1-1-2014

Proximate And Evolutionary Insights Into The Epigenetics Of Posttraumatic Stress Disorder

Levent Sipahi
Wayne State University,

PROXIMATE AND EVOLUTIONARY INSIGHTS INTO THE EPIGENETICS OF POSTTRAUMATIC STRESS DISORDER

by

LEVENT H SIPAHI

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2017

MAJOR: MOLECULAR BIOLOGY & GENETICS

Approved by:

_____

Advisor Date

_____

_____

_____

ACKNOWLEDGEMENTS

I am indebted to my advisors Drs. Monica Uddin and Derek Wildman whose guidance, insight, teaching, and thought made possible and shaped this work. It is difficult to express my gratitude for the patience, understanding, and kindness shown to me by them. It is perhaps cliché to use this space to acknowledge one's advisors via some derivation of the phrase "without whom this work would not be possible", but I doubt if it is oft written with the honesty that I write it now; all students may rightly say this in regards to the role of their advisors on the research they undertake, but I say it also in regards to my continuing to work when I was ready to quit. Derek and Monica, thank you.

Thank you to my committee members, Drs. Francesca Luca, Allison Aiello, Derek Wildman, and Monica Uddin for guiding and giving critical advice to this research project.

If I have emerged from this endeavor with any sanity intact it is thanks to the friendship and help of the Wildman and Uddin lab members. Amy Weckle and Rich Soliven were kind to me from my first day and helped in big and small ways throughout these projects. Angela Bustamante, Natalie Jameson, Amy Boddy, Will Gundling, and Michael McGowan were friends, confidants, and reassuring voices both within and outside the lab; my experience in graduate school has been improved by having friends with whom to share the experience.

Thank you to the faculty of the Center for Molecular Medicine and Genetics, from whom I've learned a great deal. Thank you also to the staff of the CMMG, particularly Suzanne Shaw. What little I have been organized is thanks to them.

A special thanks to the Wayne State University MD/PhD program and students who have supported me for many years. In particular, thank you to Dr. Ambika Mathur for having confidence in me and being both friend and mentor.

Perhaps most importantly, thank you to the participants of the Detroit Neighborhood Health Study.

Finally, thank you to my friends and family.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1. Introduction

Posttraumatic stress disorder (PTSD) is an increasingly important social and medical issue (Kessler, 2000; Druss et al., 2009). Recent United States military engagements around the globe, most notably Operation Iraqi Freedom and Operation Enduring Freedom have increased the prevalence and awareness of PTSD diagnoses attributed to exposure to military conflict in United States citizens and as a result there has been a renewed interest in the disorder popularly and within the military and research communities. For example, popular books and movies, both fictional and nonfictional, have begun to utilize PTSD as major plot points and mechanisms (Maxwell, 2011). Likewise, Governmental and military institutions have contributed considerable money and energy towards studying PTSD. As an example, the identification of reliable biomarkers of PTSD has been identified as a priority research goal by the Institute of Medicine (Institute of Medicine, 2012), Department of Defense (Congressionally Directed Medical Research Programs, 2011), and the National Institute of Mental Health (NIMH, 2008).

Descriptions of PTSD-like syndromes can be found throughout recorded history, including in classical Greek and Roman writings (Shay, 1994; Pitman, 2013). For example, Plutarch describes Cassander's response to physical and psychological abuse from Alexander in the following way (Plutarch):

> *"All which made such a deep impression of terror in Cassander's mind that, long after, when he was King of Macedonia and master of Greece, as he was walking up and down at Delphi, and looking at the statues, at the sight of that of Alexander he was suddenly struck with alarm, and shood all over, his eyes rolled, his head grew dizzy, and it was long before he recovered himself."*

Despite these ancient accounts, we can trace the contemporary origins of the concept of PTSD to the descriptions of post-railway accident syndromes by Charcot and Oppenheimer in the late 1800's (Young, 1995; Pitman, 2013). Furthermore, it wasn't until 1980, with the publishing of the Third Edition of the Diagnostic and Statistical Manual of Mental Disorders that PTSD first entered official psychiatric nosology (APA, 1980).

