased potential of a Type I error. Therefore, in addition to controlling for multiple comparisons, future research needs to attempt replication of findings among varying populations.

DISCUSSION

This study identified a statistically significant association of the mothers' trauma and fear experience prior to pregnancy on **DNA methylation** and gene expression in the newborn's umbilical cord blood. These results suggest traumatic experiences in one generation can influence gene regulation in the next generation. Moreover, the identified associated genes have been examined in previous research that highlights the broad potential for altered gene activity to influence health and behavior. We noted that BDNF is involved in overall neurogenesis and synaptogenesis. However, BDNF expression along the mesolimbic pathway has also been suggested to exert influence on the variation in dopamine neuron vulnerability to specific toxins (Hung & Lee, 1996). Furthermore, altered DNA methylation of BDNF resulted in changes in BDNF expression in the hippocampus along with deficits in memory formation (Lubin, Roth, & Sweatt, 2008). Most importantly, previous fear research indicated that increased BDNF activity in the hippocampus connections to the infralimbic medial prefrontal cortex may ameliorate learned fear disorders (Peters, Dieppa-Perea, Melendez, & Quirk, 2010). HSPA5, known mostly for involvement in monitoring protein transport through cells, has been examined in health-related research. HSPA5 activity has been associated with Ebola Virus disease progression and has been noted as a potential consideration for

countermeasures of the disease (Shurtleff et al., 2014). HOXD4, involved in stimulating transcription of androgen receptors, has also been indicated in heat disease research with hypomethylation upstream of the gene revealed in tissues with advanced atherosclerotic plaques (Nazarenko et al., 2015). It is a potential interesting coincidence that men experience heart disease more than women, and the HOXD4 association was only present in male offspring.

The sex-specific nature of the relationships is consistent with previous research showing that early-life adversity generated different **epigenetic** patterns between the sexes (Doherty et al., 2016; Roth et al., 2014). Sex-specific timing of the re-methylation of imprinted genes has been suggested for experience-by- sex interactions when investigating environmental prenatal variables (Bale, 2015), as well as sex differences in the placenta (Bale, 2016). However, this study evaluated experiential variables that occurred prior to pregnancy. It is possible that mother's trauma and fear experiences altered her state in a fashion we did not observe, or measure, creating a different prenatal environment from those mothers without trauma history, which generated the gene regulation effects in the offspring.

Generational patterns of behavior have long been investigated in interest of creating change and inhibiting unhealthy cycles. The influence of fear-evoking experiences on development, behavior, and subsequent generations has been an area of intrigue, offering insight into potential pathways for generational mental health symptoms such as anxiety and depression. This is important for social workers who are, by trade, engaged in helping populations suffering chronic discrimination, oppression,

poverty, and violence. The findings discovered in this study may be replicated in other populations served by social workers, and broaden our understanding of mechanisms generating poor quality of life.

The potential for adverse experience to influence subsequent generations should be investigated further. Future research would benefit from controlling for potential inherited epigenetic tags by including both mother's and father's **DNA methylation** and expression. Inclusion of other prenatal variables that might be influenced by trauma exposure would help refine the amount of variance accounted for by the prenatal environment. Some variables to consider include the nutrient balance of the mother's diet, household income, and social connectedness. These variables can greatly influence the prenatal environment and can be linked to trauma via previous research identifying relationship dysfunction, poorer employment status and therefore lower income (Lund et al., 1984). Ultimately, this pursuit may provide new avenues for prevention and interventions for improved quality of life.

CHAPTER III

THE INDIRECT INFLUENCE OF TRAUMA ON MOTHERS' MENTAL HEALTH AND SUBSEQUENT GENE REGULATION IN OFFSPRING

This manuscript was written independently by the student, Stefanie Pilkay. The complete draft was submitted to each committee member for feedback and the student completed appropriate changes. The final draft was re-submitted to the committee for approval. This article hasn't been published anywhere, nor will it be before I turn in the final version of my ETD.

ABSTRACT

Trauma and early-life stress have increased in the research literature to better understand the pathways to poor quality of life. Genetic research expanded our understanding of this pathway when experience was found to influence gene regulation via epigenetic marks. This study sought to explore possible relationships between the mother's trauma history, experienced fear, subsequent mental health functioning and gene regulation patterns in her newborn. Results indicate that the influence trauma exerts on mental health functioning varies according to the type of trauma experienced and is completely mediated by the degree of fear induced. Furthermore, mothers' mental health functioning predicts differential gene expression on 245 genes in males only. The sex specific nature of this relationship suggests males are more vulnerable to mother's experience during pregnancy compared to females. Future research examining prenatal variables should consider potential sex differences.

INTRODUCTION

One study has estimated that the majority of people will experience some form of trauma within their lifetimes (Breslau, 2009; Kilpatrick et al., 2013; Resnick, Kilpatrick, Dansky, Saunders, & Best, 1993; Solomon & Davidson, 1997). Moreover, a large investigation by the CDC and Kaiser Permanente discovered that as the number of adverse events experienced in childhood (ACES) increases, so do the probabilities of developing physical and mental illnesses throughout life (Felitti et al., 1998). This includes consequences such as depression, substance abuse, sleep disturbances, and cancer (Felitti et al., 1998). Further research has identified more severe mental health consequences linked to childhood trauma including posttraumatic stress disorder (PTSD) and related symptoms (Anda et al., 2006; Cicchetti & Toth, 2005; Teicher et al., 2003). Perry (2009) outlined how ongoing neurodevelopment during childhood creates a biology with increased vulnerability to environmental insults, such as stress, maltreatment, and other adversities. However, some forms of trauma in adulthood, like war, also have been linked to mental health symptoms that endured for 20 years or more (Lund et al., 1984).

Regardless of childhood or adulthood exposure, trauma-informed interventions don't appear to provide the same benefit to everyone in need. In a review of the PTSD treatment literature, Lonergan (2014) noted that individuals with more severe symptoms, possibly indicative of a controversial syndrome known as *Complex PTSD* for the combination of PTSD symptoms and additional cognitive and emotional dysregulation difficulties, appeared to experience minimal benefit from the typical

treatments (Lonergan, 2014). This knowledge is vital to social work practitioners because marginalized populations served by the profession are more likely to have experienced trauma at some points in their lifetimes. Improving our understanding of trauma's path of influence will maximize our potential to improve quality of life for suffering populations.

Neuroscience research has illuminated multiple mechanisms in the brain that help us understand how trauma can generate mental health symptoms. Recent research has uncovered how our experience can influence our genes' activity through **epigenetic** (a glossary of neuroscience terms has been included in **Table 1,** and terms are printed in **bold** in the text) marks such as **DNA methylation** (Bale, 2015). The term *epigenetic* refers to a process whereby chemical tags attached to DNA influence gene activity without changing the DNA sequence itself (Bale, 2015). **DNA methylation** is one such epigenetic mark that is the presence of **methyl groups** on the DNA strand along different points of the genes (Bagot et al., 2014).

DNA methylation is sensitive to our experiences and can appear immediately (Naumova et al., 2012), much later (Essex et al., 2013), endure a lifetime (Bagot et al., 2014), or change in response to intervention along with improved mental health (Yehuda et al., 2013). **DNA methylation** can increase or decrease gene activity (Bagot et al., 2014), affecting the proteins produced by the genes and thus the "directions" provided to cells. Changes in gene activity are theorized to explain associations between differential **DNA methylation** and early-life trauma, PTSD (Klengel et al., 2014; McGowan et al., 2009; Mehta et al., 2013; Oberlander et al., 2008; Ressler et al.,

2011; Smith et al., 2011), and mental health symptom severity (Martín-Blanco et al., 2014; Yehuda et al., 2013). One potential pathway that helps explain these connections was discovered in animal models that linked **DNA methylation** to fear experiences and identified how these **epigenetic** marks reinforced fear cues resistant to change in the **hippocampus** and **amygdala** (Zovkic & Sweatt, 2012).

Methylation of genes involved in brain development (*BDNF*), emotion (*SLC6A4*), glucocorticoid receptors (*NR3C1, FKBP5*, *STAT5B*), and other stress-response genes can potentially alter individuals' coping abilities and PTSD risk (Mill & Heijmans, 2013), subsequently affecting behaviors related to emotion regulation, cognition, and stress reactivity. It is important to note that although most of the demonstrated associations have occurred in the individuals who experience the trauma, mothers' state during pregnancy also has been connected to **epigenetic** patterns in offspring that could affect development (Babenko, Kovalchuk, & Metz, 2015; Kapoor et al., 2009; Lemaire et al., 2000).

This route of inquiry can help us understand how interactions between our genes and experience influence behavior and ultimately life quality. Since social work interventions are environmental manipulations, including therapy and social policy, social work practice can generate changes on a molecular level in the brains of clients to help alter unhealthy behaviors and symptoms. Seeking more detail about the mechanisms that allow trauma to exert harmful consequences will increase opportunities for social work to intervene. Moreover, some social work researchers promote the inclusion of **epigenetics** in social work education and investigation

(Combs-Orme, 2013, 2017). Therefore, this study addressed the questions: 1) Do mothers' mental health symptoms during pregnancy influence **DNA methylation** and gene expression in offspring? 2) do mother's mental health symptoms during pregnancy influence DNA methylation and gene expression in offspring differently for males and females? 3) do mothers' trauma histories predict mental health functioning directly? 4) do mother's trauma histories predict mental health functioning indirectly through mother's experienced fear? 5) does mother's fear moderate a relationship between trauma and mental health?

METHODS

This study employed data from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood study (CANDLE), and the University Institutional Review Board approved all measures and procedures. The cohort (*N* = 1503) consists of healthy mothers aged 18-40 years solicited in prenatal settings in Shelby County, Tennessee. This investigation used a subsample (216 mother/infant pairs) of the cohort who volunteered to provide umbilical cord blood samples immediately after birth for biological measures including **DNA methylation** and expression. Multiple studies have used umbilical cord blood to examine **DNA methylation** and expression (Adkins, Krushkal, et al., 2011; Adkins, Thomas, et al., 2011; Adkins et al., 2012; Krushkal et al., 2014).

