EPIGENETIC METHYLATION IN PTSD AS MODERATED BY TRAUMA EXPOSURE IN REFUGEES

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Dedication

To my mother, who, a war refugee herself, has taught me to be brave and resilient. Her desire to protect her children and ensure their lives were better than her own has shaped my worldview and inspired my determination for bettering the lives of others. This scientific venture is my way of honoring the lessons she has taught me and giving back to refugees who were less fortunate than my family.

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Chapter 1: Introduction

Epigenetics is a branch of biology that investigates the mechanism by which the environment modulates genes, gene expression (Toyokawa et al., 2012), and the cellular process that give rise to phenotypic expression and subsequent behavior (Stam, 2007; Ratten & Mill, 2009; Xin et al., 2012). While we are born with a fixed genetic code, genes may become activated or inactivated, via DNA methylation. DNA methylation can shed light on the molecular and environmental underpinnings of posttraumatic stress disorder (PTSD) etiology. It can aid in understanding ways long-term stress exposure effects gene transcription, both, across the genome and in candidate genes of PTSD. As such, DNA methylation will provide insight into the interaction between the environment, psychosocial stress, and the body. This is highly relevant for PTSD as the environment plays a vital role in the etiology of the disorder.

War-related trauma has adverse effects on refugee mental health and has been implicated in the dysregulation of multiple neurobiological systems in the body, including the limbic frontal system and the hypothalamic-pituitary-adrenal axis (HPA). These systems' dysregulation contribute to the development of PTSD. However, not everyone exposed to trauma develops PTSD symptoms. It remains unclear, even after considering pre-trauma, peri-trauma, and post-trauma factors, reasons a subset of individuals develop PTSD, while others do not. This is especially true for war refugees, who have PTSD prevalence rates ranging from 3% (Hauff et al., 1994) to 86% (Carlson & Rosser-Hogan, 1991). While the wide range of estimates may be due to the utilization of tools or improperly validated methods, other possibility include the role of epigenetics. DNA methylation may be the missing link in deciphering these inconsistencies. Trauma

can alter the expression of genes that are associated in the regulatory systems implicated with PTSD susceptibility. These genes include SLC6A3, a gene that codes the dopamine transporter, and SLC6A4, a gene that codes for the serotonin transporter, as well as the HPA axis-associated genes, which play an integral role in the glucocorticoid receptor complex, such FKBP5 and NR3C1.

While numerous epigenetic studies have demonstrated the link between trauma and PTSD-like symptoms in animal models (e.g., Weaver et al., 2004), fewer have shown this link in humans (Schmidt et al., 2011), and none in particular in refugees. This limits our knowledge of the molecular basis for the development of PTSD symptoms. Refugees are a particularly vulnerable group, in that they have experienced cumulative trauma ranging from war exposure to displacement and resettlement in a new country. As such, there is an urgent need for biomarkers that can be used to quantify stress, so as to eventually determine the biological effects of trauma exposure and stress on the neurobiological system.

This study sought to determine whether self-reported mental-health measures of PTSD and trauma exposure are reflected in the epigenome of Iraqi male refugees who reported varying degrees of trauma exposure and PTSD symptoms. The study aimed to determine the singular and interactive effect of DNA methylation of PTSD candidate genes and trauma on PTSD symptoms, while taking into account risk and protective factors (i.e., age and social support). The findings can shed light on molecular differences among refugees who are resilient or at risk for development of PTSD symptoms following trauma exposure, which has clinical implications for intervention development.

Chapter 2: Literature Review

Trauma

The majority of the general population will experience a potentially traumatic event in their lifetime, however, only a subset will develop posttraumatic stress disorder; similar trends are seen in vulnerable populations such as veterans, natural disaster survivors and refugees (Heinzelmann & Gill, 2013; Keane, Marshall, Taft, 2006). The disorder encompasses "a wide range of intensely stressful experiences that involve exposure to levels of danger and fear that exceed normal capacity to cope" (Fairbank, et al. 2002; pp. 183). The American Psychiatric Association (APA) defines trauma as an event that exposes an individual to actual death or threatens death, serious injury, or the physical integrity of the self or others (APA, 2013).

Researchers have attempted to create taxonomy of trauma by identifying its various dimensions (Kira et al., 2008; Kira, 2010). The dimensions of trauma range from those affecting individuals on an interpersonal level to direct trauma that is inflicted by an objective external threat. Direct trauma is defined as person-made trauma and is further divided into two types, simple and complex trauma (Kira, 2001). Simple trauma is the experience of one distinct traumatic event, whereas complex trauma is a connected series of traumas. An example of a simple trauma is an incident that occurs out of the blue, like a natural disaster or a single incident of abuse, while complex trauma include more chronic exposure to trauma, such as multiple episodes of abuse and prolonged exposure to war. A new focus of trauma research is cumulative trauma, which involves the effects of additive traumas (Kira et al., 2008; Kira, 2010). Cumulative trauma will be highlighted in this study.

Trauma causes distress that surpasses everyday stressful experiences. Psychological trauma is the mental and physical state that follows a traumatic event (APA, 2013). Although there is a consensus that traumatic events may lead to psychological trauma, it is important to note that one's response to trauma is a subjective experience (McNally, 2003). An individual's response may range from adaptation and adjustment to a state of being overwhelmed, resulting in mental and physical distress. Traumatic stress research has evolved out of studies of three types of potentially traumatic events: interpersonal violence, natural disasters, and war (McFarlane & Potts, 1999). This study will focus on war-related traumatic events and the subsequent development of PTSD in refugees.

Refugees

The plight of refugees is a well-recognized international matter. The United Nations Human Rights Commissioner for Refugees (UNHCR) in their 2012 reported that the refugee crisis has reached "levels unseen in previous decades" (UNHCR, 2013, p.5,). By the end of 2012, 15.4 million refugees were displaced worldwide. "Refugee" is a legal term recognizing individuals who flee war, economic hardships, generalized violence, or human rights violations (UNHCR, 2013). Refugees are individuals who are forcibly displaced from their home countries due to well-founded fears of persecution, usually from their own government, due to their race, religion, nationality, political opinions, or social group (UNHCR, 2013).

Research on war trauma began from an investigation into mental health among refugees (Boehnelein & Kinzie, 1995). Investigation into refugee mental health gained recognition after World War II. Follow-up studies of Jewish refugees conducted in

Europe, Israel, and the United States have provided insight into the long-term adjustment of Holocaust survivors (Boehnelein & Kinzie, 1995). The refugees experienced horrific traumatic experiences, including forced labor, starvation, mass executions, and torture. Following these experiences, it was noted that the refugees exhibited chronic responses, which included fear, depression, anxiety, and multiple somatic symptoms. Longitudinal studies of this population demonstrated that the consequences of war trauma resulted in impaired social adjustment, distrust, hostility, passive fatalistic personality style, and loss of enjoyment (Eaton et al., 1989; Frankl, 1969; Boehnelein & Kinzie, 1995). The second wave of refugee research emerged out of South Asian refugee migration in the mid-1970s. These refugees included Cambodians and Vietnamese who had experienced war, concentration camps, and starvation first-hand, as well as witnessing executions (Kinzie, 1986). Such studies have provided insight and influenced the investigation of trauma among recent refugee migrations in Central America, Africa, and the Middle East (Boehnelein & Kinzie, 1995).

The refugee experience is characterized by a host of complex stressors. Refugees are often exposed to a multitude of traumatic events, including bomb explosions, combat exposures, kidnapping, torture, murder, and rape (UNHCR, 2012). However, the stressors are not limited to their home countries. Displacements from one's home country and safety concerns in the new country are among the plethora of stressors. The subsequent effects include complex stress that results in lasting psychological consequences (Keller et al., 2006). Exposure to potentially traumatic events can lead to an array of "psychological disorders, including major depression, specific phobias, panic disorders, as well as disorders of extreme stress not otherwise specified, personality disorders, and a

range of physical symptoms" (Fairbank, et al. 2002, pp. 184). However, a hallmark of trauma exposure is post-traumatic stress disorder (PTSD).

PTSD

The impact of trauma and conceptualization of post trauma symptoms has evolved in the last century, and in particular, in the field of psychology. Sigmund Freud initially investigated trauma within the context of childhood development. He initially attributed the basis of neurosis to traumatic experiences that occurred during developmental periods in childhood (Wilson, 1994). Freud elaborated on trauma; he stated that individuals who have been exposed to a traumatic event experience neurotic illness. In this illness, the individual repeated the trauma or the context of that trauma, often times, within a pattern. Specific to war trauma, Freud proposed war neuroses after being influenced by the events of World War I (reviewed in Wilson, 1994). In the context of war, Freud believed that the threat to the ego is external in the form of physical injury (Wilson, 1994). Following the traumatic experience, the individual is in psychic disequilibrium, as the trauma is overwhelming the ego functioning. Subsequently, the individual is unable to cope and ego functioning is reduced. The symptoms are a consequence of fear and aggression in a war environment, where the individual employs repression as a defense against the anxiety experienced (Wilson, 1994).

Much like Freud's influence in conceptualizing trauma symptoms, the occurrence of wars in human history initiated an interest in post-trauma response. Initially, soldiers who were coming back from war exhibited behavior that was once thought to be due to brain damage as a result of the explosions in the field, and the symptoms were termed shell shock, war neurosis, or combat fatigue (Creamer, 2000). Over time, the response to

trauma in the civilian population was recognized to be no different than that of soldiers, propelling the psychiatric community to recognize the response as a disorder (Creamer, 2000).

Freud's conceptualization of post trauma symptoms was outlined into clusters, which heavily influenced the symptom criteria of what we know as posttraumatic stress disorder (PTSD) today. In many ways, the diagnostic criteria were based on Freud's work regarding traumatic neurosis (reviewed in Wilson, 1994). Freud identified post trauma symptoms that include intrusive thoughts and repeating the traumatic experience in their dreams and physiological hyperactivity. The individual relives the traumatic experience, as though the traumatic event has yet to be psychological processed (reviewed in Wilson, 1994). These symptoms were classified as Gross Stress Reaction (GRS) in DSM-I (1952). GSR was described as the experience of acute stress in response to unusual environmental pressure or strain, which was expected to resolve quickly. The disorder was classified as a transient situational personality disorder. The DSM-II (1968) moved the disorder from that category, reclassified it as an adjustment reaction during adult life, and provided three short illustrations of what might constitute adjustment reactions.

The term "post-traumatic stress disorder" was first introduced in the DSM-III (1980), which indicated that the symptoms manifested "after injury." It was given its own category and classified as an anxiety disorder. In essence, separating it into its own category allowed for research to be conducted specifically on post-traumatic stress disorder. The DSM-III-R (1987) specified the symptoms required for diagnosis, adding that the symptoms must persist for at least four weeks. The DSM-III-R added the four criteria that we recognize today as quintessential post-traumatic stress disorder

manifestations: Criteria A, recognizable stressor that evokes significant distress; Criteria B, reenactment and reliving the trauma; Criteria C, avoidance symptoms and numbing; and Criteria D, the introduction of physiological hyper-arousal. Although there have been only minor revisions in the DSM-IV and DSM-IV-TR, the definition of PTSD continues to evolve based on research.

The DSM-5 (2013) brought the newest revisions regarding PTSD. It is no longer classified as an anxiety disorder but as a trauma-related disorder, in which the individual is exposed to a traumatic, stressful, or life-threatening situation. PTSD continues to be characterized by several groups of symptoms, which include re-experiencing the event, avoiding cues that may remind one of the event, and hyper-arousal. The DSM-5 introduced a new cluster of symptoms. The DSM-IV-TR (2001) grouped avoidance and numbing together, whereas the DSM-5 placed them into two distinct categories. While the new category still contains numbing symptoms, this category also includes new symptoms related to persistent negative alterations of cognition and mood.

Prevalence Rate

The prevalence rate of PTSD in the general population varies from 18-36% following trauma (Heinzelmann & Gill, 2013). However, the epidemiological literature on refugee psychological morbidity has been mixed (Hollifield et al., 2002). The prevalence of PTSD in adult refugees has ranged from 3% (Hauff et al., 1994) to 86% (Carlson & Rosser-Hogan, 1991). De Jong et al. (2001) investigated the lifetime prevalence rate of PTSD in four post-conflict countries and found that the PTSD rate was 27.4% in Algeria, 28.4% in Cambodia, 15.8% in Ethiopia, and 17.8% in Gaza. The prevalence rate for PTSD among Cambodian refugees in New Zealand was found to be

12.1% (Cheng, 1994), and a similarly low prevalence was found among Vietnamese refugees in Norway, with only a 9% PTSD prevalence rate (Hauff and Vaglum, 1994). On the other side of the spectrum, 60.5% of Kosovan refugees exhibited PTSD symptoms (Ai, Peterson, & Ubeher, 2002), and 71% of displaced Bosnian women met the criteria for PTSD (Dahl et al., 1998).

In a sample of Iraqi and Kurdish refugees in Sweden, utilizing both the self-report and interview methods, Sondergaard et al. (2001) found a prevalence rate of 37% for current PTSD symptoms. A more comprehensive meta-analysis surveying 7,000 refugees resettled in western countries revealed that the refugees were 10 times more likely to have PTSD than the general populations of those countries (Fazel, Wheeler, & Danesh, 2005). PTSD symptoms are also pervasive in communities affected by war trauma. Research studies revealed prevalence rates of 11% in Yugoslavian students (Gavrilovic et al., 2002) and 20% in a community sample from Eastern Afghanistan during a one-year follow up (Scholte et al., 2004).

Etiology

Epidemiological studies have highlighted the risk factors that are associated with PTSD (Brewin et al., 2000; Kessler et al., 1999). The risk factors are categorized as pre-exposure factors, exposure factors, or post-exposure factors (Fairbank et al., 2002).

Pre-Trauma

Research has identified several pre-trauma risk factors in nonclinical samples in both community samples and refugee samples, including being a female, a family history of mental illness, age, and education (Johnson & Thompson, 2008). In a community sample, individuals with lower education level were more likely to develop PTSD after

exposure to traumatic event (Kessler et al., 1999). In terms of gender, women were more likely to develop PTSD in the general population (Brewin et al., 2000; Kessler et al., 2005; Ozer et al., 2003). Similarly, women in the refugee population were also more likely to develop PTSD (Ai et al., 2002). Interestingly, men were more likely to be exposed to trauma (Kessler et al., 1995). This finding may suggest that the nature of the trauma or the type of trauma may be indicative of the development of PTSD because men and women are exposed to different types of trauma (Johnson & Thompson, 2008). Refugee research has indicated that females at a higher risk of developing post-trauma stress due to the nature of that trauma, which includes rape, the violent loss of a spouse or children, and being widowed (Mollica et al., 1987). However, this research did not assess trauma type when examining the differences in the development of PTSD in both genders.

A family history of mental illness has been associated with the development of PTSD in both the general population and refugees (Kessler et al. 1999; Johnson & Thompson, 2002). In a sample of urban adults, the possibility of developing PTSD significantly increased in individuals who reported a family history of anxiety, depression, antisocial behavior, or psychosis (Breslau et al., 1991). Furthermore, exposure to a potential trauma increased the risk of developing PTSD twofold in individuals with preexisting anxiety disorder or preexisting major depression in a sample of urban young adults (Breslau et al., 1991).

Age plays an important role in the development of PTSD after exposure to traumatic events. Studies have shown that refugees over the age of 65 were at a higher risk of developing PTSD following the war in Kosovo (Cardozo et al., 2000). Cheng

(1994) found similar results when investigating Cambodian refugees in New Zealand. In another study, being over the age of 25 was a predictor of developing PTSD among Bosnian women who were displaced (Dahl et al., 1998). However, the studies should be interpreted with caution due to the fact that they did not investigate the type of trauma when assessing age as a risk factor.

Peri-trauma

The nature of the trauma, the types of stressors, and the characteristics of the traumatic experience can be predictive of developing PTSD (Fairbank et al., 2002; Johnson & Thompson, 2008; Hoge et al., 2007). The perceived threat and one's immediate reaction can influence the development of PTSD (Basoglu et al., 2005; Marshall & Schell, 2002**).** For example, if an individual perceives the threat to be immensely dangerous and responds with intense fear, they are more likely to develop PTSD (Basoglu et al., 2005; Marshall & Schell, 2002). Basoglu et al. (2005) also found that being prepared for the trauma psychologically can reduce the likelihood of developing PTSD (Basoglu et al., 2005; Marshall & Schell, 2002). In terms the trauma, objective characteristics of the stressor have been found to influence the development of PTSD (Fairbank, et al. 2002). For instance, the magnitude of the stressor is associated with the likelihood of developing PTSD (Carlier et al., 1997).

Post-trauma

Researchers are becoming more interested in terms of investigating post-trauma risk factors in the development of PTSD (Johnson & Thompson et al., 2002; Kessler et al., 2005). One study used structural equation modeling to assess post-trauma factors that were associated with the development of PTSD among Vietnam veterans (King,

Fairbank, Adam, 1998). The results revealed that social support and subsequent stressful life events were associated with PTSD. However**,** PTSD in men was more likely to be associated with the individuals perceiving that their socials support networks were small compared to women (King, Fairbank, & Adam, 1998). Findings regarding Iraqi refugees settled in the United States revealed that unemployment post migration was associated with poor mental health (Jamil, Aldhalimi, & Arnetz, 2012). Similarly, in Iraqi refugees, Gorst, Unsworth, and Goldenberg (1998) found that severe PTSD post-migration was associated with low levels of social support once resettled in the host country, post migration. Finally, Steel et al. (1999) found health problems, difficulties in post-migration adaptation, loss of culture, and lack of support were associated with PTSD symptoms.

Dose-Response Model

Exposure to prolonged and repeated trauma may result in mental health problems, including PTSD (Kilpatrick et al., 1998; Johnson & Thompson, 2002;). This interaction is highlighted in the dose-response model, which proposes that as an individual's exposure to trauma increases, the more likely it becomes that he or she will experience adverse symptoms in response (March, 1993; Zoladz & Diamond, 2013). A robust dose-response relationship between trauma exposure and adverse mental health results has been established in studies in refugee populations (March, 1993; Marshall et al., 2005; Mollica et al., 1998). Studies show that although symptoms appear to decrease over time, a subgroup of people with high levels of traumatic exposure remained symptomatic (Kessler, et al., 1995). Steel et al. (2002) interviewed 1,161 refugees who had experienced mass trauma and found that although most refugees were free from overt

mental health symptoms, a smaller group who had experienced a higher degree of trauma were symptomatic a decade post-exposure (Steel, et al., 2002).

Other studies show a dose-response relationship in which refugees who had experienced severe trauma experienced PTSD, after two decades of war exposure and resettlement in the United States (Marshall et al., 2005). This study took into account pre-migration trauma exposure and post-migration trauma exposure in its analysis of this dose-response relationship, both of which were associated with higher levels of PTSD and depression symptoms. Cambodian refugees reported high levels of trauma exposure during the war, during which 99% of the sample came close to death, 90% had a family member or friend murdered, and 70% were exposed to violence; these refugees developed PTSD in high proportions (Marshall et al., 2005). A dose-response relationship has been identified among Vietnamese ex-political detainees, with a correlation being found between cumulative torture and PTSD (Mollica et al., 1998).

Protective Factors

To reiterate, only a minority of trauma-exposed individuals will develop PTSD. Resiliency in refugees has generated research interest as a characteristic of those who do not develop psychological symptoms following exposure to trauma. Resiliency has been found to be an "inverse predictor of psychological distress" in refugees (Arnetz et al., 2013, p. 167), which often indicates protective factors that reduce the probability of experiencing psychological distress (Hoge et al., 2007). Resilient individuals are individuals that experience traumatic events but adapt positively despite this adversity (Luthar, Cicchetti, & Becker, 2000). Bonanno and colleagues (2006) investigated resiliency in individuals who were exposed to the September 11th terrorist attack in New

York. Resiliency was defined as existing among those individuals who reported no PTSD symptoms or, at most, one PTSD symptom. The researchers found that nearly 65% of the individuals did not develop PTSD symptoms, thus, were defined as being resilient. However, resiliency decreased as the amount of exposure increased, but it never fell below one-third of the individuals in the sample (Bonanno et al., 2006). Several protective factors emerged from this population, including marital status, ethnicity, gender, education, and income. It appears that marriage and the male gender serve as protective factors against the development of pathological symptoms following trauma. Further, individuals with higher levels of education and higher levels of income showed resiliency following trauma. Additionally, Asian Americans displayed higher levels of resiliency (Mancini & Bonanno, 2006).

Among refugees, social support has been associated with higher levels of resiliency (Willis & Fegan, 2001). However, studies revealed that social support is not easily quantified. It has been established that high levels of social support are an important protective factor in determining whether an individual will be resilient. A large quantity of social support, as defined by a large social support system, is important in the face of all stressful situations. However, a notable finding of this study is that the perception of the quality of social support is more effective than the quantity of social support in situations involving high stress levels (Willis & Fegan, 2001). Furthermore, one's cognitive state and beliefs play a protective role. Turkish ex-political-activist refugees who reported being prepared for trauma, as evidenced by their commitment to a political cause and the perceived likelihood of arrest and torture, were more resilient in the face of torture, showing lower levels of PTSD, anxiety, and depression. Their

preparation and mindset worked as a protective factor against the development of pathology following the traumatic experience (Boasoglu et al., 1997).

Making Sense of Conflicting Results: Biology of PTSD

Neurobiological Systems

Refugee and community-based research reports conflicting findings regarding the prevalence rate of PTSD, but consistently, only a subset of individuals develop the disorder (Brewin et al., 2000; Hollifield et al., 2002; Kessler et al., 2005; Ozer et al., 2003. The prevalence rate of PTSD in Refugees ranges from 3% to 86% (Hauff et al., 1994). An attempt to understand this variation has been made through the in-depth examination of pre-trauma, peri-trauma, and post-trauma factors; intrinsic risk and resiliency; and the dose-response relationship between trauma and symptoms. In addition to these factors, the biological and neurobiological implications of PTSD have been examined to explain the varying findings regarding PTSD development. Yehuda et al. (1999) have highlighted that several neurobiological systems are taxed in cases of PTSD, often leading to impairment. The brain structures that have been of primary interest in identifying the structural neuroanatomy of PTSD are the hippocampus and the limbic system (Sala et al., 2004; Stam et al., 2007)

Individuals are generally able to achieve adaptation following stress. However, chronic stress becomes debilitating and reduces one's ability to reach adaptation (Arnetz & Ekman, 2006). When the body is unable to regain proper homeostasis, an individual's stress system becomes altered and unable to respond appropriately to future stress exposure. Alterations in the brain after stressful events are reflected in the brain's structure. Animal studies have shown a relationship between stress and hippocampal

atrophy (McEwen & Magarinos, 1997) and impaired hippocampal neurogenesis (McEwen, 2001). In human studies, individuals with PTSD showed a reduction in the hippocampal volume. This was true both in veterans (Bremner et al., 1995) and in non-combat-related populations (Stein et al., 1997). However, the findings are inconclusive; other studies show no reduction in hippocampal volume among those exposed to trauma that develop PTSD as compared to those that do not develop the disorder (Bonne et al., 2001). Other explanations include the complexity of comorbid psychiatric disorders and their effects on the brain (Karl et al., 2006).

Other brain areas have been implicated in PTSD, including the amygdala (Stam et al., 2007), though these findings are also inconclusive. The functional response of the central nervous system, rather than its structures, may provide more answers regarding the development of PTSD. As parts of the limbic system, the functional responses of the dopaminergic and serotonergic systems are implicated in PTSD. The dysregulation of the serotonin receptor ligands (5HT or 5-hydroxytrpatamine) can lead to panic symptoms in individuals with PTSD and hypersensitivity (Southwick et al., 1997). The functional aspect of the dopaminergic system regulates the acquisition and consolidation of fear experiences, which may provide insight into the system's relationship with fear extinguishing and poor coping in individuals with PTSD (Pezze & Feldon, 2004; Stam, 2007).

HPA-Axis

The neuroendocrine system that is implicated in PTSD is the hypothalamic-pituitary-adrenal axis (HPA axis) (Mehta & Binder, 2013; Stam, 2007). The HPA axis is the main system that regulates stress response, and it is also involved in regulating the

immune system, mood, and emotion. A stressful exposure triggers the release of the corticotrophin-releasing hormone (CRH) from the hypothalamus, motivating the release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn triggers the release of glucocorticoids from the adrenal glands (Arnetz & Ekman, 2006; Mehta & Binder, 2012). An example of a glucocorticoid in humans is cortisol. Glucocorticoids have two receptors, mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) (Mifsud et al., 2011). MRs are involved in the appraisal of stress and the acute onset of stress. GRs are implicated in adaptation to and recovery from stress. The glucocorticoids bind to their receptors, beginning negative feedback regulation that targets the hypothalamus and hippocampus in order to regain homeostasis following stress exposure. GRs are responsible for terminating stress through this feedback regulation, making them crucial in the regulation of the HPA axis (Arnetz & Ekman, 2006; Mehta & Binder, 2012; Yehuda, 2001; Yehuda et al., 2004). Additionally, it has been shown that there is an increase in the activation of CRH and altered baseline cortisol levels in cases of PTSD (Yehuda & Seckl, 2011).