PTSD arising out of experiences of military service largely remains the driving force of discourse around trauma-related mental pathology, yet PTSD in the civilian population is also highly prevalent and highly costly, both to individuals and the state. In the United States, lifetime prevalence of PTSD is estimated to be 6.8% in the general population (Kessler and Wang, 2008) and 15-40% in inner city and combat-veteran populations (Villagomez et al., 1995; Breslau et al., 2004; Horowitz et al., 2005; Schwartz et al., 2005). Additionally, PTSD is associated with numerous comorbidities, including an increased risk of major depression (Breslau et al., 2000), substance dependence (Breslau et al., 2003), reduced life course opportunities (Kessler, 2000), and physical health problems (Farley and Patsalides, 2001; Simpson, 2002; Zayfert et al., 2002) including incident cardiovascular disease (Kessler, 2000; Kubzansky et al., 2007; Kubzansky and Koenen, 2009; Kubzansky et al., 2009). As a result, the total cost to the nation is an estimated $3.8 billion annually (Kessler, 2000), not to mention the incredible personal cost to individuals.

By definition, PTSD develops following exposure to a traumatic event (Shalev, 2001), witnessed or experienced by the individual; this traumatic event must involve the threat or actuality of death, injury, or physical integrity; and must elicit a response of

horror, fear, or helplessness (Shalev, 2001)[1]. Although a majority (50-90%) (Kessler et al., 1995) of Americans experience a qualifying trauma in their lifetime, only a minority develop diagnosable PTSD (Kessler et al., 1995; Breslau et al., 1998; Acierno et al., 2007). It should be noted, however, that while a minority develop PTSD, a higher percentage of individuals respond to traumatic exposures by developing an acute post-trauma syndrome that largely approximates PTSD but that resolves within a month, thus precluding a diagnosis of PTSD (Harvey and Bryant, 1999). What accounts for disparities in risk for PTSD is a question of intense research effort. Environmental factors at both individual and community levels, such as gender, race, educational attainment (Kulka RA, 1990; Brewin et al., 2000; Koenen et al., 2002; DiGrande et al., 2008; Galea et al., 2008; Kun et al., 2009), socioeconomic position (Kulka RA, 1990; Brewin et al., 2000; Koenen et al., 2002; Breslau et al., 2004; Koenen, 2007; DiGrande et al., 2008; Galea et al., 2008; Kun et al., 2009), and growing up in a low-income neighborhood (Breslau et al., 1991; Koenen, 2007) have been associated with increased risk of PTSD. Whereas some of this increased risk may be accounted for by individuals' increased exposure to assaultive violence (Breslau et al., 1998; Breslau et al., 2004; Coulton et al., 2007; Melzer-Lange et al., 2007; Reyes et al., 2008; Gillespie et al., 2009; Obasaju et al., 2009) (the type of trauma which carries the highest conditional risk of PTSD), biological variation may underlie observed variation in PTSD risk. Indeed, biological factors, and their relation to PTSD (Galea et al., 2006), have

[1] The diagnostic criteria detailed here is from the DSMIV-R. While completing this project, the DSM-V was published, thus altering the diagnostic criteria. The research in this dissertation was designed using the DSM-IV diagnostic criteria and utilized data that was collected in a manner consistent with DSM-IV diagnostic criteria. For these reasons, DSM-IV diagnostic criteria is used throughout this dissertation.

been given increased attention by social epidemiologists in recent years (Digangi et al., 2013; Navarro-Mateu et al., 2013).

Recently, attempts have been made to answer the question: “How does trauma get under the skin?” (Toyokawa et al., 2012). One putative, and increasingly empirically supported, mechanism by which trauma can lead to biological alterations is epigenetic variation and epigenetic modifications.