Discrepancies have been found between associations identified with **genome-wide** versus **candidate gene** analyses. **Candidate gene** studies investigate specific genes based upon previous research or theory, whereas **genome-wide** studies include

all available genes in the analyses. One type of discrepancy is that genes found to be statistically significant in **candidate gene** analysis have not shown the strongest relationships compared to other statistically significant genes, if significant at all, in **genome-wide** analysis. This could be the result of a reduction in statistical power due to the reduced alpha for multiple comparison control, or it is possible that other genes responsible for more rudimentary functions may interact to play unknown roles in behavior. Therefore, this study included both **candidate gene** and **genome-wide** analyses to examine how mothers' mental health symptoms during pregnancy influence **DNA methylation** and expression in the newborns' cord blood. Furthermore, the potential indirect influence of trauma on mothers' mental health was assessed by analyzing mediation by mothers' experienced fear, as well as an interaction between fear and trauma on mental health.

Description of variables

Detailed variable information is provided in Table 2. The Traumatic Life Events Questionnaire is a self-report measure with 23 yes/no items addressing 22 different types of trauma such as childhood maltreatment, war, and domestic assault (Kubany, 2000). It has shown good short-term stability of responses to items among several different populations, and demonstrated good content validity and discriminant validity (Kubany et al., 2000). We included timing, type, and complexity of trauma as different trauma dimensions to assess potential influences on mental health. For each item respondents are asked to indicate if the event occurred (0 = no, 1 = yes) and if the event resulted in "intense fear, helplessness, or horror" in response to the event (0 = no,

1 = yes). Respondents are asked at what age did they experience trauma (n = 1). All ages noted prior to eighteen were considered childhood exposure, and all others as adulthood exposure. Type of trauma was computed with all trauma events involving another person deemed “interpersonal” (n = 12) and all other traumas considered “non-interpersonal” (n = 10). The presence or absence of fear was identified for each event (0 = no, 1 = yes), and summed to create the cumulative fear score with higher values indicating more fear (0-22). The fear summary score was evaluated within the TLEQ for psychometric properties and determined to be an adequate fear measure (Johnson et al., 2010; Kubany et al., 2000). Complexity of trauma was constructed to assess the exposure to multiple types of trauma, such as physical or sexual abuse, during childhood by summing the total number of different types of childhood abuse exposure similar to an ACE (Felitti et al., 1998) score (no childhood abuse = 0, one type of childhood abuse = 1, two types of childhood abuse = 2). Childhood trauma was also dichotomized (no child abuse = 0, yes child abuse = 1).

The global severity index summary score from the Brief Symptom Inventory (Derogatis & Melisaratos, 1983) was used to assess mothers’ mental health status. The Brief Symptom Inventory is a shortened version of the SCL-90 mental health assessment, has been found to be sensitive to psychopathology and psychological distress (Sitarenios, Kovacs, & Maruish, 1999). The global severity index score has been investigated and found to be a more accurate assessment of overall mental health functioning than the positive symptom total score in the BSI measure (Derogatis & Melisaratos, 1983). A clinically significant cutoff score was established based on

normative population data (adults and adolescents) to determine if individuals needed additional mental health screening (score ≥ 63; (Derogatis, 1993).

DNA methylation from cord blood is widely accepted as an appropriate measure to assess associations in offspring (Boeke et al., 2012; Broberg et al., 2014; Herbstman et al. 2012; Hoyo et al., 2012; Kile et al., 2012; Koestler et al., 2013; Nomura et al., 2013; Relton et al., 2012). Cord blood, which is representative of the newborn's physiology and used to assess health in the newborn, was processed according to assay manufacturer's instructions with EZ **DNA methylation** reagents (Zymo Research, Orange CA, USA) to conduct **bisulfite conversion**. Processed blood samples were hybridized and scanned on the HumanMethylation27 **bead chip** in batches of 24. The process produced 27,578 **CpG sites** (potential methylated gene locations) for **DNA methylation** analysis. Gene expression was measured with the Illumina Human WG-6 expression array to analyze 14,495 genes available in the newborn's cord blood. These procedures are standard in genetics research.

Analyses

COMBAT is an R package that was used to adjust the **DNA methylation** data set for **chip** and **batch effects** (measurement error). **DNA methylation** beta values and gene expression probes were regressed on mothers' (*n* = 216) mental health symptoms (GSI) in the software MethLAB (Kilaru et al., 2012). We controlled for child sex, child race (African American/other minority, Caucasian), and **cell type**. As is standard in genetics research, we implemented the 95% confidence False Discovery Rate control for multiple comparisons (Storey, 2010).

This study assessed main effects of mothers' mental health symptoms during pregnancy on **DNA methylation** and expression in offspring. Furthermore, direct and indirect influence of trauma on mothers' mental health was examined. Mothers' mental health (GSI) was regressed on mothers' type, timing, and complexity of trauma. Additionally, mothers' cumulative experienced fear was assessed for moderation and mediation of association between trauma and mental health. An interaction variable for each of the trauma dimensions with fear was created and input in regression analyses with mothers' mental health to analyze moderation. Lastly, mothers' fear was assessed for mediation in Process, an SPSS package (Hayes, 2012). Figure 7 is a path diagram of the analyses conducted.

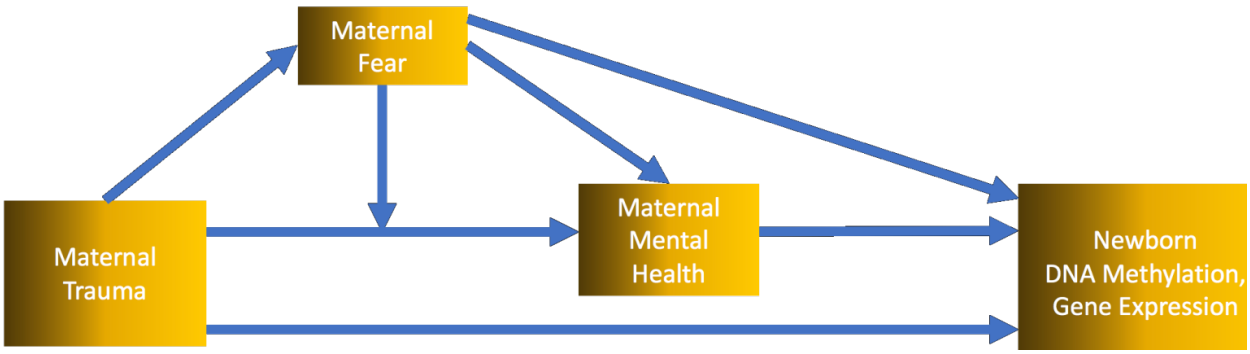


Figure 7. Analyses path diagram

Candidate gene analyses were conducted on genes identified previously in both animal and human replicated trauma research, replicated in other studies, and with suggested potential to affect development and behavior. Three genes are involved in glucocorticoid receptor production and function and thus response to stress (*NR3C1, FKBP5*, and *STAT5B*). One gene is important for neurogenesis and synaptogenesis

(*BDNF*), that is the creation and connection of brain cells. The last gene is involved in serotonin transport activity and thus can influence all things linked to serotonin such as mood, appetite, and sleep (*SLC6A4*). **Genome-wide** and **candidate gene** analyses were completed for the **DNA methylation** and gene expression data.

As is customary, we conducted quality control procedures on the gene expression data for cleaning. Identified p-values higher than .01 were set to the equivalent of not detected (NAs). Samples with fewer than 10% of detected probes were removed (134 samples remained). Probes with fewer than 10% of detected samples within each dataset were removed (12,388 probes remained). We completed quantile normalization, added 40 to all cells to adjust negative values, and conducted log2 transformation.

RESULTS

The sample is mostly African American mothers (African American 55%, Caucasian 45%) of similar age (M = 25.04, SD = 5.15 vs. M = 28.56, SD = 4.61). **DNA methylation** data were available for most of the newborns (n = 196, Male = 96, Female = 100). Gene expression data are more fragile and prone to degrade and cause data loss, yet almost half of the gene expression data remained after processing and data cleaning (n = 100, Male = 55, Female = 45).

The trauma variables were assessed for degree of correlation given their natural relatedness. Trauma complexity did not significantly correlate with trauma type (r = .13, p = .087, bootstrap CI [-.006, .238]. However, complexity of trauma did exhibit a strong negative correlation with timing of trauma (r = -.59, p < .001, bootstrap CI [-.713, -.438],

and an expected high positive correlation with the dichotomized childhood abuse variable (r = .93, p < .001, bootstrap CI [.925, .949].

Genome-wide analyses revealed mothers' mental health predicts **DNA methylation** in offspring on a gene involved in the methylation process itself (cg23855392, *MTHFS*). Specifically, as mothers' clinical severity of mental health symptoms during pregnancy increases so does the degree of methylation on *MTHFS* as seen in Figure 8 (*M* = 50.52, *SD* = 8.58, FDR *p* < .05) *B* = .002, bootstrap 95% CI (.001, .003).

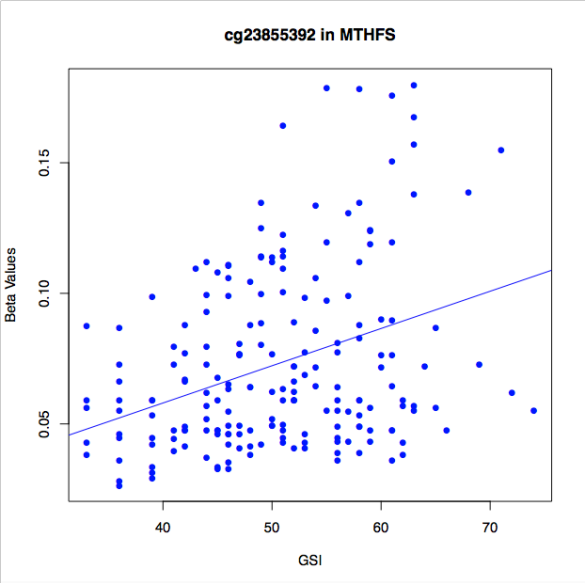


Figure 8. Scatterplot GSI

A sex-specific difference emerged with males exhibiting greater methylation as mothers' mental health severity increases, on an additional gene previously associated with neurodegeneration syndrome (cg06255524, *DNAJC3*) as seen in Figure 9 (*M* = 49.81, *SD* = 8.51, FDR *p* < .05) *B* = .0003, bootstrap 95% CI (.0002, .0004).