Individuals with PTSD often show a low urinary or salivary cortisol levels (Yehuda, 2002; Oquendo et al., 2003). However, recent research has shown that normal, as well as higher, levels of salivary and urinary cortisol have been reported in individuals with PTSD, suggesting that there is no static level of cortisol in PTSD but that the HPA is likely in a state of dysregulation, both in the upward and downward directions (Stam, 2007). Additionally, due to the fact that PTSD is manifested following trauma exposure, it remains unclear whether these changes are the consequences of that trauma or whether they are risk factors for that trauma (Radant et al., 2011). Even if they were the

consequences of the trauma, they do not provide biomarkers that would indicate the risk of developing post-traumatic stress disorder (Heinzelmann & Gill, 2013).

Molecular Genetic Studies

A particular interest of late is identifying candidate genes that individuals with PTSD share (Broekman et al., 2007). A review article by Domschke (2012) focusing on the pathogenesis of PTSD revealed that there have been nearly 40 molecular genetic studies to date that have investigated the role of single genetic markers in the development of PTSD.

The majority of the studies revealed that there are polymorphisms in specific candidate genes that are more frequent in those with PTSD (Domschke, 2012). There are discrepancies in the findings of these studies. A review of the variations in response following trauma (Yehuda & LeDoux, 2007) suggests that searching for brain mechanisms and genetic polymorphisms in PTSD is complex. Epigenetic research may provide new insight. The heterogeneity among individuals' phenotypic presentation of PTSD demands comprehensive research on traumatized populations, taking into account the environment and biology simultaneously in an effort to examine biomarkers through a comparison of trauma, individuals' self-reports, and cellular expression. Epigenetics may provide insight into how these multiple systems in PTSD are related (Yehuda & LeDoux, 2007).

The Shift from Genetic to Epigenetic Research

One of the major factors that influences epigenetic change is stress, a primary feature of PTSD. Research has linked epigenetic changes to the onset of PTSD. As such, epigenetics can shed light into the molecular underpinnings of the impact of stress

exposure on a cellular level (reviewed in Toyokawa et al., 2012). The research reviewed suggests that a comprehensive understanding of PTSD is needed in order to assess development risk (Mehta, & Binder, 2012), as well as provide insights into potential treatments (Yehuda et al., 2013). The traditional understanding of PTSD has included genetic vulnerability, such as candidate genes, environmental risk factors, such as prior trauma or stress; and trauma, such as exposure to war. Thus, the altered genes result in altered brain activity, which results in the phenotypic expression of psychiatric disorders (Toyokawa et al., 2012). A more integrative approach to studying PTSD would be examining PTSD symptoms in relation to the epigenetic modification of PTSD candidate genes while taking into account trauma exposure, as well as protective factors that may modulate symptoms.

Research has revealed new pathways of epigenetic controls in a variety of disorders (Ressler, et al., 2011; Uddin et al., 2010; Koenen et al., 2011). However, questions still remain regarding how the epigenetic process is manipulated by the environment (Allis et al., 2009). Knowledge regarding protective factors may provide more clarity. Epigenetic markers instruct the DNA to regulate the activation or silencing of genes. Epigenetic modifications, such as DNA methylation, can explain the link between pre-trauma factors and the development of PTSD by taking into account how stressful experiences may alter molecular pathways. Epigenetic modifications could play a vital role in mediating the psychosocial and biological factors in PTSD, which may help us to understand the interconnections among the systems implicated in PTSD. For instance, PTSD has been associated with the alteration of the HPA axis's activity; however, new insights have been gained from research into epigenetic modifications,

such as DNA methylation, because it plays a role in the deregulation of the genes that are responsible for regulating the HPA axis (Weaver et al., 2004; Weaver et al., 2002). Heinzelmann and Gill (2013) reviewed the epigenetic mechanisms within PTSD; six DNA methylation studies of candidate genes have reported significant aberrations in both combat-exposed veterans and community samples.

It is crucial to examine DNA methylation levels in the candidate genes of individuals who report PTSD symptoms as compared to those who do not, among individuals who have similar traumatic events or stressful experiences. A promising design would be to investigate DNA methylation changes in the same group of individuals, taking into account protective factors that may shield them from developing PTSD, while also investigating whether these factors are reflected in the methylation levels in the epigenome. Due to the fact that epigenetics does not alter the DNA sequences, it is often reversible, providing a potential new frontier in the treatment of diseases.

Epigenetics

The science of epigenetics involves the control of gene expression that is independent of the DNA sequence (Holliday, 1994). The DNA provides a blueprint in the form of genetic material, and epigenetic processes affect the expression of genes, determining whether they become active or inactive (also referred to as "silent") (Kovalchuck & Kovalchuck, 2012). Epigenetic markers represent the layer of instructional information that is "above" the genetic material (Allis et al., 2009).

The definition of epigenetics is dependent upon the differentiation between the genome and the epigenome (Allis et al., 2009). The genome is the DNA sequence, or the

double helix, while the epigenome is the overall chromatin configuration, which contains the entire genome in a cell (Allis et al., 2009). This differentiates the epigenomic cellular differentiation process, or non-Mendelian inheritance, from the classical Mendelian inheritance of phenotypic traits, which are often the result of allelic differences caused by mutations in the DNA sequence. In contrast, non-Mendelian inheritance can manifest due to the expression of one or two alleles within the same nuclear environment. Epigenetic modifications change gene transcription (Allis et al., 2009). Gene transcription is the first step in cell expression, and it involves copying segments of DNA into RNA (Kovalchuck & Kovalchuck, 2012). The epigenetic modification process is completed through the alteration of chromatin. Chromatin is the combination of DNA and the proteins that make up the nucleus of a cell (Kovalchuck & Kovalchuck, 2012); a further description will be given below.

The epigenetic process is crucial to ensuring normative cellular expression at different points of mammalian development (Kovalchuck & Kovalchuck, 2012). Epigenome diversification is a necessary process because it is responsible for cellular differentiation (Allis et al., 2012). For example, one of these points in development occurs following fertilization in mammals; the process begins with a single genome that becomes epigenetically programmed to produce a multitude of distinct epigenomes in more than 200 different cell types (Kovalchuck & Kovalchuck, 2012). The epigenetic markers are vital in the differentiation and development of various cell types because they formulate and interpret the genome. They mark the start and end of the genes, ensuring that the chromosome is folded correctly, which plays a vital role in cellular expression (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012). While there are a

number of epigenetic modifications (Holliday, 1994), the proposed study will focus on DNA methylation. Other processes, such as chromatin remodeling and histone modification, will not be reviewed.

Methylation marks or patterns are crucial to appropriate cellular transcription (Allis et al., 2009). This process is essential during gametogenesis and early post-fertilization development. DNA methylation is important in maintaining various gene expressions at certain points of development, such as tissue-specific gene expression (Stein, 2004; Kovalchuck & Kovalchuck, 2012). For example, DNA methylation occurs during embryonic development by establishing a marker or pattern that is copied during cellular division (Kovalchuck & Kovalchuck, 2012).

This process allows for epigenetic patterns in the genome to be stable for multiple cell divisions (Allis et al., 2009). The first methylation imprints occurs during embryonic development, immediately after implantation. The second wave of *de novo* DNA methylation occurs later during the post-implantation development of cells (Ling et al., 2004). After early DNA methylation waves, the DNA methylation marks are removed, which is called the de-methylation of DNA, to allow for cellular differentiation and access to more genetic material in the cell (Allis et al., 2009). DNA methylation becomes more specific throughout later development, with patterns being established in the genes of various cell types; ultimately, the goal is to have only a specific subsets of cells expressed (Kovalchuck & Kovalchuck, 2012).

DNA methylation is also important in the production and regulation of neural stem cells and their differentiation into neurons and glial cells, making it a crucial aspect of brain development and consequently psychiatric diseases (Ratten, & Mill, 2009; Xin et

al., 2012). While DNA methylation has been indicated in early development and cell differentiation (Kovalchuck & Kovalchuck, 2012; Weaver, 2009), DNA methylation can occur throughout life and is influenced by internal and external factors (Dudley et al., 2011; Jaenisch & Bird, 2003; Tammen, Friso, Choi, 2012), as described below.

While epigenetic changes are implicated in heredity (Yehuda et al., 2008), the organism's interaction with the environment, rather than DNA, is the fundamental basis for these changes (Dudley et al., 2011; Jaenisch & Bird, 2003). The epigenome shows plasticity during development, interacting with the environment, which makes it susceptible to irregular gene transcription, resulting in an array of human diseases (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012; Tammen, Friso, Choi, 2012). To better understand this process, it will be useful to review DNA structure and the role it plays in epigenetics.

DNA Structure

DNA is a macromolecule containing genetic information. It is organized into 23 chromosome pairs consisting of 25,000 genes, which generate 200 cell types in mammals (Allis et al., 2009) Chromosome pairs inherited from parents consist of 25,000 genes in mammals, which are organized into DNA sequences (Stein, 2004). These DNA sequences are composed of four bases, guanine, adenine, thymine, and cytosine, which are represented by the letters A, C, G, and T; the order of the bases gives rise to well-defined genes (Kovalchuck & Kovalchuck, 2012.

The DNA molecule is nearly 2 meters long, but is condensed down to 10 micrometers in order to fit into the cell's nucleolus, where the genetic information is stored (Kovalchuck & Kovalchuck, 2012). The DNA is wrapped around spools of

specialized proteins, histone proteins, resulting in a repeating protein structure known as chromatin (Luger et al., 1997). The smallest unit of chromatin is a nucleosome, which is wrapped around a histone octomer, which is made up of eight histone molecules. The octomer acts as a spool and is linked together by an exterior histone called H1, which is essential for locking the DNA material as it wraps around the histones (Kovalchuck & Kovalchuck, 2012). The DNA makes one and three-fourth turns around the histone octomer. The interaction between the structures is maintained by positively charged histones binding to negatively charged DNA (Kovalchuck & Kovalchuck, 2012). Histone proteins can be modified, which becomes important in cellular transcription (Allis et al., 2009).

Although the packaging of DNA enables an ordered structure, it also makes it difficult to access, which is crucial for DNA transcription, DNA replication, and DNA repair (Kovalchuck & Kovalchuck, 2012). Within the 25,000 genes, only a small subset of genes are expressed in cells. The 200 cell types contain identical genetic material; however, they are differentiated and maintain unique cellular identities (Kovalchuck & Kovalchuck, 2012). The genes expressed define a cell's type and function (Allis et al., 2009). This is achieved via cellular transcription, in which certain types of genes are made accessible by the way in which the DNA is arranged (Kovalchuck & Kovalchuck, 2012). The chromatin provides an ordered, genome-organizing platform, adding a multi-dimensional layer to the readout of the DNA (Luger et al., 1997). The folding pattern of the DNA into chromatin provides the basis for gene activity (Allis et al., 2009).

The "tightness" of the chromatin relates to the accessibility of DNA (Li, 2002; Kovalchuck & Kovalchuck, 2012). Highly condensed, or tightly held, chromatin fibers

are referred to as heterochromatin. This means that DNA is less accessible to the transcriptional machinery, such as RNA polymerase (Kovalchuck & Kovalchuck, 2012). On the other hand, euchromatin refers to the fibers being "loosely" or less compacted and is associated with the genes being more easily expressed. Thus, the tightness of chromatin compaction is negatively correlated with transcription activity (Kovalchuck & Kovalchuck, 2012). Heterochromatin has several functions, including silencing genes and aiding in the structural integrity of the genome (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012). Epigenetic components, specifically DNA methylation, provide instructions during gene expression, without altering the DNA sequence (Allis et al., 2009).

Regulation of Gene Expression Through DNA Methylation

One method of transcription control is altering the chromatin polymer through the methylation of specific DNA sites called CpG dinucleotide. They are found in areas of the DNA that are saturated in cytosine-guanine dinucleotide repeats (Laird and Jaenisch, 1996). A CpG dinucleotide is made up of cytosine and guanine separated by phosphate. CpG dinucleotide forms into CpG islands and is found in the promoter, or the start region, of the gene (Kovalchuck & Kovalchuck, 2012; Li, 2002). This is a pivotal location because the promoter site is where transcription takes place. CpG islands are present in 60% of human gene promoters (Allis et al., 2009).

DNA methylation, particularly in the brain, is catalyzed by DNA methyltransferases (DNMTs) (Li, 2002). There are three types of DNMTs: DNMT1, DNMT3A, and DNMT3B. DNMT3A and DNMT3B are important in establishing new methylation sites in embryos (Kovalchuck & Kovalchuck, 2012). DNMTs are present

throughout neural development and stimulate neuronal survival and plasticity (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012). DNMT1 is responsible for maintaining the correct methylation pattern throughout development, thus playing a pivotal role in preventing cellular mutation and disease development (Allis et al., 2009). DNMTs are enzymes that interact with DNA that is initially un-methylated; they lay down methyl marks during certain points of development. DNMTs play an important role in providing instructions for methylation. They are able to catalyze at novel sites (also referred to as *de novo*) or maintain methylation following DNA replication (Kovalchuck & Kovalchuck, 2012). This complex process is initiated by DNMT turning the cyto sine residue out of the DNA helix, allowing the interaction of the base with the methyl donor. The resulting molecule is referred to as S-adenosly-L-methionine (SAM), which forms 5-methylcytonsine (Kovalchuck & Kovalchuck, 2012).

DNA methylation requires the addition of a methyl group directly to the cytosine (c) base in the DNA template (Kovalchuck & Kovalchuck, 2012). This dynamic process provides a docking site via which proteins alter the chromatin's state. DNA methylation occurs at the CpG dinucleotide because of its symmetrical physical properties, allowing for genetic pattern maintenance throughout cell division (Allis et al., 2009). Cell expression is affected when methylation within the promoter regions of the cell results in the binding of methyl-CpG-binding domain proteins (MBDs) to the region, which results in preventing transcription factors from binding to promoter sequences (Allis et al., 2009). Therefore, DNA methylation is responsible for setting patterns for the sites of chromatin compaction and gene silencing (Allis et al., 2009). Importantly, MBDs were found to play an important role in brain development, memory, and learning (Mehler,

2008).

Methylation is present along the genome (Kovalchuck & Kovalchuck, 2012). However, the CpG islands remain neutral and are protected from methylation. Due to the fact that CpG sites occur in the promoter regions and transcription start sites, methylation deregulation may be indicative of changed transcription states for the targeted genes (Kovalchuck & Kovalchuck, 2012). Research has linked abnormally methylated genetic promoter sites to human diseases, and the environment plays an important role in DNA methylation patterns and the phenotypic expression of disease (Allis et al., 2009; Jaenisch & Bird, 2003; Dudley et al., 2011; Kovalchuck & Kovalchuck, 2012). Environmental factors and disease development in relation to epigenetics will be discussed below.

Epigenetics and Aging

Studies show that the aging process is related to specific DNA methylation patterns (Horvath et al., 2010; Zaghlool, 2015; Fraga & Esteller, 2007; Fraga et al., 2005). DNA methylation occurs at specific points in human development, starting in utero and, later, in individual's lifetime, such as in puberty and menopause (Allis et al., 2009). Genome-wide DNA methylation alterations occur to prepare the body for these vital periods in development throughout the aging process (Allis et al., 2009). As such, these genome-wide alterations allow for proper genetic pattern maintenance.

The aging process and the diseases that are related to it are often associated with hypermethylation in the promoter regions of genes, but genome-wide hypomethylation (Johnson et al., 2012). This process can also be impacted by environmental factors. A study, investigating DNA methylation and aging, found that younger twins had similar methylomes, while older twins had remarkably different methylomes in, both, genome-

wide and gene-specific DNA methylation analyses (Fraga et al., 2005). This study adds to a growing consensus in epigenetic research, suggesting that the changes occurring in the epigenome during the aging process are also a response to environmental influences (Tammen, Friso, Choi, 2012, Toyokawa et al., 2012). Therefore, one's age is not only important to include in epigenetic studies to control for age related differences, but DNA methylation can be used as a biomarker for diseases that are associated with the aging process.

Epigenetics and Disease

There are times when epigenetic modification does not follow the normal course of development, resulting in the deregulation of DNA methylation, which affects the integrity of the genome (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012). When a genome that is normally methylated becomes un-methylated, this leads to global genomic instability. The alterations are implicated in cell damage and negative physiological effects, which can be phenotypically expressed. The deregulation of DNA methylation is implicated in several diseases, such as immunodeficiency, Alzheimer's, autism, and cancer (Allis et al., 2009; Graff, & Mansay, 2009; Schaner, 2006; Petronis, 2010). The findings suggest that more research is needed to better understand the methylation patterns and the activation and silencing of candidate genes. Initially, some of these diseases were believed to be due to genetic mutations, but now, research has shown that DNA methylation impairment is linked to the genetic sites that are in question (Petronis, 2010).

Epigenetics and Environmental Effects

DNA methylation occurs throughout life, and improper methylation patterns can

threaten cellular integrity and affect phenotypic expression and disease manifestation (Kovalchuck & Kovalchuck, 2012). The study of epigenetic modification and human diseases represents a new frontier in medicine that impacts the concept of the disease progression, particularly in psychiatric disorders (Dudley et al., 2011; Galea, Uddin, and Koenen, 2011; Ratten, & Mill, 2009). While disease has traditionally been attributed to mutagenic inheritance within the DNA sequence, new research shows that abnormal DNA methylation can mimic DNA mutations. Research on monozygotic twin differences in disease phenotypes shows that there is a difference in the distribution of 5' methyl-cytosine and acetylated histones, providing insight into epigenetic variability (Fraga et al., 2005). DNA variation may not provide a comprehensive explanation of phenotypic variations. Environmental factors may play a role in disease development (Dudley et al., 2011; Toyokawa et al., 2012), a finding that has been demonstrated by examining psychiatric disorders (Petronis, 2004). The study of environment interaction remains in its infancy; however, some epigenetic research has focused on early maternal behavior, early experiences, and diet (Allis et al., 2009).

Early experiences have been indicated to be responsible for epigenetic modifications (Szyf, 2009). This relationship was first demonstrated in animal models. Weaver et al. (2004) demonstrated that mice that showed high maternal behavior, defined by frequent licking and grooming, altered the DNA methylation of their pups, specifically in the glucocorticoid receptor gene within the hippocampus. DNA methylation plays a crucial role in brain development, cognitive functioning, and neuroplasticity (Mehler, 2008; Graff & Mansay, 2009; Petronis, 2010). These pups had decreased DNA methylation and increased histone acetylation (an epigenetic process involved in cellular

regulation) in the promoter region of the GR gene as compared to the pups whose mothers were less attentive. This epigenomic change, specific to the GR region, an important region for the regulation of stress, was found to persist into adulthood (Weaver et al., 2004). Although this is a new field in human research, it may provide insight into psychiatric disorder susceptibility in humans.

Epigenetic Research on PTSD

Gene studies regarding PTSD remain inconclusive (Skelton, et al., 2012). A review article on the pathogenesis of PTSD revealed that there have been nearly 40 molecular genetic studies to date that have investigated single genes contributing to the risk of PTSD (Domschke, 2012). The majority of the studies revealed gene-specific polymorphisms in PTSD; however, the results also indicate discrepancies. Molecular genetic research has consistently identified allelic risk factors in PTSD: the s or short alleles in genes specific to PTSD predisposed individuals to developing the disorder. However, it appears that there is an interaction with the environment as well. Studies have examined the relationship between gene and environment to pinpoint risk factors (Skelton, et al., 2012). Kilpatrick et al. (2007) studied individuals exposed to the 2007 hurricanes in Florida and found that those who had an s allele genotype in the serotonin transporter gene (specifically, locus 5-HTTLPR) showed an increased risk of PTSD, but only in those who were living in an environment of high stress, which included low SES and direct exposure to the hurricane. Research conducted on the same population revealed that those with the s allele were more likely to develop PTSD when living in a high-risk environment with high levels of unemployment (Koenen et al., 2009).

One of the few studies that were conducted on a refugee sample of Rwanda

genocide survivors revealed that carriers of the s allele in the serotonin receptor gene were at a higher risk of developing PTSD, even at lower levels of exposure to traumatic events. However, those that were carriers of the l allele showed a dose-response curve with an increasing risk of developing PTSD with cumulative trauma (Kolassa et al, 2010).

While the majority of the research on PTSD has involved molecular genetics and specific polymorphisms, a smaller subset of studies are investigating the role of DNA methylation in PTSD, offering insight into the *expression* of genes that are implicated in PTSD. PTSD symptoms have been linked to abnormalities in the functions of the hippocampus, medial prefrontal cortex, and amygdala (Bremner et al., 2008). DNA methylation in the brain is important because research has shown that Methyl CpG binding domains play an important role in brain development, learning, and memory (Mehler, 2008). Similarly, DNA methylation has been indicated in synaptic plasticity, learning, and memory in the adult central nervous system (Feng et al., 2010). DNA methylation may help explain pre-trauma factors and the development of PTSD by taking into account how the environment may alter molecular pathways. For instance, PTSD has been associated with the alteration of HPA axis activity, which might involve DNA methylation playing a role in the deregulation of the genes that are responsible for regulating the HPA axis following stress exposure (Weaver et al., 2004).

Individuals with PTSD in a community sample showed various DNA methylation levels based on their exposure to potentially traumatic events in an urban environment (Uddin et al., 2010). A genome-wide analysis was conducted with data from a community epidemiological study in Detroit, MI (Detroit Neighborhood Health Study)

(Uddin et al., 2010). Levels of methylation were tested for correlation with the number of traumatic events in 14,000 genes in individuals who had reported PTSD symptoms. Individuals with PTSD had increased methylation in more genes than those who did not report PTSD symptoms. Individuals with PTSD had six times as many genes with significant negative correlation and seven times as many genes with significant positive association between the methylation level and the number of potential traumatic events (Uddin et al., 2010). Research conducted in the same epidemiological study also revealed that cumulative trauma has been identified with gene-specific methylation levels, specifically in the SLC6A4 gene (Koenen et al., 2011). Furthermore, DNA methylation patterns have been shown to change within a period of 90 minutes in individuals who were exposed to social stress in laboratory experiments, with methylation levels fluctuating between pre- and post-stress exposure (Unternaehrer et al., 2012). These results have been replicated in studies with mice as well (Roth et al., 2011).

These studies revealed the importance of environmental factors. Examining environmental factors that can influence the onset and severity of the phenotypic expression of PTSD is critical in understanding its risk factors. One important environmental factor is stress exposure. Stress has been described as eliciting psychological responses, and it increases the risk of mental illness. Exposure to stress in early development has been shown to impact development in both animal and human models (McGowen et al., 2009; Weaver et al., 2004). However, research reveals that stress effects are not confined to early life experiences (Weaver et al., 2004). Exposure to stress or potentially threatening events may involve DNA methylation in areas that are indicated in the neurobiology of PTSD. This is evident due to the allele-specific DNA

methylation in these regions. During exposure to stress, the body's HPA axis is designed to protect the organism. However, prolonged exposure to stress can led to deregulation, affecting cellular integrity, and may be expressed in the form of psychiatric symptoms (Stam, 2007).

However, only a subset of studies on trauma and stress has examined DNA methylation, and an even smaller number have tested gene-environment interaction and the implicated alteration of phenotypic symptoms in PTSD (Domschke, 2012). Therefore, it is possible that epigenetic effects may be important. Polymorphism in PTSD candidate genes and these methylation patterns may be linked (Uddin et al, 2010); it is possible that a complete understanding of PTSD risk lies in understanding these methylation patterns (Dudley, et al., 2011). As mentioned above, studies have linked candidate genes with epigenetic modifications in those same genes. Therefore, this review will expand upon the candidate genes that have been implicated in both molecular and epigenetic research involving PTSD (Meaney & Ferguson-Smith, 2010). Skelton et al. (2012) reviewed genetic variants in PTSD, of which four systems were most prevalent. The genes that have been identified were associated with the HPA axis, the dopaminergic system, and the serotonergic system, which coincide with research on the biological, neurobiological, and neuroendocrine systems.