Epigenetic mechanisms and PTSD

Epigenetics refers to the stable yet modifiable regulation of gene function that occurs through non-DNA encoded mechanisms (Feinberg, 2008). Used here, this term refers to mechanisms that cause variation by altering the *expression* of genes, rather than their sequence. Although multiple epigenetic modifications have been identified (Kim et al., 2009), all involve chemical changes that regulate chromatin structure and/or DNA accessibility, which in turn alters the transcriptional activity of surrounding genetic loci. In contrast to DNA sequences, which are largely fixed, epigenetic factors are known to change in response to individuals’ physical, biological and social exposures in a manner that influences the long-term regulation of gene expression (Weaver et al., 2004; Champagne et al., 2006; Bjornsson et al., 2008; Meaney, 2010). In addition to DNA methylation (which I describe separately below), epigenetic mechanisms include histone modifications, microRNAs, and non-coding RNAs (ncRNAs), each of which alter the expression of DNA without changing the genetic code.

DNA methylation in particular is one of the major and best-studied epigenetic mechanisms to date. DNA methylation occurs in vertebrates through covalent

modification of DNA, whereby methyl groups are coupled to cytosine residues when cytosine and guanine are separated by a phosphate (i.e., at a CpG dinucleotide site) (Figure 1) (Bernstein et al., 2007). This chemical modification at specific DNA sequences regulates DNA accessibility, which in turn alters the transcriptional activity of the surrounding loci. In many cases, increased methylation in specific gene regions (e.g. promoters) is associated with reduced transcriptional activity and, therefore, gene expression (Eckhardt et al., 2006).