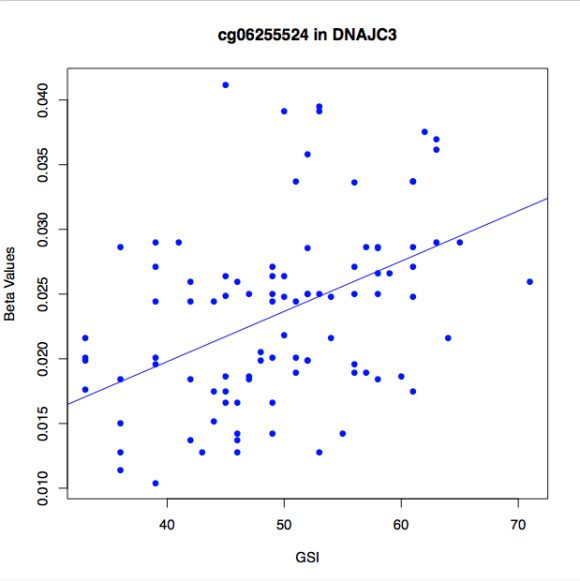


Figure 9. Scatterplot GSI males

The sex-specific trend emerged in the gene expression data also. Males exhibited statistically significant differential gene expression (some increased, some decreased) related to mothers' mental health during pregnancy on 245 **gene probes** (see Table 2).

The GSI has a clinical cutoff of ≥ 63 so we restructured GSI into a dichotomous variable (not-clinically significant = 0, clinically significant = 1). Offspring with mothers who were experiencing clinically significant mental health symptoms during pregnancy showed reduced gene expression (see Figure 10) of a gene involved in metabolism (*DGUOK*) that has been linked to **mitochondrial** disease and has a related pathway with metabolism (*M* = 1.06, *SD* = .24, FDR *p* = *.031) B* = -.668, bootstrap 95% CI (-1.413, -.119). **Candidate gene** analysis showed a positive association between mothers' clinical severity of mental health and methylation on a glucocorticoid receptor gene (cg08818984, *NR3C1*) as seen in Figure 11 (*M* = 50.52, *SD* = 8.58, FDR *p* < .05) *B* = .0002, bootstrap 95% CI (.00002, .001).

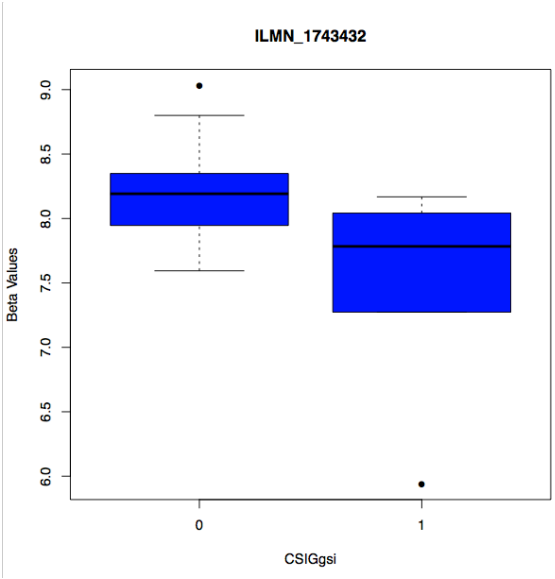


Figure 10. Boxplot Clinical Cutoff GSI gene expression

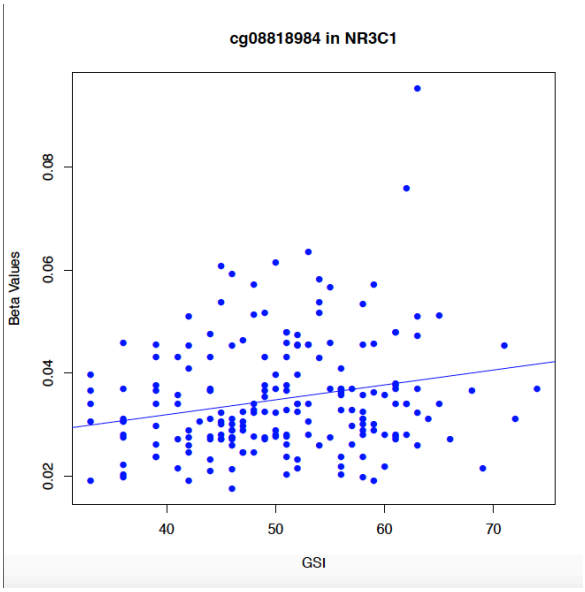


Figure 11. Scatterplot GSI candidate gene

The same relationship held for males only as seen in Figure 12 (*M* = 49.81, *SD* = 8.51, FDR $p < .05$) *B* = .0004, bootstrap 95% CI (.00002, .001), and dropped when examining females only.

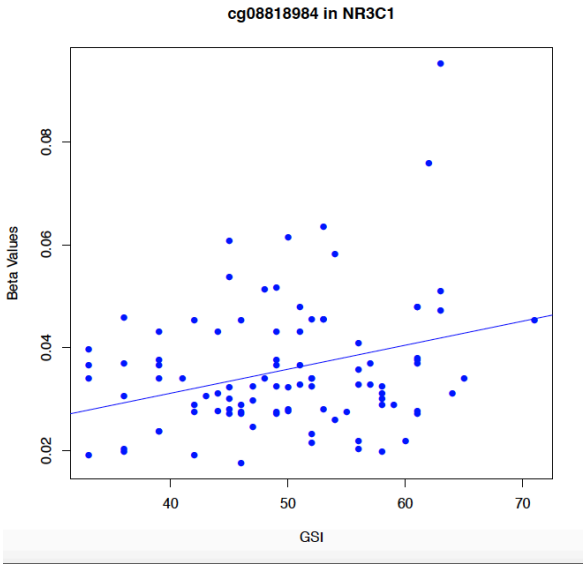


Figure 12. Scatterplot GSI candidate gene males

Exploration of the indirect effects of trauma on mothers' mental health showed no statistical significance for moderation by fear. However, a complete mediated relationship emerged. Figure 13 depicts the influence of mothers' trauma complexity on mothers' experienced fear and thereby subsequent mental health.

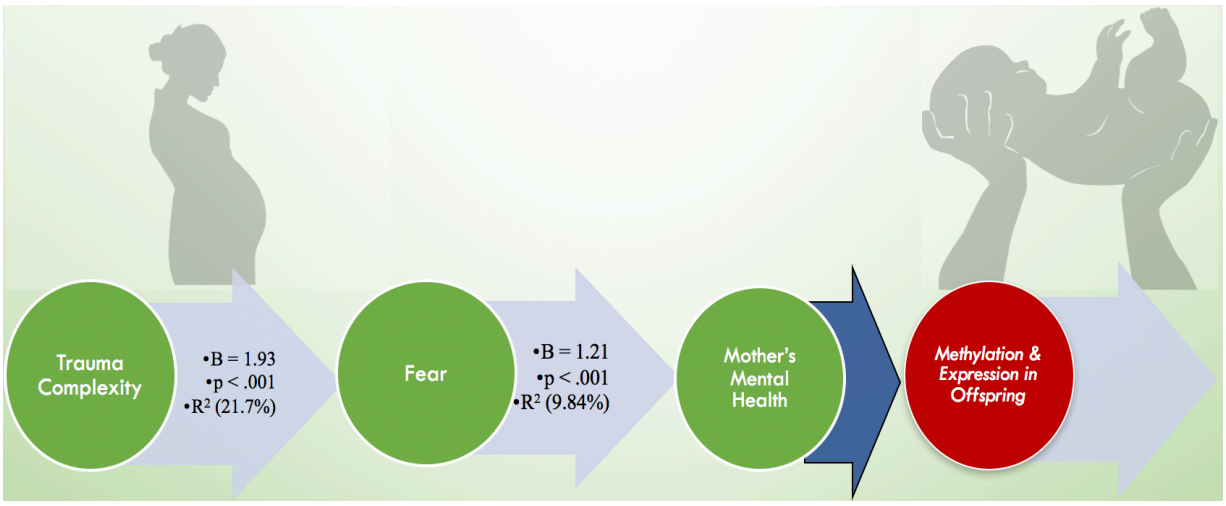


Figure 13. Trauma and mental health mediation model

Trauma complexity accounted for 21.7% of variance in mothers' experienced fear (B = 1.93, p < .001), and mothers' fear accounted for 10% of the variance in mothers' mental health symptoms during pregnancy.

LIMITATIONS

This study sampled one urban population in west Tennessee and is not necessarily generalizable to other populations without replication in more representative samples. **Genome-wide** analysis is an excellent tool to discover unknown/unsuspected relationships but it requires many statistical tests when run as individual regression analyses. Investigating the gene sites and probes individually is currently the best way to extract the most detailed information about how the **epigenetic** mechanisms may be working with the variables of interest. However, the increased number of analyses escalates probability of committing type I errors. As is typical with genetics studies, this study conducted multiple comparison control with the False Discovery Rate to address

this limitation, and calculated bootstrap 95% confidence intervals to indicate the precision of estimated relationships, yet it is important to note this risk for error.