HPA and Epigenetics

Yehuda (2010) stated that epigenetic mechanisms occur in the HPA axis following a traumatic event, are marked in the HPA system, and can become triggered later in life. Thus, the HPA axis fails to constrain stress experiences, leading to an elevation in the implicated neurotransmitters, which then leads to physiological arousal

and distress. The epigenetic changes are believed to mark a set point that becomes triggered when one experiences future trauma. Neuroendocrine studies reveal that the development of PTSD following trauma exposure is associated with pre-traumatic biological markers that reflect prior sensitization to stress (Yehuda et al., 1999). Chronic stress has been shown to result in the methylation of genes that are associated with the hippocampal structure by altering chromatin, which can become a risk factor for stress vulnerability in adulthood (Hunter et al., 2009). The modification of chromatin through methylation can decrease the plasticity of the hippocampus in the face of future stress exposure.

A dysfunction in this system may make an individual vulnerable to stress and psychiatric disorders such as PTSD because it regulates the psychological and physiological experiences of stress (Brunson et al., 2001; Stam, 2007). While previous research has suggested a downward dysfunction in the HPA axis, such as low cortisol levels, in individuals with PTSD, recent findings suggest that there is no static direction for the byproduct of the HPA axis. Rather, there is dysregulation in both directions (Stam, 2007). The direction of HPA alterations may depend on the characteristics of the stressors, the duration of stress, the type of stress, and timing (Stam, 2007; Stein et al., 1997). Genes that regulate HPA axis activity are influenced by early life events, as evidenced by maternal animal models. Weaver et al. (2004) demonstrated that pups that experienced high levels of maternal care were more resilient in the face of stress into adulthood. These early experiences altered HPA function and hippocampal GR expression (Stam, 2007; Weaver et al., 2004). These animal findings are aligned with PTSD research in humans. Research has consistently found that adverse early life events

place individuals at a higher rate of developing PTSD (Brewin et al., 2000; Breslau et al., 2009; Kessler et al., 2005; Ozer et al., 2003). This could be an important indicator in refugee research because war exposure and subsequent chronic stress may alter the HPA axis in different ways.

Relatively stable changes in methylation could explain the chronicity and persistence of the symptoms observed in PTSD (Yehuda et al., 2002). Epigenetic methylation has been implicated in the regulation of HPA axis genes, which could provide a molecular explanation for such variations in PTSD levels (Yehuda et al., 2013). In fact, early adverse experiences in humans have been found to lead to hyper-methylation, as well as the demethylation of specific genes associated with the glucocorticoid promoter in hippocampal neurons (McGowen et al., 2009). These findings suggest that environmental exposure regulates epigenetic changes. Thus, the associated epigenetic states may represent a molecular mechanism responsible for altering subsequent responses to environmental factors (Yehuda et al., 2010). Moreover, Several HPA axis genes have been shown to interact, as well as being altered by environmental factors, increasing the risk of developing PTSD. Furthermore, HPA axis reactivity has been shown to be moderated by a number of genetic polymorphisms, including variants in the gene encoding of FK506 binding protein 51 (FKBP5) and the clear receptor subfamily 3, group C, member 1 (NR3C1) (Metha & Binder, 2012 for review).

Evidence for Candidate Genes and Methylation Patterns in PTSD

HPA-Axis Genes

KFBP5. There have been studies on a number of polymorphisms of HPA axis genes, and some of these genes have been studied in regards to DNA methylation.

Several research studies have implicated a candidate gene, FKBP5, or FK506 binding protein 51, an important functional regulator in the GR complex (Grad and Picard, 2007). KFBP5 plays an important part in the stress hormone system, aiding in protein folding. The gene regulates intracellular GR signaling and helps restrict GR transcription to the nucleus, playing an important role in both the genetic and epigenetic expression of PTSD symptoms.

Studies have indicated that a polymorphism in KFBP5 is associated with PTSD, especially in adults who reported adverse early life trauma (Xie et al., 2010). Xie et al. (2010) examined European Americans and African Americans with a history of childhood abuse. The results revealed that PTSD risk among African Americans was associated with having a single-nucleotide polymorphisms (SNP) or a genetic variation in the Tolloid-Like 1 gene (TLL1), which expressed in the cerebellum. The cerebellum is implicated in HPA axis functioning. For European Americans, PTSD and SNP significance were mediated by the presence of other early stressful experiences in addition to childhood abuse. This suggests that the presence of SNPs in individuals with a history of abuse makes them more susceptible to developing PTSD following trauma in adulthood. This study points to the importance of taking environmental factors into account and examining the role of DNA methylation. Studies have shown similar results regarding stressful life experiences that occurred in adulthood (Mehta & Binder, 2012)

These findings are in accordance with research that has linked KFBP5 with symptom manifestation, in which low levels of expression of the gene were associated with current PTSD symptom severity (Yehuda et al., 2009). Yang et al. (2012) found methylation of FKBP5, as well as, a decreased Dnmt1 expression, in areas of the

hippocampus in PTSD-inflicted mice. Such methylation patterns have been confirmed in human studies, showing an interaction between trauma experienced in early life and the demethylation of FKBP5 (Klengel et al. 2013). The methylation of certain CpG sites across the FKBP5 gene interacts with the polymorphism of the gene and adverse childhood experiences (Klengel et al. 2013); this interaction modifies the sensitivity of the gene to GR regulation. This is a good example of the fact that specific polymorphisms of candidate genes have been found to show aberrant methylation patterns. Such studies demonstrate the potential for comprehensive research into the cellular changes correlated with PTSD. In an effort to understand the clinical relevance of epigenetic research, psychologists are increasingly interested in utilizing DNA methylation research to examine the association between epigenetic methylation profiles and psychotherapy (Yehuda et al., 2013).

NR3C1. Another gene that is implicated in the regulation of the HPA axis is NR3C1, the glucocorticoid receptor gene. Molecular genetic research revealed differences in the telomere length of the NR3C1 gene based on stressful life situations (Metha & Binder, 2012: Drury et al., 2011). Children who were orphaned and were placed in high-quality foster care were compared to children who remained in institutions; differences in telomere lengths were found (Drury et al., 2011). Additionally, DNA methylation has been replicated in numerous studies of the NR3C1 gene in both animal and human models (Perroud et al., 2008; Weaver et al., 2004).

Environmental factors appeared to be associated with DNA methylation at the promoter region of the NR3C1 gene in studies of mice that investigated maternal care (Weaver et al., 2004). In humans, a study of NR3C1 and mood disorders was conducted

in which mothers in their third trimester were assessed for depression. The results revealed that prenatal exposure to the mother's mood was associated with the alteration of the HPA axis in children three months after birth. Infants showed increased salivary cortisol stress and higher methylation levels at the CpG site of the NR3C1 gene than infants whose mothers were not depressed (Oberlander et al., 2008). Furthermore, victims of suicide who had a history of adverse childhood experiences or neglect had higher NR3C1 methylation in the promoter region than others (McGowen et al., 2009).

Perroud et al. (2011) investigated an NR3C1 promoter site and the severity of childhood maltreatment in adults who had been diagnosed with Borderline Personality Disorder (BPD) and major depressive disorder (MDD) A subset of individuals with BPD also met the diagnostic criteria for PTSD. It was found that those with BPD who reported repeated childhood abuse and sexual abuse had higher methylation levels of the NR3C1 gene. This suggests that the gene may be a marker for the severity of abuse. McGowen et al. (2009) also revealed that sexual abuse was associated with higher methylation levels at the NR3C1 promoter site. Essentially, these studies provide evidence that chronic stress and maltreatment alters the HPA axis throughout life via epigenetic modification.

Limbic Frontal System

As discussed above, the results remain inconclusive in regards to the neuroanatomy of PTSD. However, functional rather than anatomical changes in the brain are associated with PTSD symptoms. This is evident in the limbic frontal system, which involves complex neurocircuitory within the brain. Two parts of the limbic frontal system are the dopaminergic and serotonergic systems, which have also been implicated in epigenetic research (Skeleton et al., 2012).

Dopaminergic System Gene

SLC6A3. There appears to be literature supporting dopaminergic transmission in response to stress. Several genes have been implicated, but most evidence indicates SLC6A3 (solute carrier family 6), which is also known as DAT and DAT1. This gene is responsible for encoding the dopamine transporter, which aids in the regulation of dopaminergic neutron transmission by regulating the reuptake of dopamine from the synaptic cleft (Bonnon et al., 2001). The dopamine transporter may be important in PTSD because there is a correlation between dopamine concentration and PTSD symptoms (Yehuda et al., 1992). The polymorphism of SLC6A3 has been associated with PTSD (Skeleton et al., 2012) and combat exposure (Coming et al., 1991).

Cheng et al. (2012) conducted the only study that investigated the interaction between the genetic polymorphism and the epigenetic variation of the gene concurrently. Using an urban community sample from the Detroit epidemiological study (Cheng et al., 2012), they revealed that individuals with PTSD have a high incidence of a polymorphism of the gene (9R alleles) and have high levels of DNA methylation of the gene. The researchers noted that it is possible that the allele carriers are at a higher risk of decreased SLC6A3 transcription (Cheng et al., 2012).

Serotonergic System Gene

SLC6A4. Serotonin is another catecholamine neurotransmitter that is important in regulating activity within the limbic frontal system. Serotonin is a potent regulator of emotional behavior, as well as stress-responsive hormones in the amygdala (Wang et al., 2011). The serotonin transporter, 5HTT, is triggered by SSRI medications, common treatments for PTSD. SLC6A4 is the single gene that codes for 5HTT (Wang et al.,

2011). Studies examined a polymorphism in the promoter region of the serotonin transporter (5-HTTLPR) (Lee et al., 2007). It is believed that the s allele leads to lower levels of 5HTT expression, as well as reduced serotonin uptake, which may lead to a higher prevalence of PTSD symptoms. Shorter alleles have been found to be associated with the decoupling of the interaction between the prefrontal cortex and the amygdala. These two regions are crucial for fear response and fear extinction, dysfunctions that are hallmarks of PTSD.

One molecular genetic study linked a 5HTTLPR polymorphism to PTSD (Wang et al., 2011). This study of 212 combat veterans who were diagnosed with PTSD compared to 176 of veterans that did not have PTSD showed that the s allele was associated with the risk of developing PTSD. This study, however, did not find any influence on the part of race, age, or level of combat trauma (Wang et al., 2011). Molecular genetic studies also examined environmental exposure and PTSD risk. Adults who were exposed to natural disasters, such as hurricanes, were more likely to develop PTSD if they carried the s/s genotype, had a high level of exposure to hurricanes, or were experiencing reduced social support (Kilpatrick, 2007). Rwandan genocide survivors who carried the s/s genotype were at a higher risk of developing PTSD, even when faced with low levels of exposure to traumatic events (Kolassa et al., 2010). In a community sample, individuals with the s/s genotype were more likely to develop PTSD when living in an environment with high levels of crime than those with other genotypes (Koenen et al., 2009).

The methylation of SLC6A4 was examined in several studies. The research revealed that the differential methylation of neurotransmitter genes is linked to PTSD

onset. In two studies using samples from the urban community population from the Detroit Neighborhood Health Study, SLC6A4 methylation was implicated as a risk factor for PTSD (Uddin et al., 2011). It was also found that individuals with more traumatic events were at an increased risk of PTSD but only when there were also lower methylation levels in SLC6A4. At higher methylation levels, individuals who reported more traumatic events were protected from this disorder, suggesting that the serotonin transporter gene may also be important in trauma-related resilience (Koenen et al., 2011).

Allostatic Load Theory and Epigenetic Processes

The consequences of stress on well-being have been debated in the medical community due to the difficulty of defining stress and quantifying stressful experiences (McEwen & Gianaros, 2011). McEwen (1993; 2000) proposed a comprehensive theory involving allostasis and allostatic load, which examines stress through the interaction of biology and behavior. This interaction starts in utero and continues to influence one's health until death (McEwen, 2007). Therefore, the individual's interpretation of the event and the subsequent response to stress play an important role in how the brain and body respond to stress (McEwen & Steller, 1993). The process can lead to physiological responses, which may influence neural and neuroendocrine responses in the brain and body.

Allostasis theory argues that the body must maintain a state of homeostasis in the face of challenges in the environment and one's subjective evaluation of the stressors, as well as one's behavior, which are registered by the brain *(*McEwen & Gianaros*,* 2011*)*. Stressful experiences can threaten homeostasis and result in an increase in the internal physiological demands of the body (McEwen, 2000). The body, faced with

environmental challenges, cues physiological responses in the form of chemical mediators, including cortisol, as well as biological systems, such as the HPA axis, to face stressful challenges, integrate them, and, adapts to them (McEwen, 2000). This cascade of brain-behavior interaction, intended to aid in adaptation and maintaining the body's boundary for normal regulation, is referred to as allostasis (McEwen & Gianaros, 2011; McEwen, 1993, 2000). Setting new limits for the body is needed to appropriately regulate the stress response in these systems.

Contrastingly, allostatic load refers to the wear and tear on the body and the brain due to chronic stress (McEwen & Stellar, 1993; McEwan, 1998). Allostatic load is reflected in an imbalance in the hormones in the autonomic nervous system, central nervous system, neuroendocrine system, and immune activity (McEwen & Gianaros, 2011). Unrelenting environmental stress can alter the regulation of hormonal mediators, making the systems weaker in response to future stress. When the system is constantly responding to stress, it is taxed and thus more vulnerable to disease. Being unable to confront these stressful situations can "foster a proliferation of recursive neural, physiological, behavioral, cognitive, and emotional changes that increase vulnerability to ill health and premature death by several chronic conditions" (McEwen & Gianaros, 2011). One's reaction to environmental challenges, and subsequently, bodily response will lead to allostatic load and increase risk of disease (McEwen & Stellar, 1993).

McEwen (1993; 2000; 2007) states that the factors that contribute to allostatic load include exposure to multiple stressors, chronic or repeated exposure, lack of adaptation, and prolonged stress response due to delayed shutdown (factors which were reviewed above as related to PTSD). When the allostatic load stops the adaptation to

stress, this prevents neural plasticity in the brain. Prolonged stress can affect the hippocampus, which can affect the regulation of HPA activity, particularly the termination of stress responses. This process results in elevated HPA activity and affects the hippocampus (McEwen & Gianaros, 2011).

Epigenetic modification can help explain the transition from allostasis to allostatic load. Chronic stress affects the hippocampus by causing the plastic remolding of its circuitry. This change has been associated shown through the methylation levels in the hippocampus (Hunter et al., 2009). An animal study that examined acute versus chronic stress exposure's effects on the hippocampus revealed that chronically stressed mice showed a different type of chromatin remodeling, which was found to persist throughout adulthood (Hunter et al., 2009). This provides insight into the mechanistic process of chromatin remodeling and epigenetic modification during both adaptive and maladaptive stress responses in the brain. Chromatin remodeling affects the plasticity of the hippocampus and, in turn, the neuropathogenic effects of stress on the brain.

Much like epigenetic theory, allostatic theory proposes that changes in the hippocampus due to stress are reversible. This possibility is of particular interest in the proposed study because one's own perception of the situation and protective factors utilized may mediate of stress. Allostatic theory also suggests that stress is something that may or may not be harmful, depending on one's level of physiological arousal and the behaviors it elicits, which are influenced by one's interpretation of the situation (McEwen & Gianaros, 2011). The difference between stressors, those that are tolerable versus those that are toxic, depends on an individual's ability to utilize coping mechanisms, suggesting that one can maintain control over the toxicity of the stressor (Lazarus & Folkman, 1984).

Being able to confront stressful situations by learning to adapt can lead to growth, boosting resiliency and good health. Epigenetic processes can mediate that relationship and provide insight into how the environment can be translated into physiological functioning. Hence, epigenetic research can help explain how these diseases ultimately develop on a cellular level. On a molecular level, epigenetic research can help us understand the cascade of changes that is initiated with prolonged exposure of stress, taking into account the interaction between genetic predisposition and environmental factors. These changes have been shown to begin in utero and continue to influence one's health until death (McEwen, 2007).

Significance of the Proposed Study

Stressful experiences can modify physiological responses. Over time, chronic stressful experiences may be associated with epigenetic changes that can alter the expression of genes. While there is a fixed genetic code, genes may become activated or inactivated. This may be the mechanism for the translation of psychosocial stress into phenotypic symptoms, via epigenetics. Thus, investigating epigenetics, particularly DNA methylation, may aid in understanding the interactions between the environment and the body. Specifically, investigating the interaction between environmental exposures, such as trauma, and DNA methylation on PTSD symptoms is an important undertaking in clarifying the etiology of the disease.

PTSD prevalence rates in refugees heterogeneous; with only a subset of individuals develops PTSD following trauma exposure. Epigenetics provides important information regarding the brain-behavior relationship. While a genetic basis for PTSD has been discussed, investigating environmental interactions may provide new insight

into symptom development. DNA methylation may underlie the interactions between several biological and neurological systems that are implicated in PSTD. Gaining a better understanding of the epigenetic process can provide new diagnostic theories and the opportunity for novel PTSD treatments. The long-term goal of this study was to identify means to objectively quantify complex war and trauma exposures and incorporate such knowledge in assessing risk for disease development and developing personalized treatment. Informing intervention is essential in refugee populations because it would aid in integrating the refugees into the host country, reducing the cost burden associated with immigration, in terms of health costs and social costs.

The current study took advantage of the massive influx of Iraqi refugees in the greater Detroit region. This allowed for the opportunity to conduct a study in a population that has been exposed to war-related trauma. There is increasing evidence that war and displacement trauma, as well as psychosocial exposure, disproportionately impact refugee mental health. However, there is a scarcity in epigenetic studies related to PTSD in refugee population, with the majority of the research done in community samples. The lack of research limits our ability to discern the effects of displacement stress on long-term mental health or pathology. Further, investigating the interaction between traumatic events and DNA methylation of candidate genes of PTSD is essential in fully understanding the environmental impact in symptom development and the etiology of the disorder.

This study is novel in that is proposed to explore the utility of linking self-reported data to quantifiable epigenetics signatures in individuals having been exposed to war and subsequent stressors. It is also innovative as it was the first to apply epigenomics

in validating self-reported trauma exposure and symptom response in a refugee population in a unique group comparison design. It compared refugee groups with varying degree of trauma exposure and PTSD symptoms in, both, genome-wide and candidate genes of PTSD epigenetic analysis.

Finally, this study investigated the singular and interactive effect of trauma and DNA methylation on PTSD symptoms. Including the interaction of environmental factors and molecular procceses in predicting post trauma symptom development is a growing interest in the study of epigenetics (e.g., Uddin et al, 2011); signifiying the importance of the interplay between epiegentic and nonchemical environemntal exposure on adverse mental health symptom development.

The aims of this study were to 1) to determine whether varying degrees of self-reported mental health measures and trauma exposure measures are uniquely reflected in genome-wide epigenetic analysis and DNA methylation of candidate genes of PTSD. Self-reported measures were related to global DNA methylation at 480,000 locations in the DNA isolated from 58 refugees' blood, and 2) whether traumatic experience moderated the relationship between self-report mental health and DNA methylation. The proposed relationships among these factors are shown in Figure 1.

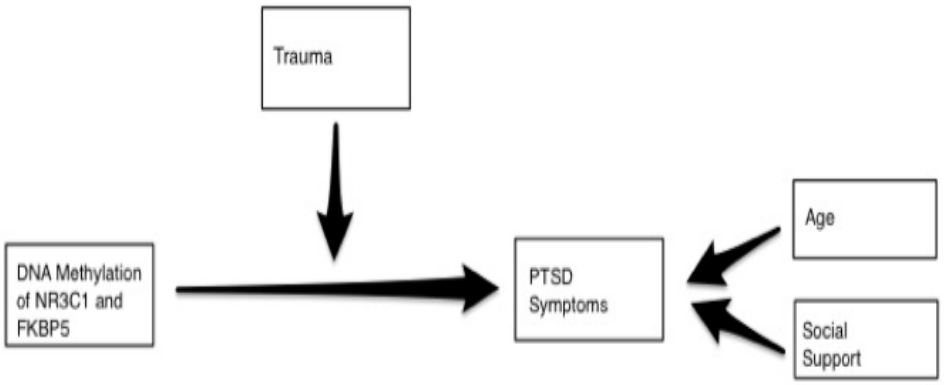

Figure 1. *Conceptual Model of the Study: Proposed Links Between PTSD and Predictors: DNA Methylation of Candidate Genes, Trauma Exposure and the Interaction of Trauma Exposure by DNA Methylation. Covariates include Age and Social Support.*

Hypotheses:

1. It is hypothesized that there will be observable DNA methylation differences in the genome-wide analysis between groups reporting variability in trauma and PTSD symptoms.

2. It is hypothesized that there will be observable DNA methylation differences in candidate genes between groups reporting variability in trauma and PTSD symptoms. (FKBP5, NR3C1, SLC6A4, SLC6A3).

3. It is hypothesized that DNA methylation β-values will be negatively associated with social support and positively associated with age.

4. It is hypothesized that PTSD symptom severity will be significantly predicted by trauma, DNA methylation β-values, and the interaction of trauma and DNA methylation β-values, while taking into account covariates of interest, age and social support.

Chapter 3: Method

Overview of Study

This cross sectional study was conducted utilizing participants, who had participated in a larger NIMH-funded longitudinal study, approved by the Wayne State University Institutional Review Board (protocol # 0902006812. Title: Mental Health in Iraqi Refugee: Importance of post-displacement social stressors and institutional resources) (Arnetz, 2012). The current study, a Wayne State University's Grants Plus-funded project, included a genetic component, to investigate epigenetic profiles of genes implicated in the development of PTSD in war refugees. It aimed to determine the relationship between self-reported trauma exposure, self-reported PTSD, and DNA methylation, both in genome-wide and in PTSD candidate genes. The measures consisted of self-report questionnaires and a blood sample via finger prick.

Participants

The participants in the original study were immigrants and refugees who fled Iraq following war in 2003 and resettled in the Detroit metro area. A subset of 79 refugees who satisfied inclusion criteria for four groups based on degrees of self-reported PTSD scores and trauma exposure were selected (Group selection is described in Research Design and Data Analysis), of which 57 refugees agreed to participate. Due to funding limitation, only three groups, consisting of 48 participants, were used for this project. Only males, 18 years or older, were sampled due to limited funding and to ensure a homogenous sample for the epigenetic analysis. Questionnaires and blood samples were collected at the ACCESS Community Medical Clinic in Macomb, MI.

Research Design

The current study is an addition of an epigenetic research component to a longitudinal study at Wayne State University School of Medicine. Within the larger refugee sample, a subset of 79 individuals who satisfy four groups based on self-reported PTSD score and trauma exposure in home country were eligible to participate in the study. Of possible participants, 57 participants consented to participant in the study. Due to limited funding, only three groups were utilized for this study, resulting in 48 participants that best fit into the three groups' criteria. The groups were compared to investigate the inverse association between trauma and PTSD on the epigenome in both genome-wide and in PTSD candidate genes DNA methylation analysis.

The groups were devised as a unique method of examining reasons a subset of refugees develop PTSD symptoms following trauma exposure, while others developed less symptoms. This group-comparison method allowed the research team to examine the differences in the epigenome of individuals who were at risk for developing PTSD as compared to those who were more resilient. The selection criteria for the groups are as follows. The first group consisted of individuals who reported high trauma score (score in the upper 25% percentile on the validated baseline trauma survey of Iraqi refugees) and high PTSD scores (PTSD score in the upper 25% of the distribution of PTSD score in the Iraqi refugee study) (HH group). The second group consisted of refugees with high trauma exposure scores (based on criteria listed for Group 1 above) but low PTSD scores (PTSD score in the lower 25% of the distribution of PTSD score in the Iraqi refugee study) (HL group). The third group consisted of low trauma exposure score (score in the lower 25% percentile on the validated baseline trauma survey of Iraqi refugees) and high

PTSD score (same as the criteria above) (LH group). The fourth group consisted of low trauma and low PTSD score (same as the criteria above) (LL group). Due to limited funding, the low trauma and low PTSD group was not included in the epigenetic analysis and therefore, eliminated from this study. The HL and LH groups were compared to HH group (in two group comparisons) to examine the differential DNA methylation patters between individuals who are at most risk of developing PTSD (HH) to individuals who are more resilient (HL) and those who are most vulnerable (LH).

Measures

Demographics Questionnaire: Archival data from NIMH longitudinal study was used to extract demographic information (age).

Trauma Exposure Questionnaire: Exposure to traumatic events was obtained from the NIMH longitudinal study to assess degrees of trauma exposure. Trauma exposure was measured with the Harvard Trauma Questionnaire (HTQ; Mollica et al., 1992; Shoeb et al., 2007). The HTQ has been utilized in war-affected communities and has exhibited high test-retest reliability (.89) and internal consistency [(.96) Mollica et al., 1992]. The questionnaire contained 40 questions, which can be endorsed by "yes" or "no" response (range 0-40). The previous research team omitted one question: "witnessed chemical attacks on residential areas or marshlands." The question was redundant as the previous questionnaire inquired about chemical exposures in greater depth.