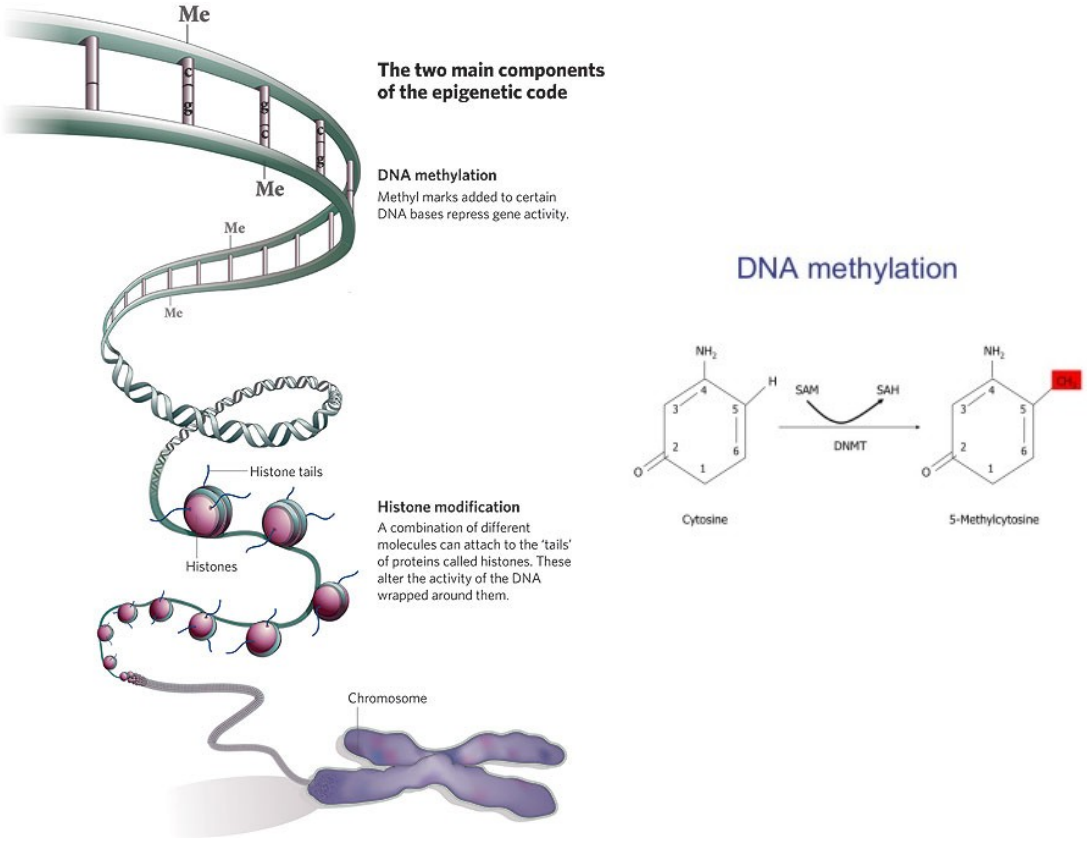

Figure 1. DNA methylation is a prominent epigenetic mechanism. DNA methylation at the 5 position of the cytosine at a CpG dinucleotide site (right panel) affects gene transcription via chromatin conformational changes (left panel). The left panel is modified from Macmillan Publishers Ltd: Nature 441:143-145.

Epigenetic factors have been the focus of increasing interest in the study of mental illness (Ptak and Petronis, 2010), including PTSD (Yehuda, 2006; Yehuda and LeDoux, 2007; Yehuda et al., 2009). This interest may be due to relatively few genes having been shown to contribute to the risk of common mood-anxiety disorders (e.g. (Lopez-Leon et al., 2008; Cornelis et al., 2010)) and evidence that epigenetic marks can change in response to external lived experiences (Weaver et al., 2004; Weaver et al., 2005; Champagne and Meaney, 2006; Champagne, 2009). Indeed, as regulators of DNA accessibility and activity, epigenetic factors offer one mechanism by which the environment can moderate the effects of genes (Rutter et al., 2006). That is, epigenetics has the potential to collapse false dichotomies of nature vs. nurture and gene vs. environment.

Evidence that DNA methylation is involved in the molecular pathology of mental health disorders, including in the developing brain, has been well documented (reviewed in (Houston et al., 2013)). As such, epigenetics offer a plausible way in which a traumatic experience may modify gene expression such that the risk of adverse psychopathological outcomes – PTSD for instance – increases (Yehuda et al., 2009). Indeed, animal models of PTSD have identified epigenetic differences that can discern rats with PTSD-like vs. non-PTSD-like behaviors (Cohen and Zohar, 2004) and, in humans expression signatures of PTSD have been reported in individuals identified in emergency rooms (Segman et al., 2005) or through long-term follow up of a traumatic experience (Zieker et al., 2007). Functional analyses of differentially expressed transcripts in these studies show a significant enrichment of genes that encode neural and endocrine proteins; genes expressed in the amygdala, hippocampus, and in the

HPA axis were found to be significantly overrepresented among the genes that distinguished trauma survivors with PTSD phenotypes (Segman et al., 2005), confirming the importance of these brain regions and biologic systems in mediating stress reactivity. Additionally, gene expression patterns associated with PTSD have been documented in postmortem brain tissue (Su et al., 2008) and peripheral blood (Segman et al., 2005; Zieker et al., 2007; Yehuda et al., 2009). It is important to note that the majority of these studies have looked at transcription changes in RNA derived from either PBMC or whole blood. Recently, Uddin and colleagues identified methylation-based epigenetic differences that distinguish trauma-exposed individuals with vs. without lifetime PTSD (Uddin et al., 2010). This work was closely followed by the publication of similar findings by Smith and colleagues (Smith et al., 2011a), with further studies further elucidating epigenetic differences associated with PTSD risk (Rusiecki et al., 2012; Rusiecki et al., 2013). Despite these advances, the underlying epigenetic changes that affect downstream gene expression and PTSD development remain largely unknown.

Elucidating epigenetic underpinnings to mental health disorders holds promise for the development of effective treatments, as their modifiability makes them potential targets for reversal. Studies in rats have demonstrated that pharmacological interventions can reverse the methylation status of the glucocorticoid receptor (GR) promoter and, consequently, the stress response, and that this reversal is possible even in terminally differentiated tissues (Weaver et al., 2005). Results from these animal studies render it plausible that pharmacologically modifiable epigenetic risk variants for

PTSD may be identified in humans. Indeed, an epigenetic signature of childhood abuse has been identified in the *NR3C1* GR promoter (McGowan et al., 2009).