DISCUSSION

This study revealed that mothers' mental health symptom severity during pregnancy is positively associated with **DNA methylation** of *MTHFS, DNAJC3* (males only), and *NR3C1* in newborns' cord blood. The genes involved in DNA methylation (*MTHFS*) and linked to a neurodegenerative syndrome (*DNAJC3*) have not been identified in previous trauma research. However, the gene involved in glucocorticoid receptors (*NR3C1*) has been acknowledged in previous research as predictive of treatment outcome (Yehuda et al., 2013), and linked to cortisol resistance (Tyrka et al., 2012), suggesting *NR3C1* methylation may be indicative of risk for trauma-related mental health consequences. Mothers' mental health symptom severity during pregnancy is also positively associated with expression of 88 genes and negatively associated with an additional 157 genes in male newborns only. Furthermore, male and female newborns showed reduced expression of *DGUOK* as mothers' mental health severity increased. The gene involved in metabolism (DGUOK) has not been noted in prior trauma research, but the ACE study showed a positive association between the number of types of traumas experienced in childhood and the development of obesity (Felitti et al., 1998). These results support previous research suggesting the in-uterine environment is manipulated by mothers' mental health and is associated with male newborn differences in **DNA methylation** that was also linked to infant stress-coping behavior that could predispose individuals to risk or resilience (Kapoor et al., 2009;

Lemaire et al., 2000; Mueller & Bale, 2008). Furthermore, the results suggest a new avenue of investigation for potential adversity variables to consider as predictors in Adverse Childhood Experiences (ACEs) research (Felitti et al., 1998). ACEs generally examine the number of adverse events as predictive of the outcome of interest. However, these findings suggest changes can occur with fewer events according to the magnitude of fear experienced. Furthermore, those changes have potential to create beneficial or derogatory health changes that could progress over time. Including DNA methylation and gene expression variables in trauma research will help us discern more points of change that will provide increased opportunities to develop interventions and preventions for better life quality.

Sex-specific epigenetic associations have been identified in other research (Doherty et al., 2016; Roth et al., 2014), and could be due to timing differences of re-methylation processes between sexes (Bale, 2015). Moreover, sex-specific differences in risk and resilience to affective disorders such as anxiety, bipolar disorder, depression, and PTSD have been found in adult populations (Altemus, Sarvaiya, & Epperson, 2014). Continuing exploration into **DNA methylation** and gene expression patterns associated with prenatal variables has potential to inform social work practice with innovative avenues for mental illness prevention. For example, prenatal screenings could include assessment for variables that increase risk for mental illness in offspring. Therefore, identified environmental interventions, such as diet or stress reduction, could be applied to foster resilience in the fetus. Ultimately, understanding more about the path of influence within and across generations to develop poor quality of life will help

social workers identify earlier signs of risk and develop interventions for earlier implementation. These study findings suggest the links are due to prenatal exposure, however we were not able to compare **DNA methylation** patterns in offspring to methylation in the mothers' DNA. As a result, we were not able to rule out any potential heritability of gene regulation differences that might be related to mothers' mental health functioning.

The inter-generational links demonstrated in this study offer a new investigative path. Social work practitioners often work with generational cycles of behavior in populations that suffer poverty, violence, and oppression. Future research may enlighten our understanding of potential mechanisms involved in these cycles, and thereby provide new opportunities to intervene for improved quality of life. This avenue of research could bolster arguments for the ongoing need for universal prenatal care, good affordable nutrition, and other services to support mothers and families. **Epigenetic** marks are sensitive to the environment and therefore interventions should address the mothers' environment and the offspring's environment. This suggests that social problems such as poverty, community violence, discrimination, and oppression have potential to exert significant influence on human development, across generations, in addition to the quality of the mother-child attachment. Therefore, interventions will benefit from environmentally-sensitive research, like **epigenetic** studies, that will help illuminate paths to risk and resilience. For example, research has identified gene variations that increase the risk for PTSD (Binder et al., 2008), as well as resilience to developing PTSD (Koenen et al., 2011). Knowledge like this could one day be used in

screening measures to aid intervention and prevention strategies and in the design and targeting of services to those who are most in need.

Mothers' mental health functioning during pregnancy should be investigated further. Future research should control for inheritance by including **DNA methylation** and gene expression from both mothers and fathers. Inclusion of the subscales from the Brief Symptom Inventory would provide a closer look at the differences between the Global Severity Index and the Positive Symptom Total. It is possible that some mental health symptoms exert greater influence than others, and the subscales would facilitate that examination. Furthermore, mental health can influence other potential covariates such as nutrition, income, and social support which could each affect the prenatal environment.

A final consideration is inclusion of epigenetic research within the social work context (teaching, practice, and research). Epigenetics is a relatively new science and we are learning in leaps and bounds, but some have expressed doubt and concern that it should be included in social work so soon (White & Wastell, 2016). The task is not to determine if we should or should not journey into the realm of epigenetics. Rather, our task is to identify the most responsible actions to explore epigenetics further. This research will continue and the populations we serve are of unique interest to epigenetic researchers. Therefore, it is imperative that a social work perspective is included.

CONCLUSION

Social Workers are ascribed profession challenges that include to advance long and productive lives, as well as ensuring healthy development for all children (AASWSW, 2017). The findings of these studies outline the relevance of epigenetic inquiry and consideration to these social work practice goals. In our examination, we found mothers' exposure to childhood trauma and interpersonal trauma both predict gene regulation patterns in the newborn. Furthermore, results indicated the mothers' mental health functioning during pregnancy appeared to exert greater influence on gene regulation in newborn males compared to females. It is likely that social challenges embedded in social work, like poverty and discrimination, also have potential to influence epigenetic marks since DNA methylation is highly sensitive to experience and the environment (Bale, 2015). Moreover, DNA methylation can alter gene activity (Bale, 2015), and thus potentially all subsequent dependent systems. The possible influence is theorized to explain the links between childhood adversity, gene regulation patterns, and mental health functioning (Murgatroyd, Patchev, et al., 2010; Prados et al., 2015). Furthermore, examination of this theorized pathway has revealed associations between psychotherapy interventions, DNA methylation, and improved mental health (Yehuda et al., 2013). Therefore, Social Work interventions have potential to influence the epigenetic mechanisms and thus probable behavior and health outcomes. Health outcomes are of great interest recently given the findings from the Adverse Childhood Experiences study conducted by Kaiser Permanente and the CDC (Felitti et al., 1998). Results from >17,000 insured mostly Caucasian adults indicated that individuals who

experienced four or more adverse childhood experiences showed increased risk for multiple poor outcomes such as heart disease, cancer, lung disease, obesity, and diabetes (Felitti et al., 1998).

This series of research identified a need to further investigate the potential for trauma or early-life stress experience to influence gene regulation across generations. Although we implemented a multiple comparison control, the large number of analyses conducted in the genome-wide approach still increases the potential to commit a type I error. Research replication is the greatest defense against spurious findings, and the relationships identified within these studies need to be replicated in a comparable cohort.

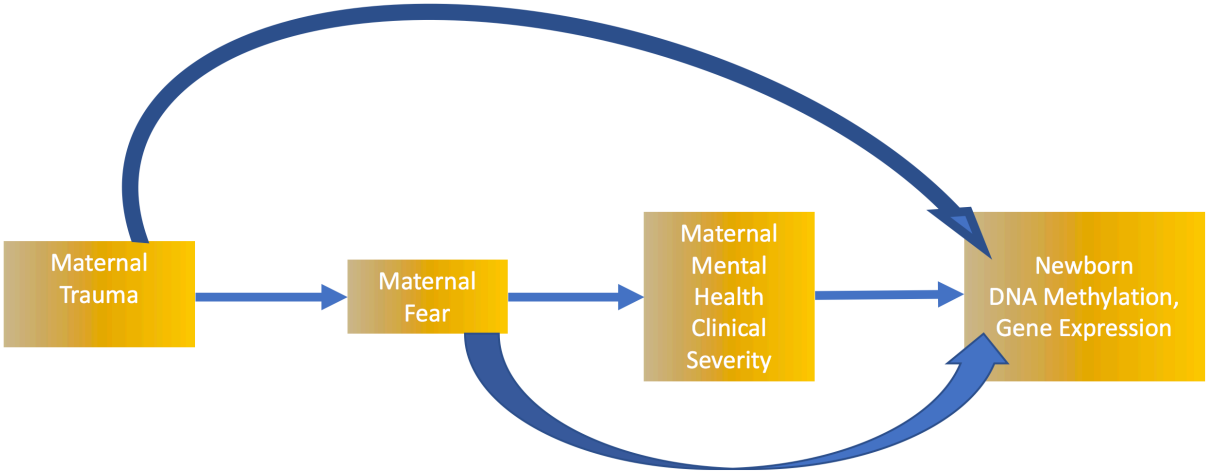


Figure 14. Results path diagram

Our inquiry discovered a link between mother's experience of interpersonal trauma and increased DNA methylation on a gene that regulates androgen receptor expression (*HOXD4*) in male offspring. If the mother experienced child abuse, female offspring revealed decreased expression of *HSPA5* (monitoring protein transport) and male offspring showed increased expression of *BDNF* (brain development). A sex difference (male/female) is not a complete surprise given previous research that revealed maternal behavior-related sex differences in DNA methylation of NR3C1 in rodent pups according to litter sex composition (Kosten et al., 2014), and female rats prenatally-stressed (PNS) showing differential BDNF expression compared to controls and PNS male rats (Luoni et al., 2015). However, sex differences in several of the analyses call for further consideration of how experience can exert varying influence across generations between the sexes.

The gene involved in neurogenesis and synaptogenesis (*BDNF*) was also confirmed to be associated with the mother's experience of fear. Trauma has long been defined as a fear evoking event (Herman, 1992), and the results support the significance of experiencing fear versus a specific type of event. These findings suggest a need to deepen our trauma investigations by including a fear measure in future research. It is highly probable that without measuring fear, trauma research is suffering type II errors from incomplete observations of the true experience of trauma. Moreover, alterations in *BDNF* gene activity could greatly affect brain development and the growth of neurons throughout the lifespan. Alterations that increase *BDNF* activity are more likely to manifest in a protective nature while facing adversity. However, reductions in

BDNF activity could result in detrimental effects on localized or overall structural and functional brain development with significant potential to influence behavior and physical and mental health.

The systematic literature review identified stress reactivity, emotionality, and brain development as the subjects of large clusters of research studies. Previous findings noted the glucocorticoid gene (*NR3C1*) is associated with exposure to early-life stress or trauma (Tyrka et al., 2015; Tyrka et al., 2012). We outlined in the third paper how poor maternal mental health can predict similar gene regulation patterns in the subsequent generation on the day of birth. Poor maternal mental health functioning, such as depression, has been proposed as a prenatal stressor (Field, Diego, & Hernandez-Reif, 2006), and our study findings suggest that may be true. This study furthers the research on trauma-related gene regulation by examining associations across generations and considering the influence of fear and maternal mental health within this context. The results indicate need for ongoing investigation of the conceivable influence of maternal experience and mental health functioning on gene regulation in offspring. Advancing our understanding of possible gene-environment mechanisms from parent to child will increase our potential to intervene for improved quality of life.