PTSD Symptoms Questionnaire: PTSD symptoms were initially obtained from NIMH longitudinal study to assess different degree of PTSD score for each possible participant, but, were re-administered for the current study. PTSD symptoms were assessed using the PTSD Checklist-Civilian version (PCL-C) (Blanchard et al., 1996;

Ruggiero et al., 2003). This scale was the most commonly used tool in assessment of PTSD and had correlations exceeding .75 with other established PTSD measures. The measure contained 17 items based on DSM-IV PTSD criteria, which included, re-experiencing, avoidance, and hyper arousal symptoms. The response scale has five points, where participants indicate their level of distress, with 1 indicating low level of distress to 5 indicating the highest (range 17-85). The total PCL-C score has excellent internal consistency ($\alpha = 0.97$) and test-retest reliability (ranging from .68 to .92). PCL-based diagnoses have been found to be moderately (kappa = .67/83% agreement) to strongly (kappa = .85/93% agreement) reliable, compared to diagnosis by clinical interview (Blanchard et al., 1996; Ruggiero et al., 2003).

Social Support Questionnaire: The social support measure was obtained from the NIMH-funded study. Social support was assessed using the Interpersonal Support Evaluation (ISEL) (Cohen, Mermelstein, Kmack & Hoberman, 1985). The scale consisted of 40 items, measuring perceived social support across four areas (belonging, self-esteem, appraisal, and tangible help). The previous research team utilized five questions from the scale, rated on a 5-point scale (1 = not at all true to 5 = extremely true) (range of items included in the current study 1-25). Studies have found that utilizing and scoring the items unidimensionally could result in loss of important information about the nature of social support. Therefore, it was suggested that using the subscales would be beneficial in gaining unique information about participants (Brookings & Bolton, 1988). Five items were chosen from the appraisal and belonging subscales. Since refugees were displaced and relocated to the U.S., it was important to include items that investigated their perceived availability to gather social support by identifying individuals to talk with

about their difficulties (appraisal) and about their perceived availability of individuals they can partake in activities with (belonging). The internal consistency reliability of the ISEL ranged from .77-.86 and internal alpha estimates of .88-.90 (Cohen & Hoberman, 1983). The ISEL demonstrated good retest-reliability for the full measure (.87) and for the subscales .71-.87 (Cohen & Hoberman, 1983).

Epigenetic Analysis: Epigenetic analysis was conducted via finger prick method and the blood spots were placed on protein cards. Dried blood spots were used to examine methylation levels across the whole genome. Dried blood spots were collected using Whatman Protein Saver Card #903 (Whatman #10534612; Fisher Scientific #NC9307519). This is an efficient way to collect and store peripheral blood specimens. The card is made out of filter paper, which included a place for participants' ID and date of collection, along with five circles, where the blood would be placed. Five dried blood spots in the designated areas of the cards were collected from each participant. The cards were then delivered to Wayne State University laboratories for analysis.

DNA material was removed from the dry blood spots on the protein cards. Analysis was conducted using The Illumina Infinium® Human Methylation 450K BeadChip (Illumina Inc., CA, USA). This Epigenotyping technology surveyed 480,000 gene locations, providing differences in the DNA methylation patterns that are gene specific as well as methylation patterns in CpG islands (Touleimat & Tost, 2012).

Several steps were taken in this analysis. DNA was bisulfate treated prior to analysis by the Infinium Human Methylation 450K BeadChips. The data were extracted using the GenomeStudio® software (Illumina Inc. CA, USA), which provided DNA methylation data, by means of βeta-values (β-values). The β-values are methylation

scores for CpG sites in genes ranging from 0, which signifies un-methylated status, to 1, signifying a fully methylated status, on a continuous scale. Background and control normalizations were done by GenomeStudio® to stabilize the variance, which yielded expected values for internal controls.

Statistical analyses from this data were calculated using R 3.0, utilizing CpGassoc statistical package to perform the analyses. A CpGassoc analysis function, cpg.assoc, was used to perform a Linear Fixed Effect Model to examine the association between the groups and DNA methylation of individual CpG sites genome-wide (Barfield et al., 2012). β-values derived from GenomeStudio® were used as input. The β-values were log-transformed. The groups were categorical variables, while age and social support were used as continuous covariates.

The output produced by cpg.assoc includes *t*-statistic, effect size, standard error, and multiple-testing-adjusted *p*-values. The estimated coefficient provided the DNA methylation difference between the groups for the single CpG site. Because the reference group was HH, a negative estimated coefficient signifies more methylation in the HH group vs. the comparison group (HL or LH). A paired *t*-test was used to evaluate the statistical significance of differential methylation between groups. Multiple hypothesis testing was conducted as a measure to avoid false positive differences. Benjamini-Holchberg procedure was utilized to control for 5% false discovery rate (FDR). A more stringent method was used as well; the Holm–Bonferroni method, a step-down Bonferroni procedure, presented significance in "True" or "False" (Barfield et al., 2012).

Procedure

Refugees from a NIMH-funded longitudinal study who fulfilled groups' criteria were selected for the current study. The groups were based on differential self-reported PTSD score and trauma exposure in their home country at two-year post arrival to the U.S. The participants had consented to be a part of additional research at Wayne State University during data collection of the NIMH-funded study. Those who have chosen to withdraw were not contacted. Refugees contact information was obtained from the Principal Investigator of the NIMH-funded study at Wayne State University.

Possible participants were contacted by mail and/or telephone calls. Refugees who were potentially interested in participating received written information about the study, which stated that study participation is voluntary and entails the collection of blood via finger prick method. Those who were interested in participation were given the option to call and set up an appointment to have their blood collected at ACCESS Medical Clinic by a licensed nurse practitioner, employed by the ACCESS Clinic. Participants were given three weeks from the time the written information flyer was sent before being contacted by telephone to receive information about the study.

A researcher met with participants at the ACCESS clinic. Participants were provided with an Informed Consent form that expanded on the details of the study, including the risks and benefits of the study. The researcher allowed time for participants to inquire about the study and have their questions answered. Once a participant agreed to participate, he signed the Informed Consent Form. Upon signing, the consent were placed in a sealed envelope and was delivered to the research team offices at Wayne State University School of Medicine, where they were kept in a locked cabinet only accessible

to the research staff. A master list containing participants' ID number and personal information was kept separately from the consent forms.

The participants were given questionnaires to complete in a private area of the clinic. Once completed, participants were directed into a private exam room at the clinic where their blood samples were collected. The licensed nurse practitioner allowed for time to answer questions from participants and gave them the opportunity to withdraw from the study. Protein cards were labeled with participants ID and were delivered by researcher to Wayne State University Lab at the Mott Center.

Data Analysis

Two separate epigenetic analyses were conducted. The first consisted of DNA methylation based on the group comparisons. The HH group was used a reference group, where the group was compared to the HL (HL vs. HH) and LH (LH vs. HH) in two group comparisons (Hypothesis 1 and Hypothesis 2). A Linear Fixed Effect Model was used to assess differences among groups across the genome. The model controlled for social support and age [Figure 2a and Figure 2b]. This analysis provided differential DNA methylation values across the genome, from which PTSD candidate genes values were derived for this project.

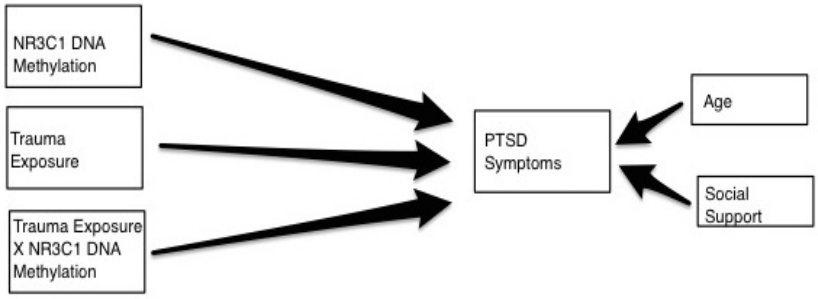

Figure 2a: *Statistical model proposing the link between PTSD (Y) on DNA methylation of NR3C1 (X), trauma exposure (Z), and the interaction of trauma exposure and NR3C1 DNA methylation (XZ), controlling for covariates of age (e) and social support (e_1).*

PTSD = b_0 + b_1 (DNA Methylation) + b_2 (Trauma Exposure) + b_3 (DNA methylation x Trauma Exposure) + e (age) + e_1 (social support).

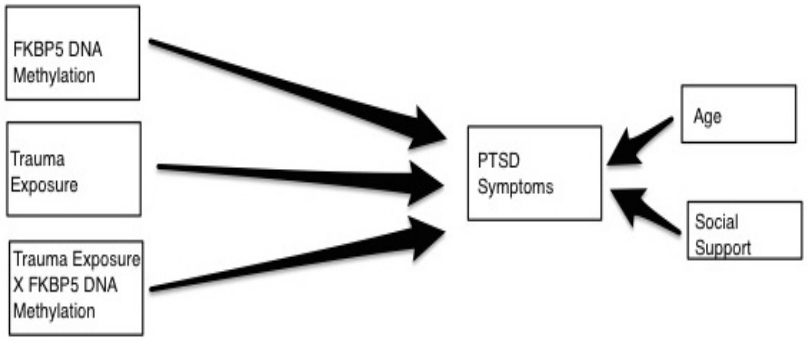

Figure 2b: *Statistical model proposing the link between PTSD (Y) on DNA methylation of FKBP5 (X), trauma exposure (Z), and the interaction of trauma exposure and NR3C1 DNA methylation (XZ), controlling for covariates of age (e) and social support (e_1).*

PTSD = b_0 + b_1 (DNA Methylation) + b_2 (Trauma Exposure) + b_3 (DNA methylation x Trauma Exposure) + e (age) + e_1 (social support).

Spearman's Correlation was used to examine the associations between variables of interest, including methylation values for candidate genes, age, social support, PTSD score, and trauma exposure. Because this study was interested in the differences between the groups, β-values of each group comparison were combined and used in correlations to better understand factors that are associated of DNA methylation of candidates genes in groups and PTSD symptoms, risk (trauma exposure) and protective factors (age and social support).

Another important aim of this study was to examine the association of PTSD symptoms with variables of interest. This was possible by deconstructing the groups and utilizing participants' epigenetic raw β-values of significant candidate genes. A Linear Regression analysis was conducted to determine predictors of PTSD symptoms; predictors included trauma, DNA methylation for each candidate gene for each participant, and the interaction between DNA methylation and CpG sites, controlling for age and social support. This analysis was computed four times, one for each gene and each CpG site of the gene. Significant interactions were graphed using Continuous Interaction 3.0 (Cohen & Cohen, 1983) to assess the interaction of trauma exposure and DNA methylation on PTSD symptoms.

Chapter 4: Results

Overview of Results

This chapter will first provide the characteristics of the participants, including demographic information, trauma exposure, and PTSD symptom scores. Second, it will report results for each hypothesis, and finally, for the conceptual model. The first section, addressing hypothesis 1, will provide genome-wide results between group comparisons, based on results from Linear Fixed Effect model. The second section, addressing hypothesis 2, will report results specific to candidate genes for group comparisons. The groups were combined and each participant's raw β-values were utilized as a continuous measure to examine the association between DNA methylation of candidate genes β-values were included as a predictor of PTSD. The third section, addressing hypothesis 3, will report results from Spearman's Correlation, which investigated the association between variables of interest and candidate genes. Four linear regression analyses were computed for each candidate gene and their CpG sites as predictors of PTSD, along with trauma exposure, and the interaction between trauma exposure and DNA methylation of candidate CpG sites, controlling for age and social support.

Sample Characteristics

The sample consisted of 48 males refugees who have emigrated out of Iraq and have resided in the United States for an average of 24.68 months (*SD* = 24.68, range 20-59). The time difference between the NIMH-funded study interviews at two-year post arrival and blood collection for the current study was 3.84 months (*SD*=3.04). The average age of the participants was 37.13 (*SD* = 11.21) and 60.4% were married. The majority of participants had less than or equal to a high school education (70.8%) and

were employed (66.7%). The refugees reported an average of 15.21 (*SD* = 3.32) trauma incidences experienced while in their home country and reported a PTSD symptom score of 21.21 (*SD* = 4.69). PTSD score t (47) = 42.36, $p < .0001$, home trauma t (47) = 42.36, $p < .0001$, months in the states t (47) = 136.30, $p < .0001$ were statistically higher than the larger group from which they were taken. However, age was statistically significantly lower than the larger group it was taken from t (47) = 22.93, $p < .0001$ [Table 1]. The characteristics of the sample are represented in Table 1.

The participants endorsed the following home traumas more often: "confined to home because of chaos and violence outside" (n = 48/48, 100%), "oppressed because of ethnicity, religion, or sect" (n = 45/48, 93.5%), "witnessed shelling, burning, or razing of residential areas or marshlands" (n = 46/48, 95.8%). Participants in the study did not endorse "witnessed sexual abuse or rape" and "forced to inform on someone placing them at risk of injury." Items that were often endorsed on the PTSD scale (PCL-C) included "repeated, disturbing memories, thoughts, or images of a stressful experience from the past" (*M* = 2.17, *SD* = .80) and "feeling emotionally numb or being unable to have loving feelings for those close to you" (*M* = 1.37, *SD* = .76), while "feeling distant or cut off from other people" (*M* = .98, *SD* = .14) and "trouble falling asleep or staying asleep" (*M* = .98, *SD* = .14) were endorsed less [Table 2].

Table 1: *Demographic Characteristic of Participants (N=48)*

Characteristics	**n**	**%**
Education		
Less than or Equal to high school	34	70.8
Greater than high school	14	29.2
Employment		
Unemployed	16	33.3
Employed	32	66.7
Marital Status		
Married	29	60.4
Single	19	39.6

Demographic and Clinical Differences Between Groups

The participants were divided into three groups based on self-reported number of traumatic experiences and PTSD symptom scores from a prior longitudinal study conducted by the same research team at Wayne State University. Selecting individuals who reported PTSD symptom scores and traumatic experiences in the upper 25% and lower 25% quartile in the prior study defined the group selection process. Groups consisted of 24 (49.0%) refugees who reported high levels of trauma ($M = 16.71$, $SD = 1.76$) and high PTSD symptom score ($M = 22.75$, $SD = 5.37$) (HH group), 14 (28.6%) refugees who reported high trauma ($M = 16.29$, $SD = 2.46$) and low PTSD symptom scores ($M = 19.57$, $SD = 3.15$) (HL group), and 10 (20.4%) refugees who reported low trauma incidents ($M = 8.30$, $SD = 4.42$) and high PTSD symptoms ($M = 19.80$, $SD = 3.76$) (LH group) [Table 3].

Table 2: *Trauma Exposure Endorsed Reported by Refugees, as presented on the Harvard Trauma Questionnaire.*

Trauma Exposure	n	%
1. Oppressed because of ethnicity, religion, or sect	45	93.8
2. Present while someone searched for people or things in your home	22	45.8
3. Searched arbitrarily	26	54.2
4. Property looted, confiscated, or destroyed	20	41.7
5. Forced to settle in a different part of the country with minimal services	41	85.4
6. Imprisoned arbitrarily	5	10.4
7. Suffered ill health without access to medical care or medicine	11	22.9
8. Suffered from lack of food or clean water	9	18.8
9. Forced to flee your country or place of settlement	42	87.5
10. Expelled from your country based on ancestral origin, religion, or sect	0	0
11. Lacked shelter	4	8.3
12. Witnessed the desecration or destruction of religious shrines or places of religious instruction	36	75.0
13. Witnessed the arrest, torture, or execution of religious leaders or important members of tribe	5	10.4
14. Witnessed execution of civilians	12	25.0
15. Witnessed shelling, burning, or razing of residential areas or marshlands	46	95.8
16. Witnessed or heard combat situation (explosions, artillery fire, shelling) or landmine	48	100
17. Serious physical injury from combat situation or landmine	3	6.3
18. Witnessed rotting corpses		
19. Confined to home because of chaos and violence outside	48	100
20. Witnessed someone being physically harmed (beating, knifing etc.)	26	54.2

Table 2: *Trauma Exposure Endorsed Reported by Refugees, as presented on the Harvard Trauma Questionnaire, Continued.*

Trauma Exposure	n	%
21. Witnessed sexual abuse or rape	0	0
22. Witnessed torture	1	2.1
23. Witnessed murder	23	47.9
24. Forced to inform on someone placing them at risk of injury or death	0	0
25. Forced to destroy someone's property	0	0
26. Forced to physically harm someone (beating, knifing, etc.)	0	0
27. Murder or violent death of family member (child, spouse) or friend	31	64.6
28. Forced to pay for bullet used to kill family member	0	0
29. Received the body of a family member and prohibited from mourning them and performing burial rites	2	4.2
30. Disappearance of family member (child, spouse etc.) or friend	26	54.2
31. Kidnapping of family member (child, spouse, etc.) or friend	31	64.6
32. Family member (child, spouse, etc.) or friend taken as hostage	27	56.3
33. Someone informed on you placing you and your family at risk of injury or death	24	50.0
34. Physically harmed (beaten, knifed, etc.	13	27.1
35. Kidnapped	9	18.8
36. Taken as hostage	9	18.8
37. Heard about frightening, dangerous events that occurred to someone else but that you did not experience yourself	48	100
38. Sexually abused	0	0
39. Coerced to have sex	0	0

Table 3: *Demographic Characteristics by Groups*

Group	HH		HL		LH	
	M	*SD*	*M*	*SD*	*M*	*SD*
Age	37.54	2.11	33.64	9.58	41.0	14.66
Home Trauma	16.71	1.76	16.29	2.46	8.30	4.42
PTSD Score	22.75	5.37	19.57	3.15	19.80	3.76
Social Support Score	19.37	2.40	22.29	1.82	19.70	3.71
	n	%	n	%	n	%
Education						
Less than or equal to high school	18	75.0	10	71.4	6	60.0
Greater than High School	6	52.9	4	28.6	4	8.3
Employment						
Unemployed	8	33.3	3	21.4	5	50.0
Employed	16	66.7	11	33.3	5	50.0
Marital Status						
Single	8	33.3	8	57.1	3	30.0
Married	16	66.7	6	42.9	7	70.0

The average age of refugees in the HH group was 37.54 (*SD* = 2.11); the average age of refugees in the HL group was 33.64 (*SD* = 9.58); and the average age of refugees in the LH group was 41.0 (*SD* = 14.66). In terms of social support, the HH group reported a mean score of 19.37 (*SD* = 2.40); the HL group reported 20.29 (*SD* = 1.82), while the LH group reported a mean social support score of 19.70 (*SD* = 3.71) [Table 3].

Testing of Hypotheses

Genome-Wide Epigenetic Analysis

Hypothesis 1: It is hypothesized that there will be observable DNA methylation differences genome-wide between groups in both group comparisons: HL vs. HH and LH vs. HH.

Genome-wide DNA methylation analysis revealed that there were significantly differentiated CpG sites in both group comparisons. Differential DNA methylation was quantified in β-values for CpG sites. After multiple hypothesis testing, the HL vs. HH group comparison yielded 5,322 CpG sites that were significantly differentially methylated at p (*FDR*) < .05, 944 CpG sites significantly differentially methylated at p (*FDR*) < .01, 91 CpG sites significantly differentially methylated at p (*FDR*) < .001, with 67 passing the threshold for Holm–Bonferroni method significance criteria. The LH vs. HH group comparison yielded 77,776 CpG that were significantly differentially methylated at p (*FDR*) < .05, 36,423 CpG significantly differentially methylated at p (*FDR*) < .01, 11,672 CpG sites significantly differentially methylated at p (*FDR*) < .001, with 1,597 passing the threshold for Holm–Bonferroni method significance criteria. The distribution of p (*FDR*) values was larger in the HL vs. HH group as shown in Figure 3a and Figure 3b. In the HL vs. HH group comparison, 58.5% of the CpG sites (3,113 out of 5,322 CpG sites) had greater DNA methylation in the HH group, while 67.1% of CpG sites (52,166 out of 77,776 CpG sites) in the LH vs. HH group comparison had greater DNA methylation in the HH group.

The distribution of the significantly differentiated CpG sites in the genome-wide DNA methylation analysis is presented in the Manhattan and Volcano plots for both group comparisons, providing a visual presentation of CpG sites that deviate from the expected null distribution (Figure 3a, b and Figure 4a,b). The Manhattan plot is a scatter plot showing genome-wide association for group comparisons. The Volcano plot shows the statistical significance versus value changes on the Y- and X-axes. There was greater significance in differential DNA methylation between LH vs. HH, as compared to HL vs. HH (Figure 3a, b and Figure 4a, b). Results confirmed our hypothesis that there was observable DNA methylation differences in both group comparisons.

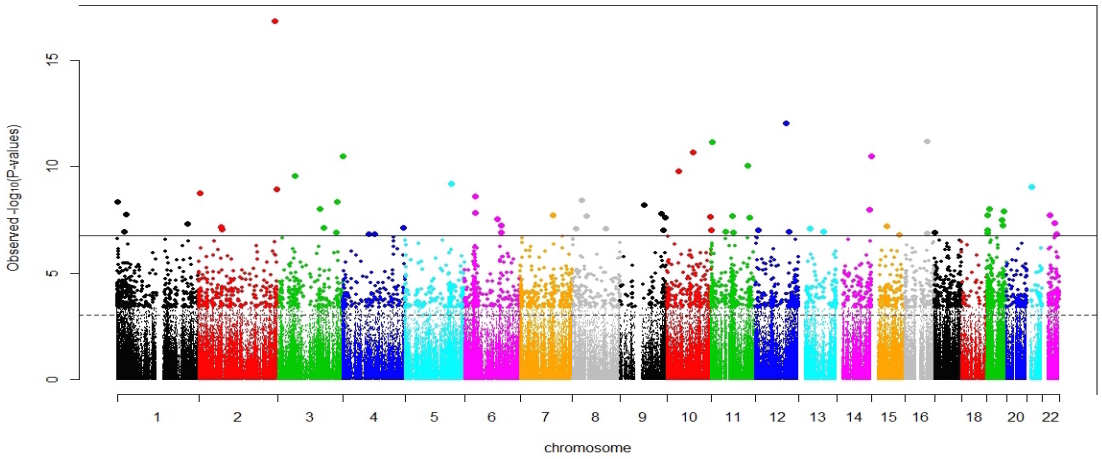

Figure 3a: *Genome-wide association between CpG methylation and high trauma/low PTSD and high trauma/high PTSD group comparison*.

Note: Manhattan plot representing methylation of approximately 480,000 CpG sites in an genome-wide analysis. The plot contains the genomic coordinates or the chromosomes on the X-axis and negative logarithms for each CpG are presented in the Y-Axis. The different colored dots help distinguish between each CpG site, presented by chromosome. The dotted line represents *FDR* statistical significance and the solid line represents CpG

sites that passed the threshold for Holm–Bonferroni method significance criteria, adjusted for age and social support. Sixty-seven CpG sites surpassed the Holm–Bonferroni significance threshold.

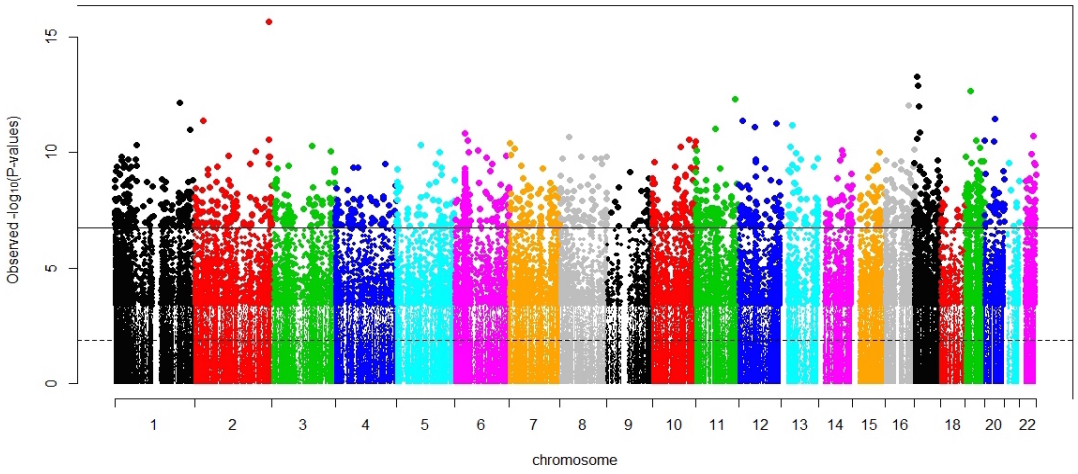

Figure 3b: *Genome-wide association between CpG methylation and low trauma/high PTSD and high trauma/high PTSD group comparison*.