PTSD as adaptive trait

Whether PTSD can be understood to be an adaptive trait is a source of current debate with biological and philosophical implications. On the one hand, PTSD is detrimental to the mental well-being of individuals suffering from it as well as to society. On the other hand, it is clear that in the context of an extremely dangerous environment the complement of symptoms that constitute PTSD can be beneficial as increased arousal and alertness, avoidance of potentially dangerous places and scenarios, and constant reminders of passed traumatic experiences can all lead to behavioral changes that serve to avoid or mediate future, potential traumatic experiences. This has led to numerous perspectives on PTSD, ranging from the conclusion that PTSD is socially and historically constructed (Young, 1995) to attempts to reduce PTSD to physical neurobiological abnormalities (Sherin and Nemeroff, 2011). In this project, I focus my attention not on PTSD, per se, but rather on the capacity to develop PTSD. I consider PTSD as the product of a potentially adaptive, epigenetically regulated process of developmental plasticity. As such, I am interested in studying this developmental process as a biological trait. As such, if the capacity to develop PTSD is an adaptive biological trait, then it can best be understood through the lens of Tinbergen's "Four Questions."

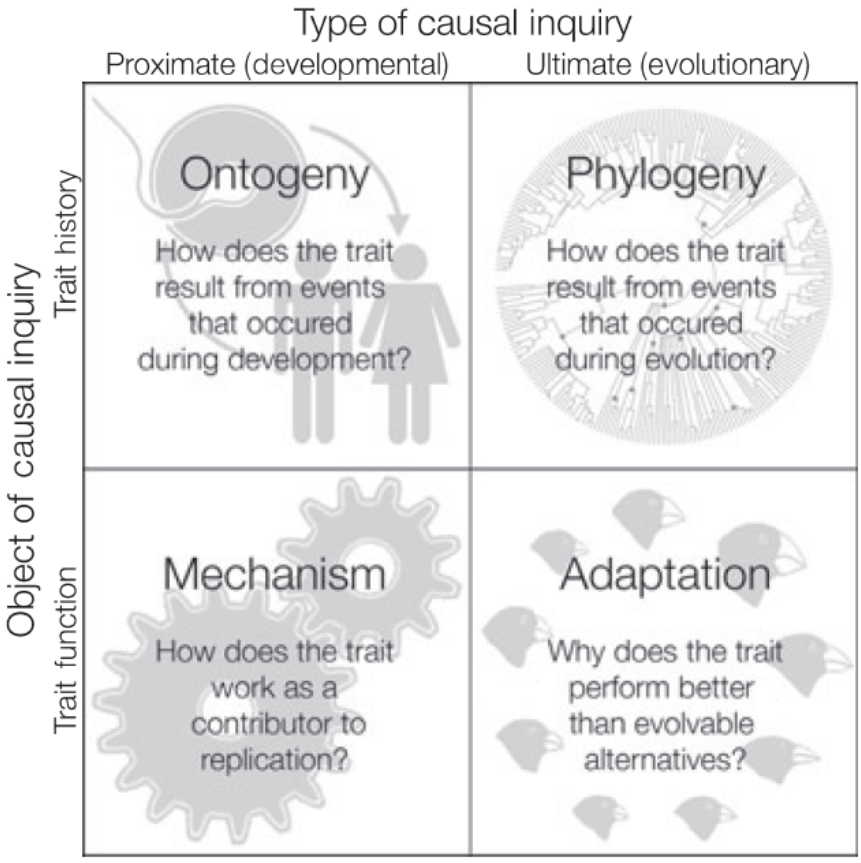

Figure 2. **Tinbergen's Four Questions.** This figure is modified from (Springer et al., 2011)

First proposed by Nikolaas Tinbergen, this perspective suggests that to understand a biological trait, one must first answer four distinct, but complementary questions concerning that trait (Figure 2):

1) What is the ontogeny?

2) What is the mechanism?

3) What is the phylogeny?

4) What is the adaptation?