It is possible that social work could one day incorporate epigenetic analysis into practice evaluation to improve clients' life quality. For example, current interventions could be assessed with pre- and post-treatment measures to identify epigenetic change associated with improved physical or mental health, as well as potential predictors of

poorer treatment outcomes. Understanding more about how intervention affects individuals' biology may provide greater insight into new avenues for intervention and prevention. If findings revealed changes to genes involved in stress reactivity, stress reduction techniques could be assessed to determine if improved health could be achieved without traditional mental health treatment requiring insurance or payment. The more options available to social workers to help their clients, the greater potential of assisting populations with diverse needs achieve desired change. Alternately, epigenetic patterns on genes identified as predictors of treatment outcome, could be a new path of intervention investigation. Researchers could assess experiences, such as early life adversity, to determine if correlates exist with treatment outcome predictors. The possible correlations are unimaginable for the conceivable practice relevance.

The potential to influence social work practice is tempered by obstacles. The largest obstacle is knowledge. This area of research is relatively new to social work and is not universally incorporated into courses with enough detail to help students make the connections among experience, epigenetic tags, and behavior. The limited understanding results in minimal social work research with these related variables. Furthermore, it is expensive to include DNA methylation or gene expression variables in research. Commercial agencies will process the tissues and generate your data at a significant cost that can limit the affordable number of subjects to a group so small you have little to no statistical power. There are some university labs that will process the tissues for much less, but the measurement tools are still expensive with some DNA methylation bead chip kits exceeding $7,000 for 24 samples. The financial burden is

often the largest contributing factor to conducting analysis of a few selected genes (candidate gene). However, finding enough willing participants to meet the ideal sample size (n > 1000) for analysis of all measured expressed genes (genome-wide) has also proven difficult when resource is available. Therefore, including both statistical approaches and acknowledging the limitations of each is the best way to maximize knowledge gains in this area of study.

All change takes time and effort to overcome obstacles. Advancing epigenetic research in social work will take time and effort, but there will never be a better time to begin than now. Epigenetic research is ongoing in multiple disciplines, and the populations served by social workers are often included in these studies. It is time for the social work perspective to be included and considered.

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APPENDIX

Appendix A

Table 1. Glucocorticoid Receptor Genes Identified in Systematic Review

Gene	Author(s)	Approach	Dependent Variable	Results
NR3C1	Yehuda et al., 2013	Candidate	Methylation, expression	(1 relationship for NR3C1) Psychotherapy reduced methylation and increased expression on FKBP5 for responsive patients. NR3C1 predicted treatment outcome and did not change following psychotherapy.
	Tyrka et al., 2015	Candidate	Methylation	(3 relationships) Mixed ancestry and sex showed positive association with child maltreatment and methylation that positively associated with individual stress
	Tyrka et al., 2012	Candidate	Methylation	(3 relationships) Maltreatment and parental loss linked to increased methylation associated with reduced cortisol response
	Romens, McDonald, Svaren, & Pollak, 2015	Candidate	Methylation	(1 relationship) Abused adolescents showed increased methylation
	Perroud et al., 2011	candidate	Methylation, expression	(2 relationships) Childhood abuse predicted increased methylation that correlated with reduced expression
	McGowan et al., 2009	Candidate	Methylation, expression	(2 relationships) Increased methylation correlated with reduced expression in hippocampus linked to childhood maltreatment
	Martín-Blanco et al., 2014	candidate	methylation	(2 relationships) Women with borderline personality disorder showed positive association between childhood physical abuse and methylation that positively correlated with mental health clinical severity
	Heinrich et al., 2015	Candidate	Methylation	(1 relationship) Those with externalizing disorders showed reduced methylation on the gene compared those with depression and controls.
	Essex et al., 2013	Genome-wide & candidate	Methylation	(2 relationships) Maternal stress during infancy and paternal stress during preschool years predicted differential methylation in adolescence
	Appleton et al., 2015	Candidate	Methylation	(3 relationships) Infants with increased methylation on both genes showed higher habituation scores after birth.
	Kosten et al., 2014*	Candidate	Methylation	(2 relationships) Sex X anxiety predicted methylation on NR3C1, litter gender composition moderated sex influence on EGR1. No findings for BDNF.

Table 1 Continued. Glucocorticoid Receptor Genes Identified in Systematic Review

Gene	Author(s)	Approach	Dependent Variable	Results
FKBP5	Yehuda et al., 2013	Candidate	Methylation, expression	(2 relationships for FKBP5) Psychotherapy reduced methylation and increased expression on FKBP5 for responsive patients. NR3C1 predicted treatment outcome and did not change following psychotherapy.
	Klengel et al., 2013	Candidate	Methylation, expression	(2 relationships) Mostly African American sample, child maltreatment linked to reduced methylation and increased expression associated with dysregulation in the stress response suggesting a dampening effect of HPA-axis negative feedback
	Binder et al., 2008	Candidate	Methylation	(2 relationships) Mostly African American sample, childhood maltreatment linked to reduced methylation that correlated with increased risk for PTSD
	Binder et al., 2004	Candidate	Expression	(1 relationship) Greater expression of gene related to two-fold increase in depression events
Whole genome	Logue et al., 2015	Genome-wide	Expression	(1 relationship) PTSD subjects showed reduced expression in genes involved in glucocorticoid signaling compared to controls.
GRa, GRb	Gola et al., 2014	candidate	Expression	(1 relationship) Trauma burden associated with reduced expression of GRa rather than specific to PTSD.
GR genes	de Rooij et al., 2012	Candidate	Methylation	(3 relationships) Lower stress reaction correlated with reduced methylation on GR genes, but education level and lifestyle accounted for some variance.
GR genes	Weaver et al., 2004*	Candidate	Methylation	(1 relationship) Nurturing could reverse methylation linked to poor caregiving
GR expression	Ridder et al., 2005*	None	Expression	(2 relationships) Reduced expression of GR in rodents predicted stress sensitivity and depressive behavior
CRF, GR	Mueller & Bale, 2008*	Candidate	Methylation, expression	(4 relationships) Mice exposed to prenatal stress showed altered stress responsivity, anhedonia, and differential methylation on the genes that correlated with expression.
No genes	Boyle et al., 2005*	None	Expression	(1 relationship) Reduced expression of GR in rodents predicted depression symptoms

*Animal studies

Appendix B

Table 2. Serotonin Transporter Genes Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
5-HTT/ SLC6A4	Vijayendran, Beach, Plume, Brody, & Philibert, 2012	Candidate	Methylation, expression	(3 relationships) Childhood abuse associated with differential methylation on one CpG site, whereas genotype and childhood abuse interacted to influence differential methylation on another CpG site. Two additional CpG locations were associated with genotype.
	Wankerl et al., 2014	Candidate	Expression	(2 relationships) Mother's stress during pregnancy OR childhood abuse associated with reduced expression. Gene variant 5-HTTLPR S showed greater reduction in expression depending on predictor variable
	Kinnally et al., 2011*	Candidate	Methylation	(2 relationships) Early-life stress related stress reactivity predicted increased methylation on 5HTT and overall global methylation
	van der Doelen et al., 2015*	Candidate	Methylation, expression	(2 relationships) Specific gene variant of serotonin gene interacts with early-life stress to predict methylation on CRF that correlated with expression
	Alasaari et al., 2012	Candidate	Methylation	(2 relationships) Work stress and burnout equally contributed to reduced methylation
	Babenko, Kovalchuk, & Metz, 2015	Candidate	Methylation	(1 relationship) Monozygotic twins showed increased methylation linked to blunted cortisol response during stress activation
	Beach et al., 2011	Candidate	Methylation	(1 relationship) Sample of women with child sex abuse history, increased methylation in region surrounding gene positively associated with antisocial personality disorder symptoms
	Booij et al., 2015	Candidate	Methylation, expression	(2 relationships) Higher methylation on the gene correlated with smaller hippocampal volume. A positive correlation emerged between amount of abuse and methylation on the gene.
	Duman & Canli, 2015	Candidate	Methylation	(2 relationships) Early and recent life stress showed increased global methylation for specific genotype (5-HTTLPR)
	Essex et al., 2013	Genome-wide & candidate	Methylation	(2 relationships) Maternal stress during infancy and paternal stress during preschool years predicted differential methylation in adolescence
	Kang, Kim, Stewart, et al., 2013	Candidate	Methylation	(2 relationships) Decreased methylation predicted poorer quality of life and greater disability

Table 2 Continued. Serotonin Transporter Genes Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
5-HTT/ SLC6A4	Khulan et al., 2014	Candidate	Methylation	(1 relationship) Men with depression histories showed depression linked to differential methylation, not early-life stress
	Koenen et al., 2011	Candidate	Methylation	(1 relationship) Positive correlation between methylation and resilence to PTSD
	Miller, Wankerl, Stalder, Kirschbaum, & Alexander, 2013	Meta-analysis	Methylation, expression	(1 relationship) Reduced SLC6A4 activity linked to increased stress reactivity
	Ouellet-Morin et al., 2013	Candidate	Methylation	(1 relationship) Monozygotic twins showed increased methylation linked to blunted cortisol response during stress activation
	Perroud et al., 2015	Candidate ($5\text{-}HT_{3A}R$)	Methylation	(2 relationships) Childhood abuse linked to increased methylation and greater severity of symptoms with specific gene variant
	Philibert et al., 2008	Candidate	Methylation	Depression did not reach statistical significance as a predictor
	Provenzi et al., 2015	Candidate	Methylation	(1 relationship) Very preterm infants exposed to high levels of pain showed an increase in methylation on the gene past discharge compared to very preterm infants without high pain exposure and full term infants.