Note: Manhattan plot representing methylation of approximately 480,000 CpG sites in an genome-wide analysis. The plot contains the genomic coordinates or the chromosomes on the X-axis and negative logarithms for each CpG are presented in the Y-Axis. The different colored dots help distinguish between each CpG site, presented by chromosome. The dotted line represents *FDR* statistical significance and the solid line represents CpG sites that passed threshold for Holm–Bonferroni method significance criteria, adjusted for age and social support. In this analysis, 1,597 surpassed the Holm–Bonferroni significance threshold.

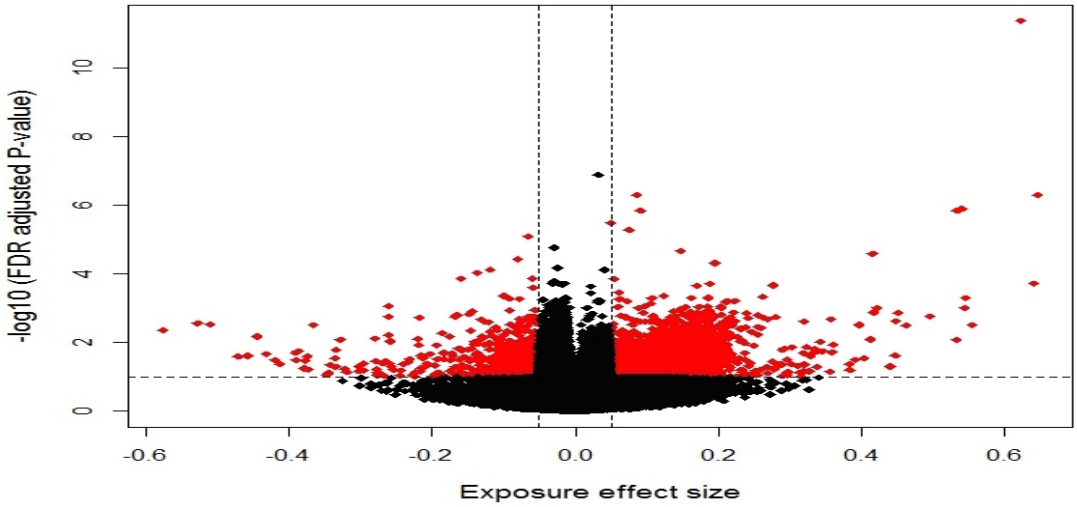

Figure 4a: *Volcano plot for association of genome-wide CpG methylation in high trauma/low PTSD and high trauma/high PTSD group comparison.*

Note: The *p* (*FDR*) values are on the Y-axis. The dots in red represent the significant differential DNA methylation between the conditions. Highly significant CpG sites are further from the center and move toward the top of the plot. The X-axis represents the value difference between the two groups for DNA methylation genome-wide.

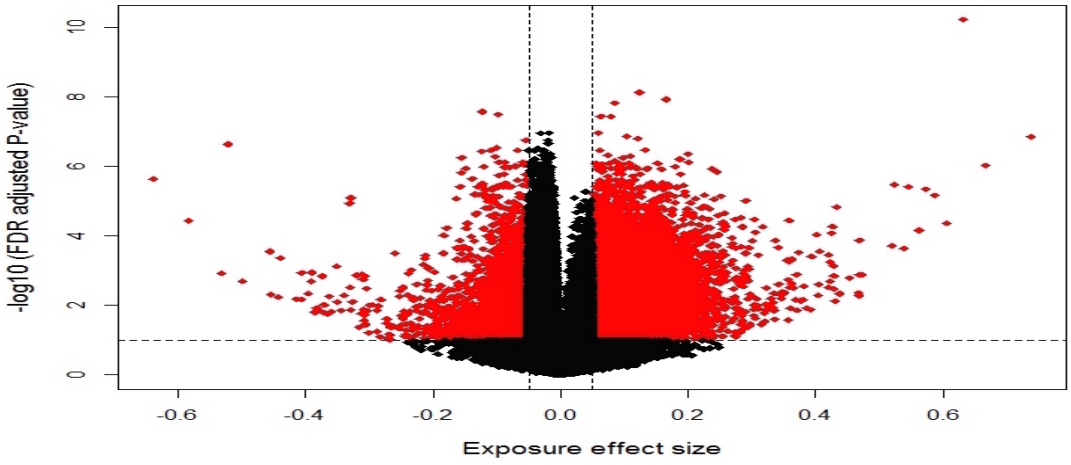

Figure 4b: *Volcano plot for association of genome-wide CpG methylation in low trauma/High PTSD and high trauma/ high PTSD group comparison.*

Note: The *p* (*FDR*) values are on the Y-axis. The dots in red represent the significant differential DNA methylation between the conditions. Highly significant CpG sites are further from the center and move toward the top of the plot. The X-axis represents the value difference between the two groups for DNA methylation genome-wide.

Table 4a: *Candidate Genes Based on Differential DNA Methylation*

Gene Name	CpG Site	Region	Chromosome Location	Relation to CpG Site
High Trauma/Low PTSD vs High Trauma/ High PTSD Group Comparison				
NR3C1	**cg00629244**	**Promoter**	**chr5:142782071-142785071**	**Island**
NR3C1	cg20753294		chr5:142782071-142785071	Island
FKBP5	**cg16012111**	**Promoter**	**chr6:35655607-35656856**	**Island**
Low Trauma/High PTSD vs. High Trauma High PTSD Group Comparison				
NR3C1	**cg00629244**	**Promoter**	**chr5:142782071-142785071**	**Island**
NR3C1	cg18019515	Promoter	chr5:142782071-142785071	Island
NR3C1	cg17860381	Promoter	chr5:142782071-142785071	Island
NR3C1	cg04111177	Promoter	chr5:142782071-142785071	Island
NR3C1	cg26464411	Promoter	chr5:142782071-142785071	Island
NR3C1	cg21702128	Promoter	chr5:142782071-142785071	Island
FKBP5	cg00862770	Promoter	chr6:35655607-35656856	Island
FKBP5	cg00140191	Promoter	chr6:35655607-35656856	Island
FKBP5	cg10913456	Promoter	chr6:35655607-35656856	Island
FKBP5	**cg16012111**	**Promoter**	**chr6:35655607-35656856**	**Island**
FKBP5	cg00052684	Unclassified_ Cell_type_specific	chr6:35695725-35696156	N_Shore
FKBP5	cg17617527		chr5:142782071-142785071	Island
FKBP5	cg20753294		chr5:142782071-142785071	Island

Note: Gene and CpG sites that were used for the analysis for Hypothesis 2 represented in bold based on a Fixed Effect Model. The table displays CpG sites, region and location of site on the gene, as well as, location on chromosome for each candidate gene (NR3C1 and FKBP5). CpG sites that were significantly differently methylated in two group comparisons (HL vs. HH and LH vs. HH) are based on Fixed Effect model, further elaborated on in Table 4b.

Table 4b: *Fixed Effect Model of Candidate Genes Differential DNA Methylation*

High Trauma/Low PTSD vs High Trauma/ High PTSD Group Comparison							
CpG Site	Adj Intercept	Effect Size	Standard Error	t Statistic	*p*	FDR Sig	Holmes Sig
cg00629244	**0.05**	**-0.03**	**0.006**	**-4.85**	**3.58E-05**	**0.001**	**FALSE**
cg20753294	0.19	-0.11	0.02	-4.98	2.43E-05	0.008	FALSE
cg16012111	**0.06**	**-0.02**	**0.004**	**-4.72**	**5.15E-05**	**0.01**	**FALSE**
cg00629244	**0.05**	**0.04**	**0.01**	**-4.92**	**4.17E-05**	**0.01**	**FALSE**
cg18019515	0.02	-0.01	0.003	-3.71	0.0009	0.008	FALSE
cg17860381	0.03	-0.02	0.002	-7.26	1.03E-07	2.36E-05	TRUE
cg04111177	0.04	-0.01	0.004	-3.87	0.0006	0.006	FALSE
cg26464411	0.09	-0.05	0.02	-3.34	0.003	0.02	FALSE
cg21702128	0.05	-0.01	0.003	-4.34	0.0002	0.002	FALSE
cg00862770	0.04	-0.03	0.007	-3.67	0.001	0.009	FALSE
cg00140191	0.07	-0.02	0.006	-3.22	0.003	0.02	FALSE
cg10913456	0.02	-0.01	0.002	-5.69	5.43E+06	0.0002	FALSE
cg16012111	**0.06**	**-0.04**	**0.004**	**-8.89**	**2.30E-09**	**3.05E-06**	**TRUE**
cg00052684	0.47	0.11	0.02	4.14	0.0003	0.004	FALSE
cg17617527	0.02	-0.01	0.002	-5.93	2.94E-06	0.0001	FALSE
cg20753294	0.18	-0.10	0.02	-4.52	0.0001	0.002	FALSE

Note: Gene and CpG sites that were used for the analysis for Hypothesis 2 represented in bold. The table displays the Fixed Effect model output for all CpG sites of candidate genes (NR3C1 and FKBP5) in two group comparisons (HL vs. HH and LH vs. HH). Those that were significantly differently methylated and were used for this study are represented bold.

DNA methylation of Candidate Genes

Hypothesis 2: It was hypothesized that there will be more methylation in the group with high trauma and high PTSD symptoms (HH) in both group comparisons (HL vs. HH and LH vs. HH) in candidate genes (FKBP5 and NR3C1).

CpG sites within genes of interest that had a significant FDR *p*-value were used for analysis; a p-value < .05 was considered statistically significant. HM450K did not include DNA methylation in the promoter regions of SLC6A3 and SLC6A4; therefore they were not included in the analysis. While NR3C1 and FKBP5 had multiple CpG sites differently methylated in each group comparison [Table 4a and Table 4b], there was one

common CpG site in each gene that was significantly differently methylated in both group comparisons, cg00629244 in the NR3C1 gene and cg16012111 within the FKBP5 gene. The two CpG sites were subjected to further analyses in this study.

With regard to DNA methylation, the HH group ($M = 0.063$, $SD = 0.012$) had more DNA methylation (β-values) on average than the HL group ($M = 0.041$, $SD = 0.009$) and LH group ($M = 0.026$, $SD = .005$) in the FKBP5 gene (cg16012111). The HH group ($M = 0.048$, $SD = 0.020$) had more DNA methylation (β-values) on average than the HL group ($M = 0.019$, $SD = 0.008$) and LH group (M=0.030, $SD = 0.028$) in the NR3C1 (cg00629244). Distribution of the average β-values for both CpG sites in each group comparison is plotted in Figure 5. Table 4a displays the CpG sites, regions, and chromosome locations of each candidate gene based on the genome-wide DNA methylation analysis. The results for significantly differently methylated CpG genes and sites were based on the output of Fixed Effect model, which is presented in Table 4b. Results revealed a statistically significant difference in DNA methylation in cg16012111 within the FKBP5 gene ($t = -4.71$, p (*FDR*) <. 01, estimated coefficient = -.02) in the HL vs. HH group comparison, indicating more methylation in HH group. In the LH vs. HH group comparison, cg16012111 ($t = -8.89$, p (*FDR*) < .001, estimated coefficient = -0.04) had more methylation in the HH group. Multiple hypotheses testing also revealed that this comparison passed the threshold for Holm–Bonferroni method significance criteria [Table 4b]. Linear Fixed Effect Model revealed a statistically significant difference in DNA methylation in cg00629244 within the NR3C1 gene ($t = -4.92$, p (*FDR*) < .05, estimated coefficient = .04) in the LH vs. HH group comparison, indicating participants with high levels of trauma and high PTSD symptom scores had higher levels of

methylation. Similarly, in the HL vs. HH group comparison, cg00629244 within the NR3C1 had more methylation in the HH group (t = -4.85, p (*FDR*) < .01, estimated coefficient = -.03) [Table 4b]. The estimated coefficient is represented by the slop, which shows the difference between groups. Results revealed that Hypothesis 2 of this study was confirmed, in that the high trauma and high PTSD group (HH) had higher methylation in candidate genes than groups used in comparison (HL and LH).

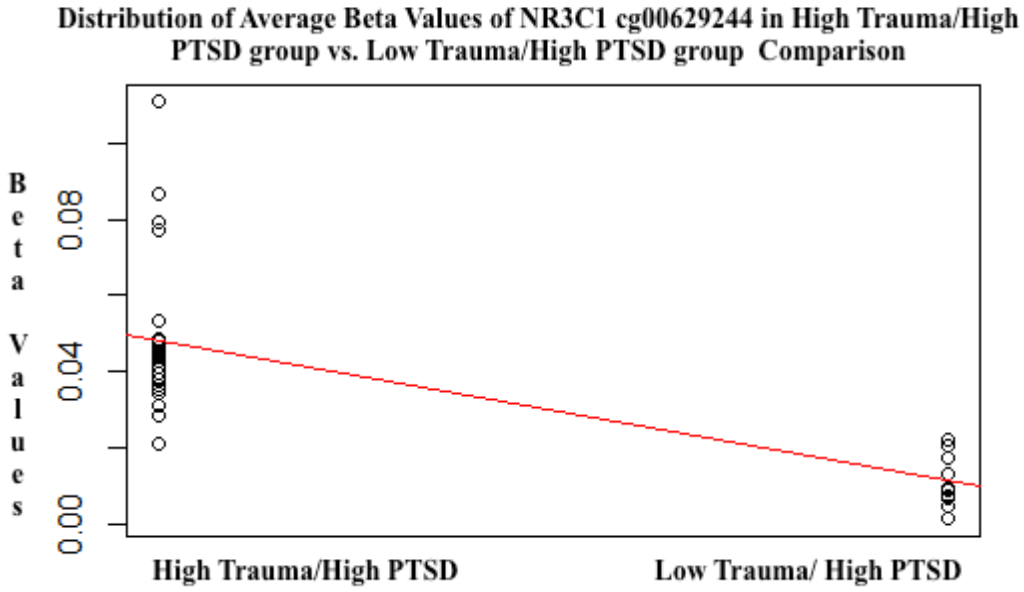

Figure 5: *Distribution of Beta Values of CpG sites in candidate genes in two group comparisons*.

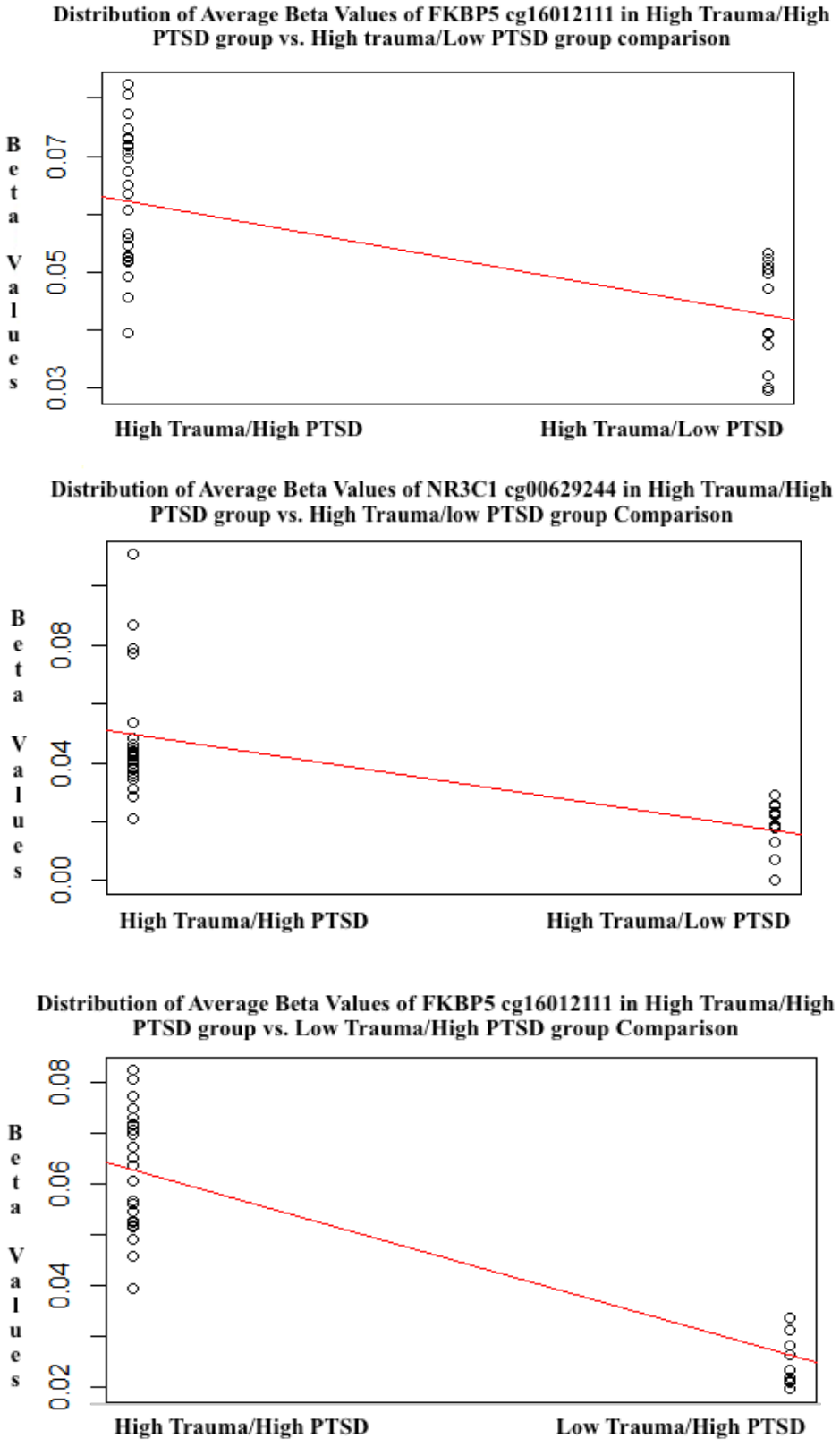

Figure 5: *Distribution of Beta Values of CpG sites in candidate genes in two group comparisons, continued*.

Correlations

Hypothesis 3: It is hypothesized that DNA methylation β-values will be negatively associated with social support and positively associated with age.

The average β-values for the CpG sites of interest of each group comparison were used to examine the association between DNA methylation of each group comparison, trauma incidents, PTSD score, age and social support. Pearson Correlations were computed; results revealed a strong positive correlation between trauma and NR3C1 methylation (cg00629244) in the LH vs. HH group ($r = .53, p < 0.01$) (total number of participants in this group comparison is 24); however, NR3C1 methylation (cg00629244) HL vs. HH was not significantly correlated (total number of participants in this group comparison is 38). FKBP5 (cg16012111) in the HL vs. HH was significantly correlated with PTSD ($r = .43, p < 0.01$) but not with trauma ($n = 38$) [Table 5].

FKBP5 (cg16012111) in the LH vs. HH ($n = 24$) was significantly correlated with trauma ($r = .75, p < 0.01$) and PTSD ($r= .37, p < 0.05$). FKBP5 in the HL vs. HH ($n = 38$) was the only CpG site that was correlated with social support ($r = -.36, p < 0.05$), indicating that lower social support is correlated with higher methylation of this area in the gene [Table 5]. In sum, PTSD and social support were significantly correlated in the DNA methylation of FKBP5 in the HL vs. HH group comparison. Trauma exposure and PTSD were significantly correlated in the DNA methylation of FKBP5 in the LH vs. HH group comparison. Trauma exposure was significantly correlated with NR3C1 DNA methylation in the LH vs. HH group comparison [Table 5].

Table 5: *Correlations between DNA methylation of PTSD candidate genes (in group comparison), risk and resiliency factors*.

		Trauma Exposure	PTSD	Age	Social Support	NR3C1 LH vs. HH	NR3C1 HL vs. HH	FKBP5 HL vs. HH	FKBP5 LH vs. HH
Trauma Exposure	Pearson Correlation	1	.308*	.025	-.061	.526**	.075	.200	.748**
	Sig. (2-tailed)		.033	.867	.681	.001	.654	.228	.000
PTSD	Pearson Correlation	-	1	.269	-.326*	.153	.166	.430**	.368*
	Sig. (2-tailed)	-		.064	.024	.381	.320	.007	.032
Age	Pearson Correlation	-	-	1	.001	-.147	.039	.257	-.009
	Sig. (2-tailed)	-	-		.993	.400	.816	.119	.959
Social Support	Pearson Correlation	-	-	-	1	-.113	-.194	-.356*	-.106
	Sig. (2-tailed)	-	-	-		.518	.244	.028	.549
NR3C1 LH vs. HH	Pearson Correlation	-	-	-	-	1	1.000**	.460*	.763**
	Sig. (2-tailed)	-	-	-	-		.000	.021	.000
NR3C1 HL vs. HH	Pearson Correlation	-	-	-	-	-	1	.640**	.417*
	Sig. (2-tailed)	-	-	-	-	-		.000	.043
FKBP5 HL vs. HH	Pearson Correlation	-	-	-	-	-	-	1	1.000**
	Sig. (2-tailed)	-	-	-	-	-	-		.000
FKBP5 LH vs. HH	Pearson Correlation	-	-	-	-	-	-	-	1
	Sig. (2-tailed)	-	-	-	-	-	-	-	

Note: * Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Hierarchical Regression Analysis Predicting PTSD Symptoms

Hypothesis 4 (Conceptual Model): It is hypothesized that PTSD symptom severity will be significantly predicted by trauma, DNA methylation β-values, and the interaction of trauma and DNA methylation β-values, while taking into account covariates of interest, age and social support.

Participants in each of the group comparisons were combined to test a linear regression mode predicting PTSD symptom severity. Four linear regression analyses were conducted for both CpG sites of interest (cg00629244 and cg16012111). Regressions were run hierarchically with age, trauma experienced in home country, social support, and the DNA methylation β-values of CpG site entered in Step 1 and the interaction of trauma and DNA methylation β-values of CpG site entered in Step 2.

β-values DNA methylation for CpG sites of interest for all participants in the study, regardless of group affiliation (HL + HH and LH + HH) was used as a continuous variable. PTSD, trauma incidents, age, and social support were continuous variables. This was done to provide a subtler and complex method to assess associations between variables of interest that may be less evident when comparing categorized groups.

A hierarchical linear regression analysis was conducted to predict PTSD symptom severity using age, trauma experienced in home country, social support, the methylation β-values of the NR3C1 (cg00629244) and the interaction between trauma and methylation in the HL vs. HH group. In Step 1, the model was statistically significant $F\ (4, 33) = 5.18, p < 0.05$. In Step 2, the inclusion of the NR3C1 by trauma interaction shows trauma, social support and the interaction of methylation and trauma were significantly related to PTSD symptoms $F\ (5,32) = 5.76, p < 0.05$, adjusted $R^2 = .39$.

Table 6.1: *One-Way Analyses of Variance (ANOVA) of PTSD symptoms by predictor variables when comparing High Trauma/Low PTSD vs. High Trauma/High PTSD groups.*

Model		Sum of Squares	*df*	Mean Square	*F*	Sig.
1	Regression	341.10	4	85.27	5.18	.002[b]
	Residual	543.64	33	16.47		
	Total	884.75	37			
2	Regression	418.52	5	83.71	5.76	.001[c]
	Residual	466.23	32	14.57		
	Total	884.75	37			

a. Dependent Variable: PTSD
b. Predictors: NR3C1 (cg00629244), Age, Trauma, Social Support.
c. Predictors: NR3C1 (cg00629244), Age, Trauma, Social Support, NR3C1 (cg00629244) x Trauma.

When adding the interaction in Step 2, the model significantly improved, R^2 change=. 08, *F Change* (1, 32)= 5.31, p<0.05**.** The analysis shows that trauma (Beta=0.05), *t* (37)=3.35, *p*<. 01, social support (Beta=-0.55), *t* (37)=-4.07, *p*<. 001, and the interaction of trauma and methylation of NR3C1 (Beta=0.37), *t* (37)=2.31, *p*<. 05 contributed most to predicting PTSD symptom severity [Table 6.2]. This indicates that approximately 39% of the variance in PTSD symptoms could be accounted for trauma, social support, and the interaction between levels of trauma and methylation values of NR3C1 (cg00629244) [Table 6.1].

Table 6.2: *Linear regression predicting PTSD symptoms when including trauma and methylation of NR3C1 (cg00629244) in High Trauma/Low PTSD vs. High Trauma/High PTSD group comparison.*

Predictors	SE	B	Beta	*t*	*p*	95.0% CI
Model 1						
Age	.10	.06	.19	1.42	.164	[-.04, .23]
Trauma	.80	.33	.33	2.39	.022	[.12, 1.48]
Social Support	-1.08	.31	-.48	-3.45	.002	[-1.71, -.44]
NR3C1 (cg00629244)	8.66	31.26	.03	.27	.783	[-54.94, 72.27]
Model 2						
Age	.06	.06	.13	1.00	.322	[-.06, .19]
Trauma	1.21	.36	.50	3.35	.002	[.47, 1.94]
Social Support	-1.22	.30	-.55	-4.07	.000	[-1.83, -.61]
NR3C1 (cg00629244)	-38.12	35.73	-.17	-1.07	.294	[-110.88, 34.65]
NR3C1 (cg00629244) x Trauma	45.73	19.84	.39	2.31	.028	[5.32, 86.15]

Note: Dependent Variable: PTSD Scores

A hierarchical linear regression analysis was conducted to predict PTSD symptoms using age, trauma experienced in home country, social support, the methylation β-values of FKBP5 (cg16012111) gene in the HL vs. HH group, and the interaction between trauma and methylation. A test of the full model was significant, indicating that the predictors would reliably predict PTSD symptom severity. In Step one, the model significantly predicted PTSD symptom severity. In Step 2, the model showed a statistical trend ($p < .01$). Trauma and social support were significantly related to PTSD symptoms in Step 2 $F\ (5, 32) = 5.77, p < 0.01$ [Table 7.2].