Two of these questions (1 and 2), are concerned with a proximate understanding of the trait, while two (3 and 4) are concerned with an evolutionary understanding of the trait. Likewise, two (1 and 3) are concerned with a trait's history, while two (2 and 4) are concerned with a trait's function.

Most of the research on PTSD has attempted to answer question 2 (What is the mechanism of PTSD?) To this end, biological correlates have been sought and biochemical, physiological, and neuroanatomical processes have been probed to reveal the factors that underlie PTSD risk. In contrast, much less has been done to answer the other three questions. The first question (What is the ontogeny?), has long been a goal of molecular epidemiologists, but has largely been precluded by the lack of large, longitudinal human cohorts with a focus on psychiatric outcome and with access to biological samples. The Detroit Neighborhood Health Study (DNHS), which I detail below, is one of the first such cohorts and opens up the possibility of testing hypotheses concerning PTSD ontogeny in human subjects. Evolutionary insights into PTSD are also relatively few in number. The question of the functional adaptation of PTSD has been addressed by only a few researchers directly (Silove, 1998; Bracha et al., 2005; Cantor, 2009; Horwitz AV, 2012; Anderson and Adolphs, 2014), while others have developed theory for the potentially adaptive benefit of what is typically considered "pathological" psychobiology (Meaney, 2010).The question of phylogeny has largely been ignored.

The work of this dissertation attempts to utilize the perspective of Tinbergen's Four Questions in order to identify and test hypotheses that are needed towards a more complete understanding of PTSD.

Outline of the dissertation

The work that follows consists of three distinct projects, which approach the epigenetic regulation of PTSD from distinct but complementary conceptual positions. Before describing the three projects themselves, it is necessary to describe the Detroit Neighborhood Health Study (DNHS) and some of the related studies that have been published to date. The DNHS is a five-wave longitudinal study. The DNHS consists of survey and biological samples from a community-based study population of 1,547 adult residents of Detroit, Michigan. One adult from homes included in a probability sample within Detroit city limits (inclusive of non-phone and non-listed-households) were recruited. Survey data includes demographics, health status, tobacco and alcohol use, exposure to trauma, and symptoms of PTSD, depression, and anxiety. Biological samples include peripheral blood and saliva samples. DNA and RNA was isolated from biological samples. Demographics of the DNHS sample are similar to the greater Detroit population in comparison to the American Community Survey (Uddin et al., 2010). Compared with NCS-R, current (11%) and lifetime (13.9%) prevalence of PTSD in DNHS at baseline is greater than national averages (current: 3.5%; lifetime: 6.8% (Kessler et al., 1995)), thus creating a unique research opportunity.

The DNHS has yielded many studies, including relating to PTSD (Koenen et al., 2011; Chang et al., 2012; Johns et al., 2012; Mitchell et al., 2013; Uddin et al., 2013; Nevell et al., 2014), depression (Uddin et al., 2011b; Uddin et al., 2013; Nevell et al., 2014). Most importantly for the work of this dissertation, in 2010 Uddin and colleagues published the results of study which compared DNA methylation epigenome-wide between trauma exposed individuals with and without PTSD (Uddin et al., 2010).

Among the primary results of that study was a list of over 600 CpG dinucleotide sites at which there was differential DNA methylation between PTSD and control groups. This study raised many interesting questions. Does differential DNA methylation pre-exist trauma exposure and PTSD development as risk and resiliency factors or do they arise following trauma exposure and PTSD development and thus represent biomarkers? What is the evolutionary history of the CpG sites at which the epigenetic regulation of PTSD via differential methylation takes place? What are the relative contributions to PTSD risk of social factors, genetic factors, and epigenetic factors? What can epigenetics tell us about PTSD, specifically, and mental health disorders, generally?