*Animal studies

Appendix C

Table 3. Brain Development Genes Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
BDNF	Ieraci, Mallei, Musazzi, & Popoli, 2015*	Candidate	Expression	(2 relationships) Stressed mice showed exercise protected against stress and increased the expression of BDNF
	Kosten et al., 2014*	Candidate	Methylation	Sex X anxiety predicted methylation on NR3C1, litter gender composition moderated sex influence on EGR1. No findings for BDNF.
	Luoni et al., 2015*	Candidate	Expression	(1 relationship) Prenatally stressed female rats did not experience the increased expression of gene in prefrontal cortex as controls following an acute stress event
	Rodrigues et al., 2015*	Genome-wide & candidate	Methylation, expression	(3 relationships) Sedentary rats exposed to acute restraint stress showed lower global DNA methylation in hippocampus and cortex, as well as increased BDNF expression in periaqueductal gray region.
	Roth, Lubin, Funk, & Sweatt, 2009*	Candidate	Methylation, expression	(2 relationships) Maltreated rats showed increased methylation on gene resulting in altered gene expression in the prefrontal cortex
	Roth, Zoladz, Sweatt, & Diamond, 2011*	Candidate	Methylation, expression	(2 relationships) Rats chronically exposed to cats showed increased methylation and reduced expression in two regions of the hippocampus (dorsal dentate gyrus and dorsal CA1).
	Roth et al., 2014*	Candidate	Methylation	(1 relationship) Caregiving predicts differential methylation
	Tsankova et al., 2006*	Candidate	Methylation	Mice that experienced chronic social defeat showed no differential methylation on gene in whole hippocampus.
	D'Addario et al., 2012	Candidate	Methylation, expression	(2 relationships) Bipolar type II showed greater methylation on promoter region and reduced expression compared to bipolar type II and controls.
	Fuchikami et al., 2011	Candidate	Methylation	(1 relationship) Hierarchical clustering analysis identified differential methylation on CpG I predicts clinical diagnosis of depression.
	Keller et al., 2010	Candidate and global	Methylation, expression	(1 relationship) Suicide completers showed greater methylation on BDNF compared to controls. Global methylation (whole genome) did not differ between groups.

Table 3 Continued. Brain Development Genes Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
BDNF	Perroud et al., 2013	Candidate	Methylation	(2 relationships) Borderline personality disorder patients showed greater methylation and the higher number of childhood traumas predicted higher methylation
	Thaler et al., 2014	Candidate	Methylation	(3 relationships) Bulimia nervosa, borderline personality disorder, and childhood abuse associated with increased methylation at region specific gene loci
Reelin	Blaze et al., 2013*	Candidate	Methylation	(3 relationships) Early-life maltreatment and nurturing outside of the home cage linked to increased methylation in males. Females who received nurturing care outside of home cage showed decreased methylation.
	Qin et al., 2011*	Candidate	Methylation, expression	(2 relationships) Maternal deprivation associates with REELIN methylation and expression in the hippocampus

*Animal studies

Appendix D

Table 4. Whole Genome Studies Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
Whole genome	Anier et al., 2014*	Genome-wide	Methylation	Male rats exposed to early-life maternal separation showed reduced global methylation in the nucleus accumbens, yet increased in promoter region of genes for sleep, neuronal activities, metabolism, and muscle contraction.
	Doherty, Forster, and Roth, 2016*	EWAS	Methylation	Caregiving predicts differential global methylation
	Rodrigues et al., 2015*	Genome-wide & candidate	Methylation, expression	Sedentary rats exposed to acute restraint stress showed lower global DNA methylation in hippocampus and cortex, as well as increased BDNF expression in periaqueductal gray region.
	Labonte et al., 2012a	Genome-wide	methylation	362 genes were differentially methylated related to abuse history compared to controls. Most significant were genes involved in cellular/neuronal plasticity.
	Mehta et al., 2013	Genome-wide	Methylation, expression	Different childhood trauma exposure related to differential methylation and expression genome-wide in a group with PTSD.
	Naumova et al., 2012	Genome-wide	Methylation	Absence of parent-child relationship linked to increased methylation on genes involved in cellular signaling, immune system response, and brain development and functioning
	Smith et al., 2015	Genome-wide	Methylation	Cross tissue examination identified greater variability in saliva methylation compared to blood, and saliva appeared to be more correlated, than blood, to brain region methylation.
	Suderman et al., 2014	Genome-wide	Methylation	Childhood abuse associated with differential methylation on 997 genes in the promoter region. 311 genes showed increased methylation and 686 showed decreased methylation. Genes were involved in pathways related to development and transcription regulation.
	Zhang et al., 2011	Genome-wide (global)	Methylation	Sex and race associated with differential global methylation.

*Animal studies

Appendix E

Table 5. Other Relevant Studies Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
CRH	Chen et al., 2012*	Candidate	Methylation, expression	Maternal deprivation linked to reduced methylation on CRH and correlated with increased CRH expression in response to stress
Dlgap2	Chertkow-Deutsher, Cohen, Klein, & Ben-Shachar, 2010*	Genome-wide	Methylation, expression	Trauma linked to reduced methylation on gene that negatively correlated with expression in rats
OPRM1	Hao et al., 2011*	Candidate	Methylation	Litter gender composition predicts methylation on OPRM1
CALCA	Jiao, Opal, & Dulawa, 2013*	Genome-wide	Methylation, expression	Gestational factors related to methylation on gene and subsequent expression in hippocampus
No genes	Kapoor, Kostaki, Janus, & Matthews, 2009*	No genes	N/A	Some spatial learning changes occurred in rats exposed to prenatal stress.
No genes	Lemaire, Koehl, Le Moal, & Abrous, 2000*	No genes	N/A	Prenatal stress reduced neurogenesis in rats in the dentate gyrus. Hippocampus related spatial tasks were also impaired.
Genes involved in stress hormones	Murgatroyd et al., 2010*	Candidate/region specific	Methylation, expression	Early-life stress related reduced methylation in specific region associated with increased expression of arginine vasopressin

Table 5 Continued. Other Relevant Studies Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
MORC1	Nieratschker et al., 2014*	Candidate	Methylation	Cross-species prediction of early-life stress related methylation on MORC1
NTSR1	Toda et al., 2014*	Candidate	Methylation	Maternal deprivation associated with increased methylation in gene promoter region in the amygdala. Reduced activity in these receptors increased freezing behaviors in rodents.
POMC	Wu et al., 2014*	candidate	Methylation	Early-life stress predicts methylation on HPA-axis gene
HSD11B2	Appleton et al., 2013	Candidate	Methylation	Infants with mothers who suffered socioexonomic adversity, especially males, showed decreased methylation on this gene that promotes an enzyme to protect fetus from increased levels of maternal cortisol.
HSD11B2	Appleton et al., 2015	Candidate	Methylation	Infants with increased methylation on both genes showed higher habituation scores after birth.
DRD2	Groleau et al., 2014	Candidate	Methylation	Women with bulimia spectrum disorder and childhood sexual abuse history showed increased methylation compared to those with no eating disorder. Those comorbid with bulimia spectrum disorder and borderline personality disorder also showed an increase in methylation
DNMT/3A, EZH2, IL-6 (regulate epigenetic functioning)	Murphy et al., 2015	Genome-wide	Methylation, expression	Mixed sex sample with anxiety showed increased global methylation. Positive correlation with anxiety scores and expression of epigenetic related genes.
MORC1	Nieratschker et al., 2014*	Candidate	Methylation	Differential methylation across species at birth and in adult brain
TPPP, GRIN1, ID3	Weder et al., 2014	Genome-wide	Methylation	Depression symptoms linked to genes involved in neural circuitry, neural plasticity, and stress activity

Table 5 Continued. Other Relevant Studies Identified in Systematic Review

Gene	Author(s)	Study type	Variable	Results
IL17RA, miR124-3, KCNQ2, EFNB1, OCA2, MFAP2, RPH3AL, WDR60, CST9L, EP400, A2ML1, NT5DC2, FAM163A and *SPSB2*	Prados et al., 2015	Genome-wide	Methylation	Genes found differentially methylation between borderline personality disorder and major depressive disorder or with severity of childhood maltreatment.

*Animal studies

Appendix F

Table 6. Glossary of Terms

Term	Definition
Epigenetic	Chemical groups that attach to the DNA, at different points on the genes influencing their activity, without changing the DNA sequence
Candidate gene	Statistical analysis of selected genes of interest only
Genome-wide	Statistical analysis of all available genes
Bisulfite conversion	Process of using sodium bisulfite to convert un-methylated gene sites (cytosines) to uracil for detection of methylation presence, absence, and variation between (Shapiro, Servis, & Welcher, 1970)
Chip effects	Variation among samples due to differences among the bead chips
Batch effects	Variation among the samples due to differences among the groups of bead chips processed

Appendix G

Table 7. Glossary of Terms

Term	Definition
Epigenetic	Chemical groups that attach to the DNA, at different points on the genes influencing their activity, without changing the DNA sequence
Candidate gene	Statistical analysis of selected genes of interest only
Genome-wide	Statistical analysis of all available genes
Bisulfite conversion	Process of using sodium bisulfite to convert un-methylated gene sites (cytosines) to uracil for detection of methylation presence, absence, and variation between (Shapiro et al., 1970)
Chip effects	Variation among samples due to differences among the bead chips
Batch effects	Variation among the samples due to differences among the groups of bead chips processed