Table 7.1: *One-Way Analyses of Variance (ANOVA) of PTSD symptoms by predictor variables when comparing High Trauma/Low PTSD vs. High Trauma/ High PTSD groups.*

Model		Sum of Squares	*df*	Mean Square	*F*	Sig.
1	Regression	360.81	4	90.20	5.68	.001[b]
	Residual	523.93	33	15.87		
	Total	884.75	37			
2	Regression	419.61	5	83.92	5.77	.001[c]
	Residual	465.14	32	14.54		
	Total	884.75	37			

a. Dependent Variable: PTSD

b. Predictors: FKBP5 (cg16012111), Trauma, Age, Social Support.

c. Predictors: FKBP5 (cg16012111), Trauma, Age, Social Support, FKBP5 (cg16012111) x Trauma.

Table 7.2: *Linear regression predicting PTSD symptoms when including trauma and methylation of FKBP5 (cg16012111) in High Trauma/Low PTSD vs. High Trauma/ High PTSD group comparison.*

Predictors	SE	B	Beta	*t*	*p*	95.0% CI
Model 1						
Age	.08	.07	.16	1.15	.257	[-.06, .21]
Trauma	.71	.34	.29	2.11	.042	[.03, .40]
Social Support	-.95	.324	-.43	-2.95	.006	[-1.61, -.29]
FKBP5 (cg16012111)	56.68	49.32	.17	1.15	.259	[-43.64, 157.02]
Model 2						
Age	.06	.07	.11	.85	.401	[-.08, .18]
Trauma	.79	.32	.33	2.41	.021	[.12, 1.41]
Social Support	-1.01	.31	-.46	-3.24	.003	[-1.64, -.39]
FKBP5 (cg16012111)	-8.97	57.38	-.03	-.15	.877	[-125.85, 107.90]
FKBP5 (cg16012111) x Trauma	34.34	17.07	.33	2.01	.053	[-.44, 69.12]

Note: Dependent Variable: PTSD Scores

The analysis revealed that trauma (Beta = 0.33), t (37) = 2.41, p <.05 and social support (Beta = -0.46), t (37) = -3.24, p <.05 were the best predictors of PTSD symptom severity [Table 7.2]. The multiple regression coefficient was .39 (Adjusted R^2=. 06), indicating that approximately 39% of the variance in PTSD symptoms could be accounted for by the model. The interaction of trauma and methylation of FKBP5 (cg16012111) showed a statistical trend and adding it in Step 2 did not significantly improve the model, R^2 change = .06, *F Change* (1, 32) = 4.05, p = .053.

A hierarchical linear regression analysis was conducted to predict PTSD symptom severity using age, trauma experienced in home country, social support, the methylation β-values of the NR3C1 (cg00629244) and the interaction between trauma and methylation in LH vs. HH group. Means and standard deviation are presented in Table 8.1. In Step 1, the model was trending to significance, F (4, 34) = 2.67, p < .051, however, in Step 2, the model was statistically significant, F (5, 34) = 3.02, p < .05. The analysis revealed that trauma (Beta = 0.91), t (34) = 2.62, p < .05 and social support (Beta = -0.66), t (34) = -2.23, p < .05 were the best predictors of PTSD symptoms [Table 8.2]. The addition of the interaction of trauma and methylation of NR3C1 (cg00629244) was not significant and did not improve the model R^2 change = .08, *F Change* (1, 29) = 3.53**,** p = 07. The multiple regression coefficient was .34 (Adjusted R^2 = .23), indicating that approximately 34% of the variance in PTSD symptoms could be accounted for by the model.

Table 8.1: *One-Way Analyses of Variance (ANOVA) of PTSD symptoms by predictor variables when comparing Low Trauma/High PTSD vs. High Trauma/High PTSD groups.*

Model		Sum of Squares	*df*	Mean Square	*F*	Sig.
1	Regression	230.47	4	57.62	2.67	.051[b]
	Residual	647.63	33	21.59		
	Total	878.11	34			
2	Regression	300.82	5	60.16	3.02	.026[c]
	Residual	577.29	29	19.91		
	Total	878.11	34			

a. Dependent Variable: PTSD

b. Predictors: NR3C1 (cg00629244), Age, Trauma, Social Support.

c. Predictors: NR3C1 (cg00629244), Age, Trauma, Social Support, NR3C1 (cg00629244) x Trauma.

Table 8.2: *Linear regression predicting PTSD symptoms when including trauma and methylation of NR3C1 (cg00629244) in Low Trauma/High PTSD vs. High Trauma/High PTSD group comparison.*

Predictors	SE	B	Beta	*t*	*p*	95.0% CI
Model 1						
Age	.07	.11	.26	1.59	.123	[-.03, .25]
Trauma	.20	.38	.34	1.84	.076	[-.04, .77]
Social Support	.29	-.47	-.26	-1.16	.115	[-1.06, .121]
NR3C1 (cg00629244)	39.78	-4.142	-.02	-.10	.918	[-85.37, 77.08]
Model 2						
Age	.07	.12	.28	1.79	.084	[-.17, .255]
Trauma	.35	.91	.85	2.62	.014	[.201, 1.62]
Social Support	.29	-.66	-.36	-2.23	.034	[.20, 1.63]
NR3C1 (cg00629244)	38.71	-15.97	-.08	-.41	.683	[-95.13, 63.20]
NR3C1 (cg00629244) x Trauma	14.95	28.11	.58	1.88	.071	[-2.47, 58.69]

Note: Dependent Variable: PTSD Scores

A hierarchical linear regression analysis was conducted to predict PTSD symptoms using age, trauma experienced in home country, social support, the methylation β-values of the FKBP5 (cg16012111) in the LH vs. HH, and the interaction between trauma and methylation. Step 2 of the analysis showed that a higher level of trauma was related to poor PTSD symptom reporting and the interaction between methylation and trauma was associated with poor PTSD symptoms. In Step 1, the overall model was significant, $F\ (4, 29) = 2.70, p \leq 0.05$. In Step 2, the model was significant $F\ (5, 28) = 3.32, p < 0.05$. The correlation regression for the model was .37 and adjusted $R^2 = .26$, indicating approximately 26% of the variance of PTSD symptom severity could be accounted by the model. The analysis showed that trauma (Beta = 0.22), $t\ (33) = 2.13, p <. 05$, and the interaction of trauma and methylation of FKBP5 (Beta = .49), $t\ (33) = 2.12, p < .05$ were the best predictors of PTSD symptom severity [Table 9.2]. The addition of the interaction of trauma and methylation of FKBP5 (cg16012111) significantly improved the model, R^2 change = .10, *F Change* $(1, 28) = 4.50, p < 0.05$.

Table 9.1: *One-Way Analyses of Variance (ANOVA) of PTSD symptoms by predictor variables when comparing Low Trauma/High PTSD vs. High Trauma/High PTSD groups*.

Model		Sum of Squares	*df*	Mean Square	*F*	Sig.
1	Regression	232.51	4	58.12	2.70	.050[b]
	Residual	622.39	29	21.46		
	Total	854.89	33			
2	Regression	318.70	5	63.74	3.32	.017[c]
	Residual	536.19	28	19.15		
	Total	854.89	33			

a. Dependent Variable: PTSD
b. Predictors: FKBP5 (cg16012111), Age, Social Support, Trauma.
c. Predictors: FKBP5 (cg16012111), Age, Social Support, Trauma, FKBP5 (cg16012111) x trauma.

Table 9.2: *Linear regression predicting PTSD symptoms when including trauma and methylation of FKBP5 (cg16012111) in Low Trauma/High PTSD vs. High Trauma/High PTSD group comparison.*

Predictors	SE	B	Beta	*t*	*p*	95.0% CI
Model 1						
Age	.10	.07	.23	1.46	.15	[-.04, .25]
Trauma	.24	.26	.22	.94	.35	[-.29, .78]
Social Support	-.42	.29	-.23	-1.43	.16	[-1.02, .18]
FKBP5 (cg16012111)	44.71	61.30	.17	.73	.47	[-80.66, 170.09]
Model 2						
Age	.1	.07	.22	1.45	.16	[-.04, .23]
Trauma	.69	.32	.64	2.13	.04	[.02, 1.36]
Social Support	-.53	.28	-.29	-1.88	.07	[-1.11, .05]
FKBP5 (cg16012111)	23.92	58.72	.09	.40	.69	[-96.38, 144.22]
FKBP5 (cg16012111) x Trauma	24.61	11.60	.49	2.12	.04	[.85, 48.38]

Note: Dependent Variable: PTSD Scores

It was observed that trauma had a moderating effect when included in an interaction with DNA methylation of FKBP5 to predict PTSD symptoms (Figure 6) as well as in DNA methylation of NR3C1 to predict PTSD symptoms (Figure 7).

Note: The moderating effect of trauma on the relationship between DNA methylation of FKBP5 and PTSD symptoms (LH vs. HH group comparison).

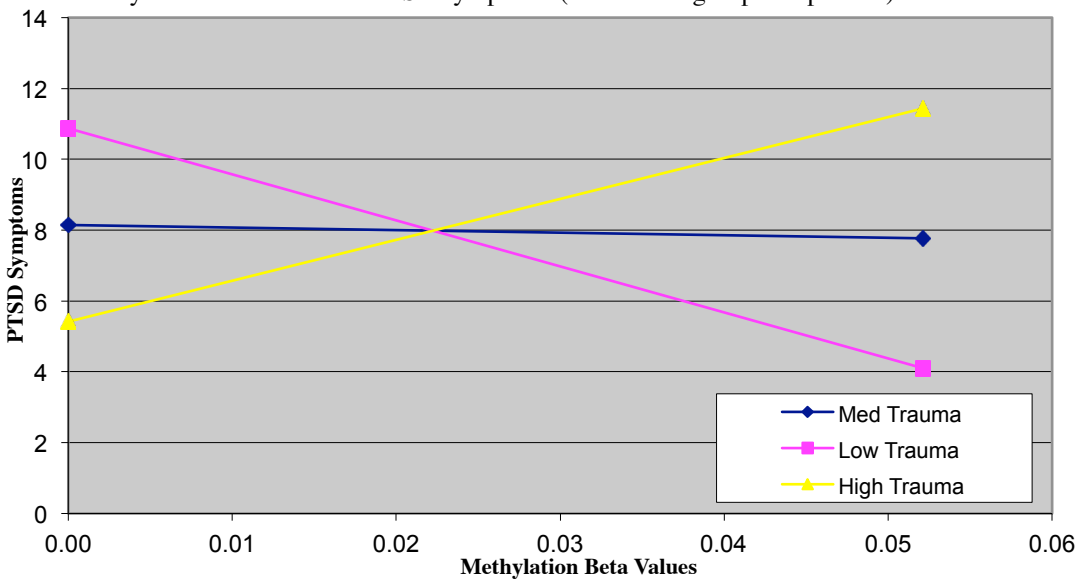

Figure 6: *Interaction graph treating trauma exposure as moderating variable between DNA methylation of FKBP5 and PTSD symptoms in the LH vs. HH group comparison.*

Legend for Figure 6: *Treating trauma as the moderator variable.*

When Trauma is High	
b-weight (for DNA Methylation for FKBP5 and PTSD symptoms)=	115.52995
A (intercept) =	5.41487
PTSD-predicted at DNA Methylation of FKBP5 average=	11.43386

When Trauma is Average	
b-weight (for DNA Methylation for FKBP5 and PTSD symptoms)=	-7.19660
A (intercept) =	8.14424
PTSD-predicted at DNA Methylation of FKBP5 average =	7.76930

When Trauma is Low	
b-weight (for DNA Methylation for FKBP5 and PTSD symptoms)=	-129.92315
A (intercept) =	10.87361
PTSD-predicted at DNA Methylation of FKBP5 average =	4.10474

Note: The moderating effect of trauma on the relationship between DNA methylation of NR3C1 and PTSD symptoms (HL vs. HH group comparison).

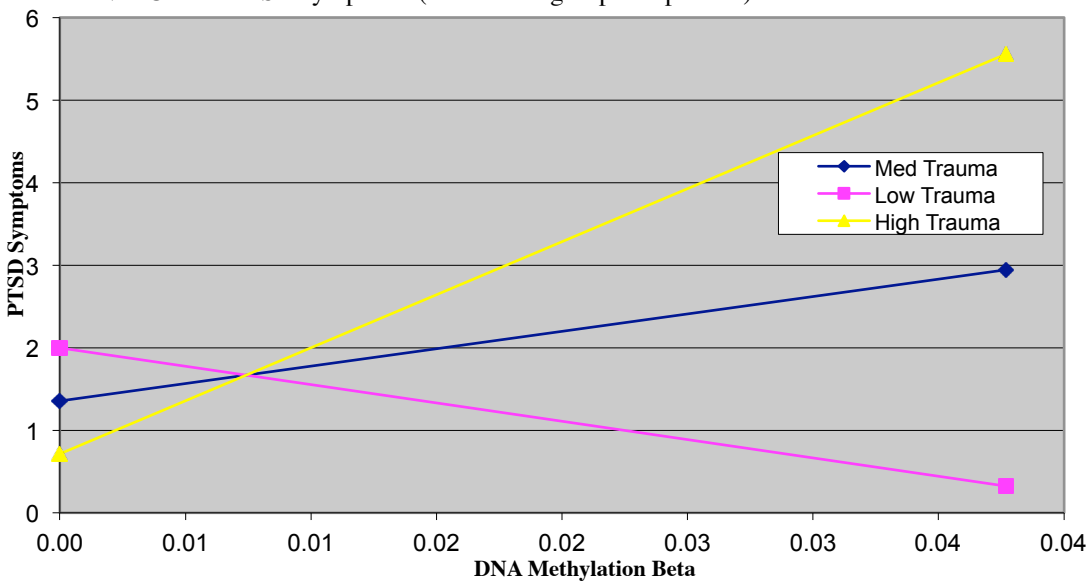

Figure 7: *Interaction graph treating trauma exposure as moderating variable between DNA methylation of NR3C1 and PTSD symptoms in the HL vs. HH group comparison.*

Legend for Figure 7: *Treating trauma as the moderator variable.*

When Trauma is High	
b-weight (for DNA Methylation for NR3C1 and PTSD symptoms)=	128.62763
A (intercept) =	0.71236
PTSD-predicted at DNA Methylation of NR3C1 average =	5.55956

When Trauma is Average	
b-weight (for DNA Methylation for NR3C1 and PTSD symptoms)=	42.12010
A (intercept) =	1.35365
PTSD-predicted at DNA Methylation of NR3C1 average =	2.94090

When Trauma is Low	
b-weight (for DNA Methylation for NR3C1 and PTSD symptoms)=	-44.38743
A (intercept) =	1.99494
PTSD-predicted at DNA Methylation of NR3C1 average =	0.32225

Summary of Hierarchical Regression Results

Of the four models, two were found to be significant and two showed trending significance. In all models computed, trauma was a significant predictor of PTSD symptoms, as would be expected. Age was not a significant predictor in any of the four models. Social support was a significant predictor in three models, and was trending significance in one model (FKBP5 in LH vs. HH). DNA methylation was not a significant predictor of PTSD; however, the interaction of DNA methylation and trauma was significant predictor of PTSD symptoms in two of the models, while two models showed a statistical trend. The regression models accounted for adjusted R^2 (.23 to .39). It was found that that trauma moderated the association between DNA methylation and PTSD symptoms. Hypothesis 4 (conceptual model) was partially supported by these results.

Chapter 5: Discussion

Overview

The aims of this study were to 1) to determine whether varying degrees of self-reported mental health measures and trauma exposure measures are uniquely reflected in genome-wide epigenetic analysis and DNA methylation of candidate genes of PTSD and 2) whether traumatic experience will moderate the relationship between self-report mental health and DNA methylation.

Traumatic events can modify gene expression by altering gene transcription via DNA methylation (Mifsud, 2011; Fraga et al., 2005 Yehuda, & Bierer, 2009). This molecular process plays a role in dsyregulating the body's stress system, the HPA axis (Yehuda, 2009). Such changes are associated with behavioral and symptomatic changes (Oitzel et al., 2010), and in particular, PTSD (Yehuda, 2009). This study utilized groups consisting of war-refugee with varying degrees of trauma exposure and PTSD symptoms to investigate genome-wide and PTSD candidate genes DNA methylation. Findings of this study may have important clinical implication, as DNA methylation is reversible (Weaver et al., 2009).

This chapter will present findings briefly for each hypothesis and for the conceptual model, as well as consider important literature relevant to the findings of this study. The chapter will conclude with a discussion of the study's limitations and expand on clinical implications of epigenetics in psychology.

Hypotheses:

The first hypothesis investigated whether there would be an observable DNA methylation difference in a genome-wide analysis in two group comparisons: HL vs. HH and LH vs. HH. This hypothesis was supported. There were significantly differentially methylated CpG sites in both group comparisons. It was observed that there was more differentially DNA methylated CpG sites in the LH vs. HH group than the HL vs. HH group comparisons.

The design of this study allowed for PTSD symptoms to be held constant in the HL vs. HH group comparison, while trauma was held constant in the HL vs. HH group comparison. It was found that more CpG sites were significantly differentially methylated across the genome in the group comparison that had PTSD symptoms held constant, but allowed variability in traumatic exposure. Such finding suggests that trauma exposure may play a role in genome-wide dysregulation of DNA methylation.

The findings of this study and those of others illustrate that trauma exposure is related to aberrant DNA methylation in both human and animal models (Uddin et al., 2011; Weaver et al., 2007; McGowan et al., 2009). Much like this study, a prior study confirmed this in a male population. Labonté et al. (2012) demonstrated that there are genome-wide DNA methylation differences in men with histories of childhood abuse when compared to males with no childhood abuse. Another possible explanation for more differentially methylated CpG sites in this study is that PTSD symptoms are a consequence of traumatic exposure; thus, trauma may elicit a physiological response that gives way to epigenetic aberrations (Yehuda & Bierer, 2009), and subsequently, to

behavior change and symptomology (Fraga et al., 2005; Yehuda & Bierer, 2009; Bagot et al., 2010; Boulle et al., 2011).

The second hypothesis investigated whether the refugee group who reported higher trauma exposures and higher PTSD symptoms (HH) would display elevated DNA methylation in HPA axis-associated genes, when compared to refugees who reported higher trauma exposure and lower PTSD symptoms (HL vs. HH) and to refugees who reported lower trauma and higher PTSD symptoms (LH vs. HH). This hypothesis was supported. An elevated level of DNA methylation was found in CpG sites of candidate genes, FKPB5 (cg16012111) and NR3C1 (cg00629244), in the HH group in both group comparisons (HL vs. HH and LH vs. HH). Both CpG (cg16012111 and cg00629244) sites are located in the promoter region of their respective genes.

The two candidate genes examined in this study are integral to the function of the body's stress response system, the HPA axis, and have been implicated in PTSD symptom development (Yehuda, 2009; Binder, 2009; de Kloet et al., 2007; Yehuda et al., 2009; Mehta et al., 2011; Sarapas et al., 2011). Specifically, they play a role in the GR complex. The NR3C1 gene encodes GRs, which bind to glucocorticoids (Lu et al., 2006) to inhibit the HPA-axis activity during post-stress exposure (Webster et al., 2002). The binding of glucocorticoids to GRs results in the transcription of the FKBP5 (FK506 binding protein), building a feedback loop between cells that regulate GR sensitivity (Binder, 2009) by regulating the GR signals between the cells (Stechschulte et al., 2011). While these genes are related, the epigenetic mechanisms involved in their relationship have yet to be thoroughly investigated (Yehuda, 2013).

In the current study, refugees who reported higher traumatic exposure incidences and higher PTSD symptoms had elevated DNA methylation at promoter regions of both genes. The promoter regions of genes are the genetic regulatory sites, where transcription occurs. More methylation of cytosine, termed as "hypermethylation," in the candidate genes, as was the case in the HH group, implies that the transcriptional factors and RNA polymerase are circumvented from accessing the DNA of the gene. The consequence of this is gene silencing and reduction in gene expression (Brenet et al., 2011; Yehuda & LeDoux, 2007; Strathdee & Brown, 2002; Turner, 2002).

Hypermethylation of the promoter regions of the candidate genes is often associated with those genes being less expressed, contributing to hyperactivity of the HPA axis (Sapolsky et al., 1990; Champagne et al., 2008; Teicher et al., 1997). DNA methylation can interfere with the signals of the HPA axis, as the increase of methylation of the genes implicated in the regulatory function of the HPA axis can result in reduced efficiency of the feedback loop that is involved in the HPA axis (Kappeler et al., 2010). Specifically, excessive methylation alters normal glucocorticoid feedback on adrenocorticotropic hormone (Perroud et al., 2008).

Hypermethylation of NR3C1 in the promoter region in humans and mice has been associated with prenatal stress, early life stress (such as childhood abuse), and poor maternal care (Mueller & Bale, 2008; Turner & Muller, 2005; Weaver et al., 2007; Oberlander et al., 2008; McGowan et al., 2009; Perroud et al., 2011). Perroud et al. (2011) found an increase in methylation, in peripheral blood, at the promoter region of NR3C1 in adults with childhood sexual abuse. Similarly, McGowan et al. (2009) demonstrated similar results when the team sampled tissues from the hippocampus of

suicide victims with childhood abuse. It was revealed that there was hypermethylation in NR3C1 when compared to suicide victims with no history of childhood abuse. Furthermore, Weaver et al. (2004) found that there was a decrease in the transcription activity in the regulatory sites of the NR3C1 gene in mice that were given less attention by their mother at a young age. The associated consequence is dysregulation of the HPA axis in these mice into adulthood. Similar studies have found that mice with less maternal attention had a decreased expression of the NR3C1 gene and lower availability of glucocorticoid receptors in the brain. This was associated with higher hormonal response to stress compared to mice with more material attention in the first week of their lives (Francis et al., 1999; Lui et al., 1997). Additionally, refugees who reported higher traumatic incidences in this study had the highest methylation in both of the candidate genes. Much like prior studies, the number of traumatic events played an important role in the methylation at NR3C1 (Perroud et al., 2011).

With regard to FKBP5, studies have found the alterations in DNA methylation and polymorphism in FKBP5 to be related to early life trauma and PTSD risk (Xie et al., 2010; Klengel et al., 2013; Mehta et al., 2011; Mehta & Binder, 2012). Mehta and Binder (2012) found an association between a polymorphism of FKBP5 and HPA axis function in 219 individuals with a history of trauma in adulthood, utilizing whole-blood genetic analysis, as well as, cortisol levels. The study included a sample of African Americans who reported a high rate of trauma exposure. To reiterate, FKBP5 plays an important role in the glucocorticoid complex of the HPA axis. The results of the study revealed that there was a difference in glucocorticoid receptor sensitivity between those with PTSD and those without it. Similar methylation pattern show a connection between childhood

trauma and the demethylation of FKBP5 (Klengel et al., 2013).

Within the framework of allostasis theory, prolonged stress can impact the hippocampus, which can affect the body's ability to terminate stress response. This process results in elevated HPA activity (McEwen & Gianaros, 2011). It is well established that prolonged exposure to glucocorticoids in the body is associated with adverse effects on the brain and subsequent behavioral problems, including risk of PTSD (Champagne et al., 2008; Oitzel et al., 2010; Yehuda, 2009). This is followed by deregulation of stress response when there are higher levels of glucocorticoids released at every subsequent stress response (Klengel, 2014). Epigenetic alterations, and especially DNA methylation, can help explain the alterations found in the HPA axis and the association between glucocorticoid alterations and PTSD risk (Yehuda, 2009).