In the dissertation that follows, I report research that was designed in order to address several of these questions. In Chapter 2, I report research that sought to address whether PTSD-associated differential methylation represents pre-trauma risk factors or post-trauma biomarkers in order to shed light on Tinbergen's Question #2: What is the ontogeny? To do so, we took advantage of the DNHS in order to identify thirty incident cases of PTSD. These individuals were matched on the basis of age, gender, and trauma exposure to controls without a history of PTSD. DNA methylation was then examined before and after trauma exposure. Among the differentially methylated sites study by Uddin and colleagues (Uddin et al., 2010) were CpG dinucleotides at *DNMT3B* and *DNMT3L* loci. For these reasons, we chose to examine DNA methylation at four DNA methyltransferase genes: *DNMT1*, *DNMT3A*, *DNMT3B*, and *DNMT3L*. The work detailed in Chapter 3 is also based on this foundational work by Uddin and colleagues (Uddin et al., 2010). In this chapter, I report the results of a study designed to elucidate and characterize the evolutionary history of the CpG sites at

which Uddin and colleagues reported PTSD-associated differential methylation. By doing so, I contribute to Tinbergen's Question #3: What is the phylogeny? To do so, we utilized phylogenetic methods within the context of mammals in order to infer the period of human evolutionary descent during which various CpG sites evolved. This evolutionary history was then biologically characterized using functional annotation clustering and identification of transcription factor binding sites. In Chapter 4, I present preliminary data on the association between hypothalamic-pituitary-adrenal axis SNPs and PTSD risk, in a contribution to answering Tinbergen's Question #1: What is the mechanism? Finally, in Chapter 5, I attempt to bring all of these data together in order to propose that PTSD be understood to be the product of an evolutionarily conserved, epigenetically regulated program of developmental plasticity, thus addressing Tinbergen's Question #4: What is the adaptation?

Chapter 2. Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder (PTSD)

This chapter published as:
Sipahi L, Wildman DE, Aiello AE, Koenen KC, Galea S, Abbas A, and Uddin M. Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder. *Psychological Medicine* (2014) DOI: http://dx.doi.org/10.1017/S0033291714000968.

Abstract

BACKGROUND: Epigenetic differences exist between trauma-exposed individuals with and without posttraumatic stress disorder (PTSD). It is unclear whether these epigenetic differences preexist, or arise following, trauma and PTSD onset.

METHODS: In pre- and post-trauma samples from a subset of Detroit Neighborhood Health Study participants, DNA methylation (DNAm) was measured at *DNMT1*, *DNMT3A*, *DNMT3B*, and *DNMT3L*. Pre-trauma DNAm differences and changes in DNAm from pre- to post-trauma were assessed between and within PTSD cases (n=30) and age-, gender-, and trauma exposure-matched controls (n=30). Pre-trauma DNAm was tested for association with post-trauma symptom severity (PTSS) change. Potential functional consequences of DNAm differences were explored via bioinformatic search for putative transcription factor binding sites (TFBS).

RESULTS: *DNMT1* DNAm increased following trauma in PTSD cases (p=0.001), but not controls (p=0.067). *DNMT3A* and *DNMT3B* DNAm increased following trauma in both cases (*DNMT3A*: p=0.009; *DNMT3B*: p<0.001) and controls (*DNMT3A*: p=0.002; *DNMT3B*: p<0.001). In cases only, pre-trauma DNAm was lower at a *DNMT3B* CpG site that overlaps with a TFBS involved in epigenetic regulation (p=0.001); lower pre-trauma *DNMT3B* DNAm at this site was predictive of worsening of PTSS post-trauma

(p=0.034). Some effects were attenuated following correction for multiple hypothesis testing.

CONCLUSIONS: DNAm among trauma-exposed individuals shows both longitudinal changes and preexisting epigenetic states that differentiate individuals who are resilient vs. susceptible to PTSD. These distinctive DNAm differences within *DNMT* loci may contribute to genome-wide epigenetic profiles of PTSD.