Appendix H

Table 8. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_2185264	ZNF461	-0.078886042	-.119, -.038	0.041379732		ILMN_2406532	F11R	-0.033700911	-.051, -.011	0.043466784
ILMN_1891924	N/A	-0.071855826	-.101, -.037	0.041723664		ILMN_2263236	HFE	-0.033652648	-.053, -.014	0.043466784
ILMN_1762573	LOC401630	-0.069570218	-.108, -.035	0.041723664		ILMN_2367300	ANKRD5	-0.032971488	-.052, -.012	0.049899687
ILMN_2096442	LOC260339	-0.065750621	-.097, -.032	0.041379732		ILMN_2299795	CPM	-0.032837325	-.045, -.010	0.041723664
ILMN_1746917	LOC729843	-0.064957403	-.084, -.038	0.042316501		ILMN_1728957	ANKRD5	-0.032553034	-.049, -.012	0.042316501
ILMN_2175737	ZNF826	-0.064650594	-.099, -.023	0.043466784		ILMN_1711994	TCIRG1	-0.032527688	-.051, -.016	0.047682533
ILMN_2198823	H6PD	-0.063303215	-.098, -.031	0.043466784		ILMN_1746923	LOC646443	-0.03245525	-.047, -.011	0.043466784
ILMN_2404320	SNTN	-0.06312612	-.095, -.033	0.041723664		ILMN_1758812	LDHAL6A	-0.03204024	-.048, -.013	0.043466784
ILMN_1698766	PYCARD	-0.063042137	-.082, -.041	0.041379732		ILMN_1775919	C6orf79	-0.031994525	-.049, -.013	0.043466784
ILMN_2141523	MRPL44	-0.06294615	-.097, -.029	0.042316501		ILMN_1686514	LOC642267	-0.031885579	-.049, -.012	0.047682533
ILMN_2362439	C19orf12	-0.062051098	-.097, -.029	0.049899687		ILMN_2329744	PMS2	-0.031842404	-.044, -.012	0.047682533
ILMN_2402499	SC4MOL	-0.06135017	-.089, -.035	0.041723664		ILMN_1699476	RPE	-0.031564525	-.040, -.007	0.041723664
ILMN_2203876	CCDC68	-0.060809072	-.094, -.027	0.042316501		ILMN_1759585	CHEK2	-0.03152546	-.049, -.008	0.045952824
ILMN_1876838	N/A	-0.060756232	-.088, -.039	0.041379732		ILMN_2323302	SON	-0.031503972	-.051, -.018	0.043466784
ILMN_1715635	ATP6V0E1	-0.060589336	-.095, -.033	0.042316501		ILMN_1810488	NFYC	-0.030915679	-.044, -.012	0.041723664
ILMN_2049417	TMEM86B	-0.06026815	-.095, -.035	0.04732714		ILMN_1712400	SERPINB6	-0.030091528	-.042, -.017	0.041379732
ILMN_2357377	TERF1	-0.059190782	-.091, -.027	0.041723664		ILMN_1770673	AKNA	-0.029749607	-.043, -.009	0.044240905
ILMN_2208491	RPLP0P2	-0.058546721	-.089, -.036	0.041379732		ILMN_1887008	N/A	-0.029710248	-.042, -.014	0.041723664
ILMN_2066249	RPP30	-0.058426512	-.095, -.031	0.041723664		ILMN_1678957	WDR55	-0.029212956	-.050, -.020	0.049899687

Table 8 Continued. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_2217955	TTC21B	-0.058323114	-.091, -.029	0.042316501		ILMN_1811644	FAM106A	-0.02919367	-.041, -.010	0.043466784
ILMN_2281089	STEAP3	-0.057859572	-.092, -.025	0.043466784		ILMN_1761865	LOC641704	-0.029013221	-.041, -.012	0.045952824
ILMN_1748090	SLC2A11	-0.057746703	-.091, -.023	0.046928677		ILMN_2144116	CPSF2	-0.02838741	-.040, -.012	0.043466784
ILMN_1679809	GSTP1	-0.057681223	-.088, -.031	0.041723664		ILMN_1723567	C1orf118	-0.028275993	-.044, -.010	0.046928677
ILMN_1733985	SIRT3	-0.057181346	-.089, -.029	0.041723664		ILMN_1688996	LOC643060	-0.027311467	-.043, -.004	0.049899687
ILMN_1651358	HBE1	-0.056699663	-.081, -.020	0.043466784		ILMN_1685239	GABPAP	-0.027235348	-.039, -.008	0.047327114
ILMN_1698519	LOC642267	-0.056541588	-.086, -.019	0.049899687		ILMN_1730879	CBY1	-0.027131446	-.039, -.014	0.044240905
ILMN_2346562	ZNF273	-0.056319333	-.090, -.029	0.043466784		ILMN_1744113	TNFAIP8L2	-0.026052743	-.034, -.008	0.041723664
ILMN_2382657	ARHGAP9	-0.056298906	-.086, -.027	0.043466784		ILMN_1760513	DYDC2	-0.025691897	-.035, -.012	0.049044604
ILMN_1791147	YPEL3	-0.056271359	-.089, -.032	0.049899687		ILMN_2122022	ZNF639	-0.024844573	-.038, -.010	0.043466784
ILMN_2049228	NUDT4P1	-0.055937291	-.086, -.028	0.041723664		ILMN_1807649	SPOPL	-0.024839798	-.029, -.007	0.043466784
ILMN_2180997	GTF2IRD2B	-0.055805828	-.088, -.031	0.043466784		ILMN_1797964	ARL6IP6	-0.024273555	-.030, -.004	0.045141267
ILMN_1728083	EIF4EBP2	-0.054931752	-.080, -.032	0.043466784		ILMN_1773850	FXC1	-0.022685488	-.035, -.011	0.041723664
ILMN_2126802	RPS27L	-0.054882677	-.084, -.029	0.041723664		ILMN_1749327	MAPK13	-0.022513271	-.039, -.009	0.049899687
ILMN_2058841	LILRA6	-0.054787834	-.083, -.025	0.041723664		ILMN_1794143	Rg9mtd1	-0.022451343	-.032, -.008	0.048276252
ILMN_2380101	PHACTR4	-0.053983378	-.070, -.030	0.041379732		ILMN_1682736	LOC643452	0.01550105	.004, .024	0.045199051
ILMN_2190850	PPID	-0.053745272	-.086, -.025	0.043466784		ILMN_1675852	LOC650518	0.016336257	.008, .026	0.042316501
ILMN_2179579	SNHG3	-0.053519627	-.085, -.024	0.044980779		ILMN_1721713	EXOSC9	0.016725851	.006, .025	0.043064722
ILMN_2106002	ACBD7	-0.052888042	-.085, -.027	0.044980779		ILMN_1788689	PHIP	0.016802624	.009, .028	0.047327114
ILMN_2395496	KLK7	-0.052570497	-.087, -.020	0.043466784		ILMN_1659523	USP39	0.018544909	.003, .024	0.043466784

Table 8 Continued. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_1752028	PMS2L1	-0.052357113	-.078, -.025	0.041723664		ILMN_1776347	TCP1	0.018823076	.009, .026	0.043466784
ILMN_2115011	FGD2	-0.052162454	-.082, -.024	0.044980779		ILMN_1767992	SLC12A6	0.019306512	.009, .029	0.041379732
ILMN_2084489	ZNF595	-0.051316734	-.082, -.019	0.043466784		ILMN_1704206	NPSR1	0.019418619	.0003, .024	0.041723664
ILMN_2215965	CYP2B6	-0.050994618	-.074, -.024	0.041379732		ILMN_2192683	DHX37	0.020070541	.007, .026	0.043466784
ILMN_1712357	HNRPK	-0.050745076	-.074, -.025	0.042316501		ILMN_1662896	BRWD2	0.0209141	.009, .030	0.041723664
ILMN_1776995	LOC651192	-0.050474837	-.079, -.022	0.043466784		ILMN_1771949	TAF4B	0.021219752	.008, .038	0.046928677
ILMN_1757914	C19orf56	-0.050123353	-.072, -.027	0.044980779		ILMN_1776147	C21orf59	0.021299857	.008, .031	0.043466784
ILMN_1726175	ITGAX	-0.049805107	-.070, -.018	0.045952824		ILMN_1727761	GMEB1	0.021302431	.006, .026	0.043466784
ILMN_1741491	ZNHIT1	-0.049742067	-.083, -.026	0.044240905		ILMN_1693421	RPN2	0.022035022	.010, .032	0.043466784
ILMN_1681490	ZNF568	-0.049566005	-.079, -.017	0.043466784		ILMN_1725169	INTS12	0.022214042	.005, .034	0.041723664
ILMN_1675249	SGTB	-0.049205886	-.075, -.017	0.042316501		ILMN_1698304	LOC642969	0.02271069	.012, .031	0.043466784
ILMN_1708296	DEAF1	-0.04912634	-.079, -.020	0.043466784		ILMN_1737413	MSH2	0.023029546	.005, .030	0.043466784
ILMN_2178186	PIGW	-0.04887585	-.076, -.022	0.043466784		ILMN_1658182	MEX3C	0.023083471	.007, .036	0.047327114
ILMN_2287707	POT1	-0.047957948	-.075, -.019	0.04768253		ILMN_1916094	N/A	0.023133907	.009, .035	0.043466784
ILMN_2127416	GSR	-0.047914114	-.073, -.028	0.043466784		ILMN_1897536	N/A	0.023158648	.011, .036	0.043466784
ILMN_2362832	STAG3L1	-0.047421685	-.076, -.021	0.049899687		ILMN_1677376	CHD7	0.023791824	.012, .037	0.043466784
ILMN_1671494	USP5	-0.047160548	-.068, -.032	0.041379732		ILMN_1774974	CLUAP1	0.023952943	.011, .030	0.041379732
ILMN_2281529	STAP2	-0.047150205	-.075, -.018	0.046928677		ILMN_1702244	TMEM68	0.024139182	.010, .038	0.043466784
ILMN_1810424	HRH4	-0.047118688	-.065, -.025	0.041379732		ILMN_1748018	GORASP2	0.02441163	.010, .032	0.043466784
ILMN_1689710	C16orf50	-0.047007495	-.069, -.026	0.041379732		ILMN_1801833	ARHGAP24	0.024525524	.015, .038	0.041379732
ILMN_2102580	UTP20	-0.046813344	-.071, -.021	0.041723664		ILMN_1771801	SIRPG	0.024716906	.012, .036	0.043466784