The findings of this study may have important psychological implications. It was observed that refugees who reported high trauma exposure, yet developed less PTSD symptoms had less methylation in HPA axis-associated genes. This implies proper HPA axis functioning (Yehuda, 2009). Even at higher levels of stress, this group was resilient. Important factors, such as one's interpretation of stressful events and coping strategies, may explain such differences. Richard Lazarus's (1984; 1993) Stress Appraisal Theory can provide insight into these findings. In his theory, Lazarus stated that one's stress is impacted by their cognitive appraisal and evaluation of their ability to cope with the situation. In terms of cognitive appraisal, multiple factors can influence the amount of stress an individual experiences. For example, if an individual is unsure of how to avoid a harmful or stressful situation, they are more likely to experience higher levels of distress (Lazarus and Folkman, 1984). Such conditions are similar to a war environment, where

refugees are faced with harmful situations and may not know how to escape. Additionally, coping strategies can help mediate stressful exposures. Coping efforts can influence physiological changes, which can impact long-term health and well-being (Lazarus, 1991). It is plausible that refugees who experience less PTSD symptoms are employing mental strategies that can aid in overcoming stress. Future epigenetic studies should incorporate coping strategies as they may mediate the relationship between trauma exposure and DNA methylation of genes associated with the body's stress response system.

The third hypothesis investigated whether DNA methylation β-values were negatively associated with social support and positively associated with age. The refugee group comparisons were utilized to provide insight into the association between these factors and group affiliation. It was found that age was not correlated with DNA methylation values, contrary to studies that have found epigenome-wide alterations associated with aging (Horvath et al., 2010; Zaghlool, 2015). This finding has been reproduced in an Arab community sample (Zaghlool, 2015). A difference in study design may clarify this discrepancy. The current study focused on candidate genes implicated in PTSD risk, controlling for age in the genome-wide analysis, while the cited studies examined clusters of CpG markers found to be related to aging. Knowing that age is a protective factor in PTSD, investigating the role of age and PTSD and its impact on the epigenome offers a unique endeavor for future studies.

With regard to social support, FKBP5 in the HL vs. HH study was correlated with social support. Specifically, lower social support was correlated with higher methylation at this locus of the gene. This is the first study to our knowledge that has included social

support when investigating HPA axis-associated genes. Social support has been implicated in physiological regulatory processes, and particularly, in the HPA axis. It has been linked to lower HPA-axis activity in children (Gunnar et al., 1992) and adults (Seeman & McEwen, 1996). Social support has been found to play a role in the effects of other genes, like the serotonin transporter gene. Kilpatrick (2007) found that adults who were exposed to natural disasters, i.e., hurricanes, were more likely to develop PTSD if they had reduced social support.

In the current study, DNA methylation is associated with social support in the group comparison that combined refugees with high trauma, but had variability in PTSD symptomology. This finding provides a possible mechanistic explanation for findings that find social support to be an important factor in the development of PTSD in refugees (Lie, 2002). It is possible that social support can buffer against stress, thus impacting DNA methylation; however, more in-depth epigenomic study is important to elaborate on this finding.

Conceptual Model

The conceptual model for this study investigated whether PTSD symptom severity would be significantly predicted by: trauma exposure, DNA methylation β-values of candidate genes, and the interaction between trauma and DNA methylation β-values, taking into account covariates of interest (age and social support). This was computed by four linear regression models for each gene and their associated CpG sites. Foremost, it was observed that trauma predicted PTSD symptoms, confirming that the model is theoretically sound with respect to trauma being a prerequisite to the development of

PTSD symptoms (APA, 2013). Furthermore, social support predicted PTSD symptoms in three of the models. Refugees who perceived greater social support were less likely to report elevated PTSD symptoms. This finding is in accordance with studies that found social support to be as an important protective factor for PTSD, especially in Iraqi refugees (Gorst, Unsworth, & Goldenberg, 1998).

With respect to epigenetics, DNA methylation was not a predictor of PTSD symptoms in any of the models tested; however, the interaction of DNA methylation and trauma was a predictor of PTSD symptoms in two of the models. While works of Sarapas et al. (2011) and Yehuda et al. (2014) have found a significant association between DNA methylation and PTSD symptoms in these genes, findings in the current study revealed that the interaction between DNA methylation and trauma better accounted for PTSD symptom severity. This finding demonstrated that trauma moderates the association between DNA methylation and PTSD symptoms.

The interaction between DNA methylation of NR3C1 and trauma exposure significantly predicted PTSD symptoms in the HL vs. HH group comparison. The interaction between FKBP5 and trauma exposure predicted PTSD symptoms in the LH vs. HH group comparison. Studies have found that elevated DNA methylation in both candidate genes can interfere with HPA axis regulation, due to alterations in the glucocorticoid receptor complex (Yehuda & Ledoux, 2007). As such, the HPA axis fails to confine stress brought forth by traumatic events; the byproduct of this is associated with distress and PTSD symptomology (Yehuda, 2009).

The interaction between DNA methylation and traumatic exposure in predicting PTSD symptoms is important because it reveals that the environment does impact the

effects of DNA methylation status. In turn, it may effect gene transcription, gene expression, and ultimately, behavioral and mental health consequences (Stam, 2007; Ratten & Mill, 2009; Xin et al., 2012). It was found that being exposed to more traumatic events and having higher DNA methylation is related to reporting higher PTSD symptoms. This finding is accordance with research that has taken into account the interaction effect of the environment, and in particular, trauma, on DNA methylation (Uddin et al., 2011; Klengel et al., 2013). In particular, Uddin et al. (2011) have found an interaction between the methylation of a candidate gene of PTSD (*MAN2C1*) and trauma exposure, where higher methylation and greater number of trauma exposure increased risk of lifetime PTSD (Uddin et al., 2011). The findings of this study confirm this interactive effect in HPA axis-associated genes.

Different glucocorticoid alterations in the HPA axis functioning are related to distinctive aspects of PTSD manifestation; some of which are due to traits, while others correspond to symptom severity (Yehuda, 2009). Even though both genes are implicated in the function of the GR complex, their impact is not uniform. Because the genes have different jobs in the regulation of the HPA axis, being less expressed can impact the HPA axis differently. The results of this study allude to these findings. Differential DNA methylation in the regulatory region of NR3C1 was significant when comparing individuals with high trauma exposure, but who varied in degree of PTSD symptoms, whereas, differential DNA methylation in the regulatory region of FKBP5 was significant when comparing individuals who reported higher PTSD symptoms, but varied in degree of trauma exposure. This suggests that PTSD-glucocorticoid alterations are not confined to one aspect of HPA axis functioning.

Yehuda et al. (2013) further investigated this by examining the changes in DNA methylation status of both candidate genes during psychotherapy as a predictor of PTSD in Veterans. After 12 weeks of prolonged-exposure therapy, Yehuda et al. (2013) found that the methylation of the NR3C1 promoter region assessed before the start of therapy predicted treatment outcome (whether a veteran responded to therapy after 12 weeks). However, the DNA methylation of the gene at the promoter site did not change following assessment at the end of therapy (12 weeks of therapy) or at follow-up (3 months after end of therapy). Yehuda et al. (2013) suggested that these findings point to NR3C1 being related to prognosis of PTSD. In the current study, DNA methylation of NR3C1 gene was significantly different in HL vs. HH group comparison. In this group comparison, the trauma level was constant, however, there was a variation in PTSD symptoms. Refugees who reported higher trauma exposure and elevated PTSD show more methylation in the promoter regions of this gene. Findings, much like Yehuda et al. (2013), suggest that that DNA methylation of this gene may be associated with predicting the likelihood of individuals developing PTSD symptoms.

Furthermore, it was found that methylation of the FKBP5 gene promoter decreased in Veterans who were recovered by end of psychotherapy, which Yehuda et al. (2013) defined as no longer meeting the diagnostic criteria for PTSD after a three-month follow-up. Yehuda et al. (2013) suggested that this gene was, therefore, associated with severity of PTSD symptoms (Yehuda et al, 2013). In the current study, there was significantly differential DNA methylation of FKBP5 in the LH vs. HH group comparison; both refugee groups compared had elevated levels of PTSD symptoms, yet varying degrees of traumatic exposure. High levels of PTSD symptoms can provide insight into FKBP5's

association with symptom severity. While Yehuda et al (2013) and current study utilized different epigenotyping technologies to assess DNA methylation, the commonalities of aberrant DNA methylation in promoter regions associated with the risk, prognosis, and symptom severity in PTSD points to an important finding that should be investigated more comprehensively. This finding may address a critical research question regarding the underpinning of PTSD development.

The current study confirms that DNA methylation can aid in understanding the interactions between the environment, psychosocial stress, and the body. It is the first study to demonstrate the utility of linking self-reported data to quantifiable epigenetic signatures in refugees having been exposed to war and who have developed subsequent PTSD symptoms. The findings of this study contribute to a new research area that can shed light on the varying prevalence rate of PTSD among refugees by providing an epigenetic association of PTSD symptoms in vulnerable populations. It also opens up the possibility of utilizing DNA methylation as a biomarker to quantify stress, and so determine eventually the biological effects of trauma exposure and stress on the epigenome in individuals at high risk for developing PTSD.

Limitations of the Study

There were several limitations in this study, while beyond the researcher's control, must be addressed. The study design called for a two-group comparison, based on varying degrees of trauma exposure and PTSD symptoms. The groups' inclusion criteria were based on self-report measures collected from archival data. Even though some of the measures were re-administered (PCL and Harvard Trauma Questionnaire), the group affiliation criteria were pre-selected based on prior data collection of the

NIMH-funded longitudinal study. The use of archival measures is limiting, as it requires refugees to provide retrospective information regarding their mental health. Furthermore, PTSD symptoms were measured using the PCL-C, which is an inventory of symptoms and not a diagnostic measure of PTSD. The measure defined PTSD within the Western culture, possibly hindering our abilities to understand the construct of within the Arab culture. This can lead to inaccurate results if not interpreted with caution. Furthermore, the HTQ assessed trauma on a continuous scale and was utilized to examine the impact of cumulative trauma on health outcomes in the current study. However, Arnetz et al. (2014) has found that utilizing subtypes of trauma improve the predictive validity of the HTQ in Iraqi refugees. It is likely that more can be gleaned from investigating specific trauma or trauma types, such as trauma involving the individual refugee compared to trauma or threat imposed on others, and their association to health outcomes, including PTSD and DNA methylation.

Another limitation in the current study is that data collection did not account for refugees' level of acculturation. Refugee research has pointed to the importance of acculturative stress on refugees' health (Sundquist et al., 2000; Palinkas & Pickwell, 1995). Stress is associated with the adjustment and adaptation to the culture of the host country (Sundquist et al., 2000). In the current study, refugees have resided in the U.S. for an average of two years at the time of data collection. It is conceivable that their stress level is related to the acculturation process, possibly impacting mental health outcomes. Future studies would gain a more comprehensive assessment of refugee mental health and associated DNA methylation by assessing level of acculturation.

This study is a cross research design, limiting the researcher's ability to infer a cause and effect relationship between variables of interest. As such, it provided only a snapshot the population's symptom presentation rather than a comprehensive examination of symptoms within a longer period of time. Additionally, the study utilized a convenient sample of an ethnic group concentrated in a metropolitan area. This posed a threat to the generalizability of the sample to a larger population of refugees. Only males were selected to participate due to limited funding, which may have threatened the external validity of the study. Further, inclusion of men made the study less generalizable, especially in terms of epigenetic findings, as women and men's physiological response to stress differs.

With regard to epigenetics, this study investigated only one out of three possible epigenetic mechanisms. While DNA methylation is widely investigated, more insight can be gained from investigating other epigenetic mechanisms, such as histone modification. This is especially true in psychiatric research. Epigenetic analysis is made possible by the development of array-based technologies, such as the 450K BeadChip. This technology has allowed for large-scale epigenotyping studies, like genome-wide epigenetic analysis. While there are major advantages to this technology, such as being cost efficient compared to previous models (Touleimat & Tost, 2012), having a strong test retest reliability ($r > .98$) (Illuminia, 2012), and replication of results in different populations (e.g., Joubert et al., 2012), it is not without its limitations. The 450K BeadChips surveys only 2% of the CpG sites in the genome (approximately 450,000 single-nucleotide), providing only a small snapshot of DNA methylation activity (Morris & Beck, 2015). Furthermore, the statistical analyses computed from the resulting dataset are often

complicated due to the complexity and size of the datasets produced by the bead chip (Fazer & Greatly, 2004).

Another area of limitation is the technology's inability to provide possible association of DNA methylation to genetic information, such as risk alleles (Morris and Beck, 2015). As such, it is difficult to determine the association between functional and genetic molecular underpinning of disorders (Fazer & Greatly, 2004). Furthermore, other environmental exposures may play a role in the progression of disease (Rayan et al., 2012). For such reason, it is difficult to infer causation in epigenetic results provided by this technology. Additionally, epigenetic variations may be present prior to disease onset; however, it may not be causative for the development of disease. Studies in model systems are needed to clarify pathways of development associated with epigenetic findings (Holbrook, 2015).

In the current study, DNA methylation data was collected from whole blood, whereas PTSD is considered a brain-based disorder. However, more recent studies have found that blood samples have the ability to detect DNA methylation differences in candidate genes in disorders likely to be expressed throughout the body (Rakyan et al., 2012). Specific to PTSD, NR3C1 gene DNA methylation has been established in peripheral blood (Yehuda et al., 2015; Zeiker et al., 2007). Another limitation is that this study did not control for single-nucleotide polymorphisms (SNPs) in the epigenetic analysis. While these genetic variations are important to account for in such analysis, the lack of an annotated SNP database specific to the sample's ethnic background was an obstacle. However, to minimize possible genetic variations, the sample used in this study is of refugees from a similar area of Iraq and who are predominately of the same religious

background. It behooves future researchers in this area to conduct studies that would identify single-nucleotide polymorphisms specific to ethnic groups that are not accounted to date.

Finally, various factors can confound genome-wide epigenetic analysis, including age, idiosyncratic traits, and behaviors. The age range of the refugees in this study is fairly restrictive (20-59 years). It would be important to include a wider range of age in future studies to ensure genome activity is accounting for age-related DNA methylation changes. Furthermore, factors, such as smoking behaviors, can impact the epigenome and should be controlled for future studies (Rakyan et al., 2012).

Clinical Implications

Mental illnesses, such as PTSD, are complex, with inconclusive bases for etiology. As such, a multifaceted approach in understanding disease susceptibility and development of symptoms is necessary. Much like this multifaceted approach, a new field in epigenetics—Behavioral Epigenetics—is calling for an interdisciplinary involvement from psychologists, psychiatrists, physicians, and geneticists, to explore the underpinnings of mental health disorders (Holbrook, 2014). Working across disciplines would better inform theory and practice, specifically, in the areas of disease development, diagnosis, and intervention.

Although the majority of epigenetic research has been devoted to cancer progression, a new frontier of psychiatric research has emerged that examines the association of epigenetic modifications and mental health symptoms (Galea, Uddin, & Koenen, 2011; McGowan & Szyf, 2010; Dudley et al., 2011; Yehuda et al., 2011; Yehuda & Bierer, 2009). The study of epigenetics shows promise in its ability to provide

biomarkers that may confirm the cellular mechanisms involved in the phenotypic expression of psychiatric disorders following environmental stress. Prior studies have found that even minor changes to either genetic or environmental factors are associated with resilience or vulnerabilities in the face of stress (Sanchez, 2006). Thus, environmental factors may play an important role in gene expression (Dudley et al., 2011; Jaenisch & Bird, 2003; Petronis, 2004). This is of relevance as the current study has demonstrated that traumatic events or the environmental stress moderates the association between DNA methylation and PTSD symptoms.

Epigenetics may have important implications for psychology and may shed light on the inter-individual differences between mental illness and behavior (e.g., Yehuda & Bierer, 2009). PTSD prevalence rates are heterogeneous, with only a minority of trauma-exposed individuals developing PTSD. DNA methylation may help explain the varying prevalence of PTSD and the reasons some individuals have post-trauma symptoms, while others are resilient (Yehuda & Bierer, 2009). Research findings in areas of diet, psychotherapy, and psychopharmacological can help demonstrate the clinical importance of epigenetics.

The Dutch Famine study, conducted by Roseboom and colleagues (2001) has demonstrated the importance of environment on human molecular development in early life. During World War II, the Germans place an embargo on goods entering the Netherlands between 1944 and 1945. Consequently, a population that had been well nourished became nutritionally deprived. Of particular interest to the researchers was the impact of the famine on fetal development during the famine and subsequent health in later life in these individuals. Roseboom and Colleages (2001) were able to track 2414

individuals who were still alive to assess their current health. The findings suggested that the period when a fetus was exposed to the famine was related to their health later in life. Specifically, mortality and risk for chronic disease development, such as high blood pressure, impaired glucose tolerance, and obesity in adulthood was related to the period an individual was exposed to the famine during gestation. A fetus exposed to the famine in early part of the gestational period was more likely to have poor health outcomes in later life, suggesting that there is a link between developmental programming and nutrition (Roseboom et al., 2001).

Epigenetics can help clarify the findings from the Dutch Famine study. Diet has been well-studied in terms of its effects on human disease through epigenetic changes (Allis et al., 2009; Kovalchuck & Kovalchuck, 2012). Several neuropsychiatric disorders that have been associated with alterations of methylation patterns in the central nervous system were also linked to B12 and folate deficiencies; these are factors that are important in the methylation process. For example, depression symptoms in participants improved when they were given higher doses of folate in their diets (Bottiglieri et al., 1992; 2000). Furthermore, adult males suffering from uremia were shown to have reduced global and locus-specific DNA methylation, which was reversible via supplementation with high doses of folic acid (Ingrosso et al., 2003). Additionally, mice that were carriers of the agouti variable gene, which made them more susceptible to diabetes, were given a diet of folate, choline, and betaine while pregnant (Waterland & Jirtle, 2003). Their offspring demonstrated a shift of coat color, which was correlated with higher methylation levels in the *agouti* genes. It was believed that the diet provided the mice with extra methyl donors, aiding in the DNA methylation of a gene associated

with disease. This study provided insight into the epigenetic-phenotype relationship and the possibility of such relationships being related to stable changes in the epigenome (Waterland & Jirtle, 2003). More research is needed to explore the possibility of identifying diets that target PTSD symptoms.

Specific to clinical psychology, epigenetics may have important implications for understanding mental health disorders (Pardo & Alvarez, 2013). This is due to the fact that the epigenome is highly dynamic and DNA methylation can be reversed or restored to regain proper and efficient cellular functioning (Weaver et al., 2009; Gregg et al., 2010). Understanding of epigenetic processes may inform new diagnostic theories and initiate the opportunity for novel PTSD treatment to develop. Studies have demonstrated that therapeutic modalities have been associated with alterations in DNA methylation levels in HPA axis-associated genes in trauma-exposed Veterans (Yehuda et al., 2013). Yehuda et al. (2013) further established that genes, specifically NR3C1 and FKBP5, were associated with different PTSD presentations. NR3C1 were associated with the prognosis of PTSD, whereas FKBP5 were associated with symptom severity. Such finding may be the first step in the possibility of utilizing DNA methylation as an additional means to aid clinicians in conceptualizing PTSD. DNA methylation can be used as a biomarker in noninvasive epigenetic screenings for individuals' pre- and post- therapy (Holbrook, 2014), providing another source of information, in addition to self-report measures. However, more research will be vital in this area.

Furthermore, one's interpretation of a 'stressful event' is important in how each individual processes traumatic events (McEwen & Gianaros, 2011; Lazarus, 1991), and whether these events initiate the body's physiological stress response. Even if individuals

are pre-disposed to having a vulnerable HPA axis, the interpretation of the event can help individuals cope with the stressor and alleviate the body's stress response. Interpretations, which can be processed in psychotherapy, can mediate the body's response to stress.

The utilization of epigenetic mechanisms in psychopharmacological therapy is a growing research area (Powledge, 2011). Insight gained from epigenetic analysis may help identify pharmacological approaches to alleviate symptoms by targeting the dysregulated system in the body. For example, PTSD is associated with reduced cortisol signaling and increased exposure to endogenous catecholamine; it is believed that the HPA axis can be regulated by introducing cortisol to those effected (Yehuda, 2009). This has been demonstrated in several studies of trauma survivors, in which low-dose glucocorticoids were associated with a reduction of chronic PTSD symptoms, (Schelling et al., 2006). Other studies have shown that administering high levels of glucocorticoids (cortisone) is associated with decreased PTSD risk (Schelling et al., 2001). Such findings demonstrate that glucocorticoids can aid in decreasing PTSD, by decreasing the traumatizing effects of the memory associated with the traumatic event (review in Yehuda, 2009).

Recommendation for Future Research

Several important implications are highlighted from the findings of the current study. It behooves researchers to include other forms of epigenetic modification, besides DNA methylation, in order to elucidate the underpinning of disease development, especially for mental illness. At this time, we are unable to determine, with full certainty, that the epigenetic information obtained from peripheral blood can reflect the status of DNA methylation associated with brain-based disorders, like PTSD. As such, future

research must broaden the ability to access more tissue in human studies (Powledge, 2011). Furthermore, conducting longitudinal studies to investigate epigenetic modification is essential in providing a compressive examination of DNA methylation over time. Specific to psychology, it is important to include diagnostic measures of PTSD, rather than only utilizing symptom inventory measures. Furthermore, obtaining more information regarding the genetic factors associated with disease susceptibility can be an important addition to epigenetic analysis. It can shed light on ways these factors may interact in candidate genes (e.g., Klengel et al., 2013). It may also provide information on the association between genotype and phenotype, and in particular, the role of the environment in this relationship.

Conclusion

This study set out to explore DNA methylation differences in PTSD candidate genes in a genome-wide DNA methylation analysis. The overall goal of the study was to utilize a unique group comparison of war refugees with varying degrees of trauma exposure and PTSD symptoms manifestation to investigate DNA methylation differences associated with risk and resiliency of PTSD symptoms in a vulnerable population. Findings of this study suggested that higher trauma exposure was likely to play a role in the genome-wide dysregulation of DNA methylation. Furthermore, higher DNA methylation was found in regulatory regions of HPA axis associated genes. The findings are in accordance with prior studies, suggesting that traumatic events may modify gene expression and give way to dysregulation in the body's stress response system. Additionally, results revealed that DNA methylation did not predict PTSD, however, the interaction of DNA methylation and trauma exposure was a significant predictor of

PTSD. This suggested that trauma moderates the association between DNA methylation and PTSD symptoms. Epigenetics findings may have important clinical implications, as they may provide insight in disease risk, prognosis, and symptom severity. Moreover, findings can aid in informing the development of new diagnostic theories and development of novel PTSD treatments.

Appendix A: Research Informed Consent in English

Title of Study: Mental Health in Iraqi Refugees: Importance of post-displacement social stressors and institutional resources

Principal Investigator (PI): Bengt B. Arnetz
Family Medicine and Public Health Sciences
(313) 577-2644

Funding Source: Grant Plus Program

Purpose

You are being asked to be in a research study of whether certain environmental exposures during wartime may have lasting effects on your genes because you are an Iraqi refugee who has previously participated in our study. This study is being conducted at Wayne State University and ACCESS Medical Clinic (Macomb). The estimated number of study participants to be enrolled at Wayne State University and ACCESS Medical Clinic is about 60 participants. **Please read this form and ask any questions you may have before agreeing to be in the study.**

In this research study, the researchers are collecting blood samples and a brief survey to learn more about a variety of environmental factors and chemical exposures that may have occurred in your home country, which may have affected hormones, and therefore, stress levels in the United States. Certain environmental exposures during wartime may have lasting effects on one's genes, through the modification of the DNA. This in turn will affect how our genes work and how our bodies respond to our current environment. Researchers are interested in investigating the information you have provide us through the brief survey regarding the prevalence of stress and environmental exposures to petrochemicals and metals.

Study Procedures

If you agree to take part in this research study, you will be asked to give a blood sample.

1. If you agree to be in this study, you will go to *ACCESS* Medical Clinic and complete a simple blood draw and a brief survey.
2. The blood will be drawn by placing a needle into a vein in your arm. One small tube of blood will be taken.
3. This will be done in one visit and the visit will take about twenty minutes.
4. You are free to choose the date of your clinic visit.
5. Wayne State University will keep the questionnaire and the blood results in a locked cabinet where it will be inaccessible except for research purposes supervised by the principle investigator. Each questionnaire and blood results will be assigned an identification number rather than using the name of the participant. The master list linking your name and ID number will be kept in a locked cabinet in a secure office. This list will be destroyed at the end of data collection.

Benefits
As a participant in this research study, there may be no direct benefit for you; however, information from this study may benefit other people now or in the future.

Risks
By taking part in this study, you may experience the following risks:
Physical Risks:
1. Slight discomfort or bruising from the blood draw.
2. The needle stick may hurt.
3. There is a small risk of bruising.
4. A rare risk of infection.
5. You may feel lightheaded.

Social/Economic Risks:
1. Possible Loss of Confidentiality

Emotional Risk
1. Feeling of anxiety

There may also be risks involved from taking part in this study that are not known to researchers at this time.

Study Costs
Participation in this study will be of no cost to you.

Compensation
For taking part in this research study, you will be paid for your time and inconvenience. Each participant will receive a gift certificate to a local store in the amount of $35.00 after their blood draw at the clinic.

Research Related Injuries
In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Care for such will be billed in the ordinary manner to you or your insurance company. No reimbursement, compensation, or free medical care is offered by Wayne State University or ACCESS Medical Clinic. If you think that you have suffered a research related injury, contact the PI right away at (313) 577-2644

Confidentiality
All information collected about you during the course of this study will be kept confidential to the extent permitted by law. You will be identified in the research records by a code name or number. Information that identifies you personally will not be released without your written permission. However, the study sponsor, the Institutional Review Board (IRB) at Wayne State University, or federal agencies with appropriate regulatory oversight [e.g., Food and Drug Administration (FDA), Office for Human Research Protections (OHRP), Office of Civil Rights (OCR), etc.) may review your records.

When the results of this research are published or discussed in conferences, no information will be included that would reveal your identity.

Voluntary Participation/Withdrawal

Taking part in this study is voluntary. Donation of blood for research is voluntary and you should not be placed under any pressures to do so. You have the right to choose not to take part in this study. If you decide to take part in the study you can later change your mind and withdraw from the study. You are free to only answer questions that you want to answer. You are free to withdraw from participation in this study at any time. Your decisions will not change any present or future relationship with Wayne State University or its affiliates, or other services you are entitled to receive.

The PI may stop your participation in this study without your consent. The PI will make the decision and let you know if it is not possible for you to continue. The decision that is made is to protect your health and safety, or because you did not follow the instructions to take part in the study

Questions

If you have any questions about this study now or in the future, you may contact Dr. Bengt Arnetz or one of his research team members at the following phone number (313) **577-2644**. If you have questions or concerns about your rights as a research participant, the Chair of the Human Investigation Committee can be contacted at (313) 577-1628. If you are unable to contact the research staff, or if you want to talk to someone other than the research staff, you may also call (313) 577-1628 to ask questions or voice concerns or complaints.

Consent to Participate in a Research Study

To voluntarily agree to take part in this study, you must sign on the line below. If you choose to take part in this study you may withdraw at any time. You are not giving up any of your legal rights by signing this form. Your signature below indicates that you have read, or had read to you, this entire consent form, including the risks and benefits, and have had all of your questions answered. You will be given a copy of this consent form.

_____	_____
Signature of participant / Legally authorized representative	Date
_____	_____
Printed name of participant / Legally authorized representative	Time
_____	_____
Signature of witness**	Date
_____	_____
Printed of witness**	Time
_____	_____
Signature of person obtaining consent	Date
_____	_____
Printed name of person obtaining consent	Time

*Remove LAR reference if you don't intend to consent participants that have or may have a LAR.

**Use when participant has had this consent form read to them (i.e., illiterate, legally blind, translated into foreign language).

_____	_____
Signature of translator	Date
_____	_____
Printed name of translator	Time

Appendix B: Research Informed Consent in Arabic

موافقة على المشاركة في بحث
عنوان البحث : الصحة النفسية للاجئين العراقيين: اهمية الضغوط الاجتماعية والخدمات الحكومية لما بعد التوطين
الباحث الرئيسي : الدكتور بينغت آرنيتز
قسم طب العائلة وعلوم الصحة العامة
3135772644

اهداف الدراسة
يقوم الباحثون في هذه الدراسة بجمع عينات من الدم لمحاولة ايجاد العلاقة مابين التعرضات الكيمياوية والسايكو اجتماعية المرافقة للعمليات الحربية في بلادك الاصلية مع المستوى الحالي لهورمونات الضغوط النفسية ، ومؤشرات الالتهابات والتمثيل الجيني وهو مايسمى بالمؤشرات الايبي جينية. ان الدراسات الايبي جينية سواء كانت الجينات في الحمض النووي ناشطة او معتمدة على اضافة او انتزاع نواتج كيمياوية معينة يمكن ان تلتصق او لاتلتصق بجيناتك .
الباحثون مهتمون بدراسة العلاقة بين الاقرار الشخصي المتعلق بمعدل الاضطرابات النفسية له علاقة ببعض التعرضات البيئية للمواد البتروكيمياوية والمعدنية وانطباعها في بعض جينات الحمض النووي
ان بعض التعرضات البيئية خلال فترة الحرب قد يكون لها تاثير طويل الامد على جينات الشخص المتعرض من خلال تحوير الحمض النووي. وهذا سينعكس بدوره على الكيفية التي تعمل بها الجينات وكيفية استجابة الجسم للبيئية الحالية .

خطوات الدراسة
(1) بما انك قد اكملت ملأ استبيانين في هذه الدراسة فلديك الخيار في المشاركة في الملحق الطبي لهذه الدراسة
(2) مطلوب منك اعطاء عينة من الدم
(3) اذا وافقت على المشاركة في هذا الملحق سيتعين عليك الذهاب الى عيادة اكسيس واتمام سحب الدم .
(4) سيتم سحب الدم من الوريد وبمقدار لايزيد عن 20 سي سي اي مايعادل 4 ملاعق من الشاي وتستغرق العملية 5 دقائق ، كما سنأخذ بضعة قطرات اخرى من الدم من وخزة ابرة من اصبعك وتوضع القطرات على ورقة خاصة
(5) انت مخير في اختيار موعد الذهاب الى العيادة
(6) سيكون بوسع الباحثين بربط عينة دمك باسمك والاستبيانات التي سبق لك ملؤها ، وبعدها سيتم رفع اسمك لالغاء اية امكانية في المستقبل لربط معلوماتك بشخصيتك من قبل اناس غير فريق البحث للمحافظة على حقوقك في سرية المعلومات.
(7) ستحتفظ جامعة وين بالاستبيان ونتائج عينة الدم في خزانة مقفلة حيث لايمكن الوصول اليها الا لاغراض البحث المشار اليه وفقط تحت اشراف الباحث الرئيسي الدكتور آرنيتز . وسيخصص رقم استدلالي بدلا من وضع اسم المشارك . وسيتم الحفاظ على سرية القائمة التي تربط الارقام الاستدلالية بالاسماء في خزانة مقفلة في مكتب مؤمن وسيتم اتلافها حال انتهاء من جمع المعلومات .

المنافع
سيحصل كل مشارك بعد اتمام سحب الدم في العيادة على كارد تسوق بمبلغ 35 دولارا
لاتوجد منافع اخرى للمشاركين في هذه الدراسة ، لكن نتائجها ربما سيكون لها فائدة كبيرة للمشاركين ولاشخاص آخرين في المستقبل

المخاطر
لاتوجد مخاطر من الدراسة سوى الازعاج البسيط الذي يسببه سحب الدم، او احتمال حصول ازرقاق لبضعة ايام او تورم في المنطقة او الشعور بالدوخة ولكلها احتمالات نادرة

التكلفة
المشاركة لن تكلف المشارك شيئا

اصابات متعلقة بالمشاركة

احتمال حصول مثل هذه الاصابات نادرة جدا ولكن في حالة وقوع اصابة لها علاقة بالبحث سيتم توفير العلاج كالاسعافات الاولية واسعاف الطواريء في العيادة التي سيتم فيها سحب الدم في اكسيس

المشاركة والانسحاب اختياري

ان المشاركة في هذه الدراسة طوعي تماما. واعطاء عينة دم لاغراض البحث العلمي قرار اختياري بمطلق الحرية ودون اية ضغوط . لست مجبرا باية حال على المشاركة باعطاء عينة الدم وليس مطلوبا منك اعطاء تفسيرات لعدم رغبتك في المشاركة . ومن حقك الانسحاب من المشاركة في اي وقت تشاء ، وقرارك سوف لن يؤثر على علاقتك الحالية او المستقبلية بجامعة وين او الكادر المرتبط بها او اية خدمات من حقك ان تحصل عليها. للباحث الرئيسي الحق في ايقاف مشاركتك بالدراسة واعلامك بذلك اذا لم يكن الاستمرار بها في صالحك او اذا كان استمرارك سيعرضك الى مخاطر صحية او لانك لم تتبع الاجراءات الخاصة بالمشاركة . خلال مشاركتك سيتم اعلامك باية مستجدات ربما تغير استعدادك للاستمرار في الدراسة .

الاسئلة

اذا كانت لديك اية استفسارات حول هذه الدراسة الان او في المستقبل يمكنك الاتصال بالدكتور بينغت آرنيتز او احد اعضاء فريق البحث على الرقم التالي 3135772644

اذا كانت لديك استفسارات حول حقوقك كمشارك في بحث يمكن الاتصال بلجنة البحوث البشرية على الرقم 313 5771628

الموافقة على الاشتراك

من خلال توقيعي على هذه الموافقة اقر باني قد قرأت محتويات هذه الوثيقة او انها قرأت على مسامعي من قبل شخص اثق به وباني قد حصلت على اجابات على كل استفساراتي .

توقيع المشارك **التاريخ**

توقيع الباحث **التاريخ**

رقم استمارة الاستبيان **تاريخ سحب الدم**

Appendix C: Participant Recruitment Letter in English

Dear Participant,

You are being contacted by our research group at Wayne State University because you have previously participated in the following study: Mental Health in Iraqi Refugees: Importance of post-displacement social stressors and institutional resources. You have completed two interviews that consisted of questionnaires thus far. We are adding a medical component to our study and are inviting you to participate. This is separate from the follow-up questionnaire portion of the study. In this study, our research team is collecting blood samples to learn more about the possible biological effects from war-related trauma and chemical exposures. Certain environmental exposures during wartime may have lasting effects on one's genes, through the modification of the DNA. This in turn will affect how our genes work and how our bodies respond to our current environment. Our research team is interested in investigating whether self-reported war-related environmental exposures as well as self-reported exposures to metals and chemicals during the war are reflected in our DNA.

Taking part of this medical component of the study will require you to complete a simple blood draw. If you agree to be in this study, you will go to *ACCESS* medical clinic and complete the blood draw. The blood will be drawn by placing a needle into a vein in your arm as well as through a finger prick. One small tube of blood will be taken as well as a few drops of blood will be collected from your fingertip. This will take about five minutes. You are free to choose the date of your clinic visit. The risks associated with this study are slight discomfort or bruising from the blood draw. The needle stick may hurt. There is a small risk of bruising; a rare risk of infection, and you may feel lightheaded. However, no more of a risk than having your blood drawn for your regular doctor's physical. Research related injuries are extremely unlikely; however, in the event that this research related activity results in an injury, treatment will be made available including first aid and emergency treatment provided at the sight of the blood draw, at ACCESS medical clinic. Once completing the blood draw, you will receive a gift certificate to a local store in the amount of $35.00.

Your participation will be confidential. Wayne State University will keep the questionnaire and the blood results in a locked cabinet where it will be inaccessible except for research purposes supervised by the principle investigator. Each questionnaire and blood results will be assigned an identification number rather than using the name of the participant. The master list linking your name and ID number will be kept in a locked cabinet in a secure office. This list will be destroyed at the end of data collection.

Taking part in this study is voluntary. Donation of blood for research is voluntary and you should not be placed under any pressures to do so. You do not have to agree to give a blood sample nor need to explain why you should choose not to donate. You are free to withdraw from participation in this study at any time. Your decisions will not change any present or future relationship with Wayne State University or its affiliates, or other services you are entitled to receive. If you are interested, please feel free to contact our research group at (313) 577-2644. You may also contact Dr. Yousif Rafa at (248) 252-1562 or through email at jopsych55@yahoo.com.

Appendix D: Participant Recruitment Letter in Arabic

المشارك العزيز
يسرفريق البحث في جامعة وين ان يتصل بك كونك قد شاركتنا سابقا في الدراسة الموسومة : الصحة النفسية لدى اللاجئين العراقيين ، اهمية الضغوط الاجتماعية والخدمات المؤسساتية.
لقد اكملت مقابلتين خلال تلك الدراسة اقتصرت على الاجابة على استبيان ومازالت امامك مقابلة ثالثة تجيب فيها على اسئلة الاستبيان ايضا. وقد قمنا باضافة جانب طبي الى الدراسة ندعوك للمشاركة به. اذا يقوم فريق البحث الخاص بالدراسة بجمع عينات من الدم لغرض دراسة التاثيرات البايولوجية لما يمكن ان تكون قد تعرضت له من مخلفات الحرب والمواد الكيمياوية. فبعض العوامل البيئية والتعرضات الكيمياوية خلال الحرب يمكن ان يكون لها تاثير طويل الامد على الجينات من خلال تحوير الحامض النووي في الخلايا. وهذا بدوره يمكن ان يؤثرعلى طريقة عمل الجينات واستجابة الجسم للبيئة الحالية. والفريق الباحث مهتم بدراسة كيف تنعكس بيئة الحرب والتلوثات الناتجة عن المعادن والمواد الكيمياوية على الاحماض النووية للمشاركين بهذه الدراسة

ان المشاركة في الملحق الطبي لهذه الدراسة سيتطلب منك اجراء فحص دم بسيط. فاذا وافقت على المشاركة ستذهب الى عيادة اكسيس الطبية لاتمام الفحص. سيتم سحب الكمية المطلوبة وهي مقدار انبوبة صغيرة من الدم من الوريد وعن طريق شكة في الاصبع ، حيث تستغرق العملية بكاملها 5 دقائق . انت مخير في اختيار الوقت المناسب لك للذهاب . مخاطر عملية السحب لاتكاد تذكر وهي الم الوخزة ونادرا ما تحدث زرقة في مكان السحب يبقى اثره اياما قليلة وهي نفس الاثار عندما يسحب منك الدم لاغراض اجراء التحليل الطبي. واذا
حدثت اية اضرار من عملية السحب فسيتم توفير العلاج اللازم في نفس عيادة اكسيس الطبية،الا ان احتمالية حدوث ذلك لاتكاد تذكر.
مقابل هذه المشاركة ستحصل على هدية عبارة عن كارد تسوق بقيمة 35 دولارا.

مشاركتك ستكون محاطة بالسرية .ستحتفظ جامعة وين باجوبة الاستبيان ونتائج فحص الدم في خزانة مقفلة لايصل اليها الا فريق البحث وتحت رقابة الباحث الرئيسي. سيتم اعطاء كل استمارة استبيان ونتيجة فحص الدم رقم استدلال حيث لايتم استخدام اسماء المشاركين والتي ستبقى سرية ومحفوظة في مكتب آمن. وسيتم اتلاف كل المعلومات في نهاية الدراسة.

ان المشاركة في هذه الدراسة طوعي تماما. واعطاء عينة دم لاغراض البحث العلمي قرار اختياري بمطلق الحرية ودون اية ضغوط . لست مجبرا باية حال على المشاركة باعطاء عينة الدم وليس مطلوبا منك اعطاء تفسيرات لعدم رغبتك في المشاركة . ومن حقك الانسحاب من المشاركة في اي وقت تشاء ، وقرارك سوف لن يؤثر على علاقتك الحالية او المستقبلية بجامعة وين او الكادر المرتبط بها او اية خدمات من حقك ان تحصل عليها.
اذا كنت مهتما بهذا البحث ، يمكنك الاتصال بفريق البحث على هاتف 3135772644
او يمكنك الاتصال بالدكتور يوسف روفا على الرقم 2482521562 او على البريد الالكتروني الخاص به وهو jopsych55@yahoo.com.

Appendix E: Posttraumatic Stress Disorder Questionnaire-PCL-C

PCL/PTSD (21-37) Please select the box which best corresponds to how much you have been bothered by each listed problem *in the last month:*	[1]	[2]	[3]	[4]	[5]
Repeated, disturbing memories, thoughts, or images of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Repeated disturbing dreams of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Suddenly acting or feeling as if a stressful experience were happening again?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling very upset when something reminded you of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Having physical reactions (heart pounding, trouble breathing, sweating) when something reminded you of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Avoid thinking about or talking about a stressful experience from the past or avoid having feelings related to it?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Avoid activities or situations because they remind you of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Trouble remembering important parts of a stressful experience from the past?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Loss of interest in things you used to enjoy?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling distant or cut off from other people?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling emotionally numb or being unable to have loving feelings for those close to you?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling as if your future will somehow be cut short?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Trouble falling asleep or staying asleep?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling irritable or having angry outbursts?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Having difficulty concentrating?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Being super alert or watchful or on guard?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely
Feeling jumpy or easily startled?	[1] Not at all	[2] A little bit	[3] Moderately	[4] Quite a bit	[5] Extremely

Appendix F: Trauma Exposure Questionnaire-Harvard Trauma Questionnaire

TRAUMA (124-162) Please circle "YES" or "NO" for each question to indicate whether or not you have experienced any of the following events "Before coming to the U.S." and/or while "In the U.S."	Before coming to the U.S.	
1. Oppressed because of ethnicity, religion, or sect	[2] No	[1] Yes
2. Present while someone searched for people or things in your home	[2] No	[1] Yes
3. Searched arbitrarily	[2] No	[1] Yes
Please circle "YES" or "NO" for each question to indicate whether or not you have experienced any of the following events "Before coming to the U.S." and/or while "In the U.S."	Before coming to the U.S.	
4. Property looted, confiscated, or destroyed	[2] No	[1] Yes
5. Forced to settle in a different part of the country with minimal services	[2] No	[1] Yes
6. Imprisoned arbitrarily	[2] No	[1] Yes
7. Suffered ill health without access to medical care or medicine	[2] No	[1] Yes
8. Suffered from lack of food or clean water	[2] No	[1] Yes
9. Forced to flee your country or place of settlement	[2] No	[1] Yes
10. Expelled from your country based on ancestral origin, religion, or sect	[2] No	[1] Yes
11. Lacked shelter	[2] No	[1] Yes
12. Witnessed the desecration or destruction of religious shrines or places of religious instruction	[2] No	[1] Yes
13. Witnessed the arrest, torture, or execution of religious leaders or important members of tribe	[2] No	[1] Yes
14. Witnessed execution of civilians	[2] No	[1] Yes
15. Witnessed shelling, burning, or razing of residential areas or marshlands	[2] No	[1] Yes
16. Witnessed or heard combat situation (explosions, artillery fire, shelling) or landmine	[2] No	[1] Yes
17. Serious physical injury from combat situation or landmine	[2] No	[1] Yes
18. Witnessed rotting corpses	[2] No	[1] Yes

19. Confined to home because of chaos and violence outside	[2] No	[1] Yes
20. Witnessed someone being physically harmed (beating, knifing etc.)	[2] No	[1] Yes
21. Witnessed sexual abuse or rape	[2] No	[1] Yes
22. Witnessed torture	[2] No	[1] Yes
23. Witnessed murder	[2] No	[1] Yes
24. Forced to inform on someone placing them at risk of injury or death	[2] No	[1] Yes
25. Forced to destroy someone's property	[2] No	[1] Yes
26. Forced to physically harm someone (beating, knifing, etc.)	[2] No	[1] Yes
Please circle "YES" or "NO" for each question to indicate whether or not you have experienced any of the following events "Before coming to the U.S." and/or while "In the U.S."	Before coming to the U.S.	
27. Murder of violent death of family member (child, spouse) or friend	[2] No	[1] Yes
28. Forced to pay for bullet used to kill family member	[2] No	[1] Yes
29. Received the body of a family member and prohibited from mourning them and performing burial rites	[2] No	[1] Yes
30. Disappearance of family member (child, spouse etc.) or friend	[2] No	[1] Yes
31. Kidnapping of family member (child, spouse, etc.) or friend	[2] No	[1] Yes
32. Family member (child, spouse, etc.) or friend taken as hostage	[2] No	[1] Yes
33. Someone informed on you placing you and your family at risk of injury or death	[2] No	[1] Yes
34. Physically harmed (beaten, knifed, etc.)	[2] No	[1] Yes
35. Kidnapped	[2] No	[1] Yes
36. Taken as hostage	[2] No	[1] Yes
37. Heard about frightening, dangerous events that occurred to someone else but that you did not experience yourself	[2] No	[1] Yes
38. Sexually abused or raped	[2] No	[1] Yes
39. Coerced to have sex for survival	[2] No	[1] Yes

Appendix G: Social Support Questionnaire: Interpersonal Support Evaluation (ISEL)

Social Support Scale						
163.	When I feel lonely, there are several people that I can talk to.	[1] Strongly Agree	[2] Agree	[3] Undecided	[4] Disagree	[5] Strongly Disagree
164.	There is no one that I feel comfortable talking to about intimate personal problems.	[1] Strongly Agree	[2] Agree	[3] Undecided	[4] Disagree	[5] Strongly Disagree
165.	I often meet or talk with family or friends.	[1] Strongly Agree	[2] Agree	[3] Undecided	[4] Disagree	[5] Strongly Disagree
166.	There are several different people I enjoy spending time with.	[1] Strongly Agree	[2] Agree	[3] Undecided	[4] Disagree	[5] Strongly Disagree
167.	There is at least one person I know whose advice I really trust.	[1] Strongly Agree	[2] Agree	[3] Undecided	[4] Disagree	[5] Strongly Disagree

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ABSTRACT

EPIGENETIC METHYLATION IN PTSD AS MEDIATED BY TRAUMA EXPOSURE IN REFUGEES

By

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War-related trauma has adverse effects on refugee mental health and has been implicated in the dysregulation of multiple systems in the body. Trauma can alter genes associated in these systems, contributing to PTSD symptoms via DNA methylation. While there are exceptions, hypermethylation in regulatory regions of genes are associated with poor mental health, e.g., PTSD. The present study examines differential DNA methylation in an HPA axis associated gene in comparisons utilizing three groups: those with high levels of trauma and high PTSD symptom scores (HH) (n=24), those reporting high levels of trauma but low PTSD symptom scores (HL) (n=14), and those with low levels of trauma, but high PTSD symptoms (LH) (n=10); HH group was used as a reference. Self-report questionnaires and blood samples were collected from Iraqi male refugees. Genome-wide analysis revealed more differentially methylated CpG (CpG dinucleotide-specific DNA) sites in the LH vs. HH group comparison, than the HL vs. HH group comparison. Genes associated with HPA-axis function, NR3C1 and FKBP5, showed significant methylation differences in the regulatory region of the genes. There

was a statistically significant difference in DNA methylation in cg16012111 within the in FKBP5 gene and a statistically significant difference in DNA methylation in cg00629244 within the in NR3C1 gene separately, in both group comparisons (HL vs. HH and LH vs. HH). It was observed that individuals with high trauma and high PTSD symptoms were more likely to have higher methylation in these loci. Furthermore, the interaction of DNA methylation of these CpG sites and trauma was the best regression predictor of PTSD symptoms. Epigenetics has great clinical implications, providing valuable information on disease risk, prognosis, and symptom severity.

Autobiographical Statement

At the age of five, I had no country, no nationality, and no place to call home. I learned I was a minority at an early age. I was born in Iraq, but my family and I became refugees after being exiled for religious and sociopolitical reasons amidst war-torn Iraq. The only source of shelter was refugee camps midst endless desert, becoming our home for two years. Sand and tents characterized safety, however, it was from these tents that my mother taught me to read and write. One of my earliest childhood memories is of my mother creating a homemade chalkboard where she taught me the alphabets, and more importantly, a life lesson of being resourceful in the face of adversity. The steps she took to ensure learning in these circumstances fueled an intellectual curiosity, and concurrently, provided a source of normalcy in my childhood.

While at the camps, I became inquisitive about human behavior and interactions, as it was a crucial aspect for survival. Amidst the confusion at the refugee camp, I witnessed the wide range of responses different refugees had in the face of adversity. This curiosity continued even after my family moved to the United States. I noticed that while some families have trouble adapting and integrating post-displacement in the United States, other families were resilient. Further, individuals who had trouble integrating also expressed both psychological and physical health ailments, including anxiety, depression, and psychosomatic symptoms. While I recognized this paradigm in refugees first, I later learned that sociopolitical and cultural factors influence how all humans cope and adapt, impacting psychological and physical health.

This became a compelling force in understanding mental health. Something that started as an observation and my own nurtured curiosity has taken me on a path toward investigating differences in behavior, thought, and health. This has taken the form of pursuing a doctorate in clinical psychology, where I have devoted the majority of my graduate training to understanding stress in a cultural context, particularly as it relates to mental and physical health. In particular, my clinical research to date has encompassed a wide range of projects investigating psychosomatic health in minorities and my clinical experience speaks to interest in health psychology, as I trained in several hospital settings with a diverse, underserved populations, treating victims of crime, veterans, and patients with behavioral comorbidities secondary to medical illness.

While the memories may be distant, the mantra of resilience I learned with every step through endless desert is deeply rooted in my worldview. The triumphant and tragic moments I saw as a child in the refugee camp taught me to be an empathic, flexible, and adaptable individual. Such values inform my clinical skills. As I further my training, I hope to continue serving underserved populations and work in settings that emphasize the interaction of environmental and cultural factors on mental and physical health, which I see as a crucial part of the human experience and understanding human behavior.