Table 8 Continued. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_1731931	LOC727773	-0.046789815	-.076, -.017	0.045493915		ILMN_1684724	CR2	0.024839519	.012, .037	0.041723664
ILMN_1686360	ZNF124	-0.046355415	-.070, -.025	0.04802105		ILMN_1816646	N/A	0.02485684	.015, .037	0.041723664
ILMN_2409720	SLA2	-0.04625212	-.070, -.018	0.043466784		ILMN_2136133	PABPC1	0.024864516	.010, .033	0.041723664
ILMN_1712435	LOC644294	-0.046079677	-.082, -.022	0.046928677		ILMN_1720270	CDR2	0.025226775	.006, .032	0.041723664
ILMN_2245686	GYG2	-0.045899964	-.073, -.018	0.045141267		ILMN_1838113	N/A	0.025329166	.009, .038	0.047327114
ILMN_1765621	HDGF	-0.045702693	-.068, -.020	0.043466784		ILMN_1789653	PBLD	0.025480103	.010, .038	0.044980779
ILMN_1776956	LOC400221	-0.04560514	-.065, -.020	0.041379732		ILMN_2048822	NUDCD2	0.025886067	.013, .033	0.041379732
ILMN_1917044	N/A	-0.045493069	-.072, -.030	0.043466784		ILMN_1894862	N/A	0.025948733	.013, .042	0.048021005
ILMN_1773238	XCR1	-0.044549281	-.068, -.018	0.041723664		ILMN_2087575	ZC3H4	0.026027863	.013, .035	0.047682503
ILMN_1756942	SP3	-0.044384942	-.056, -.025	0.041379732		ILMN_1685661	RRP15	0.02636941	.004, .040	0.047327114
ILMN_1703433	PLSCR3	-0.043624803	-.066, -.020	0.049044604		ILMN_1655625	GPATCH1	0.026500147	.007, .041	0.041723664
ILMN_2151168	SLC30A6	-0.043380056	-.065, -.019	0.041723664		ILMN_1725175	FOSL2	0.02654905	.010, .039	0.042316501
ILMN_2378670	SNX15	-0.043006845	-.067, -.016	0.043466784		ILMN_1828967	N/A	0.026727936	.017, .041	0.047327114
ILMN_1760757	BRIP1	-0.04272931	-.064, -.018	0.043466784		ILMN_1749586	LOC642914	0.026746809	.016, .046	0.043466784
ILMN_2070477	TAF8	-0.042691067	-.064, -.020	0.041723664		ILMN_1717852	USH1G	0.026754287	.016, .039	0.041723664
ILMN_1749417	LOC649495	-0.042509765	-.053, -.017	0.041723664		ILMN_1774369	LOC200420	0.026771862	.010, .044	0.049899687
ILMN_1715698	MGC71993	-0.042485825	-.064, -.020	0.043466784		ILMN_2156953	ZFAND6	0.026820464	.015, .036	0.043466784
ILMN_2292696	COX15	-0.041623601	-.062, -.028	0.041379732		ILMN_2186482	TMED7	0.026990153	.015, .043	0.043466784
ILMN_1694136	LOC649144	-0.041081248	-.063, -.018	0.043466784		ILMN_1672446	RPL11	0.027072111	.008, .040	0.041723664
ILMN_1742400	CEP350	-0.040846365	-.054, -.022	0.041379732		ILMN_1868058	N/A	0.027351792	.012, .038	0.043466784
ILMN_2280441	PACRG	-0.040702491	-.059, -.017	0.041723664		ILMN_1830425	N/A	0.027459213	.012, .040	0.043466784

Table 8 Continued. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_2042941	TMEM159	-0.040579203	-.059, -.019	0.041723664		ILMN_1704956	SMTNL1	0.027710644	.007, .039	0.041379732
ILMN_1758100	GALR3	-0.040217106	-.060, -.019	0.041723664		ILMN_1915722	N/A	0.027866159	.013, .044	0.043466784
ILMN_2224300	SNAPC3	-0.040163335	-.063, -.012	0.049899687		ILMN_1818935	N/A	0.028159219	.016, .044	0.043466784
ILMN_1739792	RHOG	-0.040075792	-.061, -.018	0.043466784		ILMN_1692535	DPP4	0.028160224	.012, .036	0.045094286
ILMN_2410362	ACBD5	-0.039916622	-.059, -.018	0.041723664		ILMN_1683487	ZNF154	0.028435486	.010, .044	0.047682533
ILMN_1745154	PARD6B	-0.039480076	-.059, -.017	0.041723664		ILMN_2114876	RPL11	0.028446346	.009, .043	0.041723664
ILMN_2055271	A1BG	-0.039415396	-.056, -.017	0.041723664		ILMN_1764323	LOC124512	0.028469498	.016, .040	0.041379732
ILMN_2358652	NXF1	-0.039356826	-.062, -.019	0.041723664		ILMN_2410771	KEAP1	0.028499536	.008, .033	0.041379732
ILMN_2374383	TSPAN17	-0.038850669	-.059, -.021	0.043466784		ILMN_2352295	PRDM10	0.028756246	.013, .043	0.046928677
ILMN_1684032	ZNF613	-0.038656076	-.055, -.015	0.041723664		ILMN_1722662	RAD23B	0.028974988	.008, .043	0.048021005
ILMN_2162972	LYZ	-0.03863507	-.055, -.016	0.041723664		ILMN_1854664	N/A	0.029103749	.011, .039	0.041723664
ILMN_1690545	TAF11	-0.038611833	-.057, -.020	0.041723664		ILMN_1853160	N/A	0.029498432	.017, .044	0.043466784
ILMN_1771066	PCDHB16	-0.038603209	-.059, -.020	0.041723664		ILMN_1741640	ZNF675	0.029522593	.015, .044	0.048021005
ILMN_1673171	LOC651268	-0.038544075	-.063, -.020	0.046928677		ILMN_1670218	EXOSC6	0.029789366	.007, .045	0.043466784
ILMN_1651506	NCOA6IP	-0.038395972	-.049, -.019	0.041379732		ILMN_1726516	SCRIB	0.029952126	.014, .047	0.048276252
ILMN_1773042	SGEF	-0.038384503	-.059, -.008	0.047190517		ILMN_1653129	CSTF2	0.030208328	.013, .039	0.042316501
ILMN_1682938	ARF3	-0.038045144	-.060, -.014	0.043466784		ILMN_1861772	N/A	0.030223967	.016, .045	0.041723664
ILMN_2277252	PPFIBP1	-0.038031935	-.055, -.017	0.041723664		ILMN_1892010	N/A	0.030370307	.018, .043	0.041723664
ILMN_2178201	ZNF43	-0.037734631	-.055, -.018	0.041723664		ILMN_1730791	LOC646783	0.030379729	.015, .043	0.041723664
ILMN_2255142	TRIM34	-0.037587268	-.057, -.013	0.043466784		ILMN_2151048	STAG1	0.030436997	.019, .041	0.041723664
ILMN_1800020	GART	-0.037315162	-.058, -.014	0.043466784		ILMN_1880113	N/A	0.030540174	.013, .045	0.041723664
ILMN_2115974	GSDM1	-0.037211655	-.055, -.014	0.041723664		ILMN_1879078	N/A	0.031327435	.013, .047	0.043466784

Table 8 Continued. GSI, Mothers' Mental Health, Expression, Males only

Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR		Probe	Gene	Beta Coefficient	Bootstrap 95% CI	FDR
ILMN_1660869	LOC643438	-0.037161222	-.056, -.020	0.043466784		ILMN_1888252	N/A	0.031513924	.015, .048	0.041723664
ILMN_1724555	LOC651959	-0.036980819	-.052, -.015	0.041723664		ILMN_1662845	NBPF11	0.031580898	.018, .049	0.041723664
ILMN_1750805	ARHGAP30	-0.036936857	-.053, -.023	0.043466784		ILMN_1748141	AMOTL1	0.031599523	.015, .048	0.043466784
ILMN_2261600	FCGR1B	-0.036794728	-.046, -.017	0.041379732		ILMN_2130525	TSPAN13	0.031815095	.015, .043	0.043466784
ILMN_1684434	SLC17A5	-0.036576672	-.057, -.014	0.045141267		ILMN_2381064	TPD52	0.032692639	.017, .050	0.043466784
ILMN_2190851	PPID	-0.036289407	-.052, -.014	0.043466784		ILMN_1652085	MPHOSPH10	0.033498448	.017, .048	0.041723664
ILMN_2196232	C1orf210	-0.036197628	-.052, -.016	0.041723664		ILMN_1657873	XPO4	0.034105854	.017, .054	0.044267941
ILMN_2137464	DVL3	-0.035973219	-.055, -.017	0.043466784		ILMN_2200636	KIAA1267	0.035372831	.017, .048	0.044980779
ILMN_2145143	FKBP9	-0.035961146	-.052, -.012	0.041723664		ILMN_1909223	N/A	0.035757529	.017, .050	0.042316501
ILMN_2331658	C3orf17	-0.03585831	-.054, -.017	0.043466784		ILMN_2103774	PIP5KL1	0.036060422	.014, .047	0.044267941
ILMN_1794522	EIF5A	-0.035854562	-.061, -.019	0.043466784		ILMN_1875380	N/A	0.038354853	.016, .054	0.043168509
ILMN_1901394	N/A	-0.035816176	-.052, -.008	0.049899687		ILMN_1837286	N/A	0.038877769	.019, .055	0.041723664
ILMN_2141030	LOC641522	-0.035638955	-.054, -.014	0.043466784		ILMN_1804421	AREG	0.039712051	.010, .053	0.042324396
ILMN_1768855	C15orf37	-0.035564497	-.055, -.014	0.043466784		ILMN_1819251	N/A	0.039768665	.019, .053	0.041379732
ILMN_2149400	SPC25	-0.035074611	-.053, -.015	0.049899687		ILMN_2379788	HIF1A	0.041570256	.028, .062	0.041723664
ILMN_2116242	ZNF808	-0.035034596	-.056, -.013	0.049899687		ILMN_1798874	TMEM85	0.042248923	.018, .057	0.041723664
ILMN_2359096	SS18	-0.034992783	-.053, -.015	0.042316501		ILMN_1696675	CES2	0.042250107	.019, .064	0.045952824
ILMN_1794379	DHX40	-0.034748991	-.055, -.009	0.049899687		ILMN_2319000	MATK	0.046472902	.019, .068	0.047682533
ILMN_2307025	CPNE1	-0.034511714	-.054, -.013	0.049899687		ILMN_2379762	NPM1	0.055644091	.027, .087	0.043466784
ILMN_2252136	YWHAE	-0.034150242	-.060, -.021	0.043466784						

VITA

Stefanie R. Pilkay received her B.S.S.W., double major in Psychology, and M.S.S.W. from the University of Tennessee, Knoxville, where she also completed the Trauma Treatment Graduate Certificate program. Her research interests focus on utilizing a collaborative cross discipline approach to identify the mechanisms involved in traumatized populations that interfere with recovery and perpetuate poor overall quality of life. Her current investigations are examining potential epigenetic mechanisms for the mother's trauma history to influence the subsequent generation development.