Introduction

PTSD is a prevalent and debilitating mental health disorder that may arise following exposure to a potentially traumatic event (Association, 2013). While the lifetime prevalence of traumatic exposure is 50-90% (Kessler et al., 1995), PTSD in the general U.S. population is estimated to be only 6.8% (Kessler and Wang, 2008). Although the majority of persons exposed to trauma display resiliency (Kessler et al., 1995; Breslau et al., 1998; Acierno et al., 2007; Kessler and Wang, 2008), the molecular underpinnings of risk remain poorly characterized. The identification of risk markers, and particularly biomarkers, that distinguish between persons at high and low risk of developing PTSD following trauma exposure has been identified as a priority research goal by the Institute of Medicine (Institute of Medicine, 2012), Department of Defense (Congressionally Directed Medical Research Programs, 2011), and the National Institute of Mental Health (NIMH, 2008). Ideally, the ability to identify persons at high risk of developing PTSD would enable providers to target evidence-based interventions to high-risk groups (Andrews and Neises, 2012). The identification of robust predictive

biomarkers may also improve our understanding of the pathophysiology of PTSD and lead to more effective pharmacological interventions.

Although much work has been done to identify social and environmental factors that contribute to PTSD risk [e.g. (Kulka RA, 1990; Breslau et al., 1991; Brewin et al., 2000; Koenen et al., 2003; Breslau et al., 2004; DiGrande et al., 2008; Galea et al., 2008; Kun et al., 2009)], the biological undergirding of differential PTSD risk and resiliency remains to be more fully elucidated. Twin studies have demonstrated heritability and genetic contribution to PTSD risk (True et al., 1993; Koenen et al., 2002; Stein et al., 2002) and targeted gene and GWAS approaches have identified both genetic risk loci (Lu et al., 2008; Ressler et al., 2011; Chang et al., 2012; Logue et al., 2013) and important gene-by-environment interactions (Binder et al., 2008; Xie et al., 2010; Uddin et al., 2013) that contribute to risk for the disorder; nevertheless, a substantial proportion of biologically mediated variance in PTSD risk has yet to be explained.

Epigenetic variability is considered a plausible and increasingly empirically supported contributor to the etiology of phenotypes with marked genetic and environmental influences (Meaney, 2010), including certain psychopathologies (Toyokawa et al., 2012). Indeed, recent advances have revealed that PTSD risk and resiliency is associated with differential epigenetic variation (El-Sayed et al., 2012). Epigenetic mechanisms – including histone modifications, non-protein coding RNAs, and, most notably, DNA methylation (DNAm) – affect gene expression and cellular phenotype without altering the underlying DNA sequence (Feinberg, 2008; Meaney, 2010). DNAm is stably heritable across mitotic replications, but is modifiable throughout

the life course in response to lived experiences and environmental exposures (Bird, 2002). In primordial mammalian germ cells, global DNAm is removed (with the exception of imprinted loci) (Reik et al., 2001), with new patterns established by de novo methyltransferases DNMT3A, DNMT3B, and DNMT3L following fertilization (Bourc'his et al., 2001; Bourc'his and Bestor, 2004; Kaneda et al., 2004; Kato et al., 2007; Ooi et al., 2007). These reprogrammed DNAm patterns are largely maintained throughout mitotic DNA replication by the action of the maintenance methyltransferase DNMT1 (Li et al., 1992; Seisenberger et al., 2013).

Although influenced by other variables, global DNAm patterns are largely established and maintained by the activity of the DNA methyltransferases, DNMT1, DNMT3A, DNMT3B, and DNMT3L (Feng and Fan, 2009). Gene expression evidence suggests that these DNMTs may be active throughout the life course (Robertson et al., 1999; Feng et al., 2005; Siegmund et al., 2007), including in brain tissue (Goto et al., 1994; Veldic et al., 2004; Feng et al., 2005) and in association with mental disorders (Veldic et al., 2004; Veldic et al., 2005). In addition, protein-level expression of DNMT1 (Inano et al., 2000; Veldic et al., 2005) and DNMT3A (Feng et al., 2005) has been demonstrated in the mouse and human brain. With respect to PTSD, recent work confirms that DNMT activity plays a role in mediating risk for PTSD-related phenotypes, including fear conditioning and memory consolidation (Miller and Sweatt, 2007; Feng et al., 2010). Together, these findings suggest that DNAm and DNA methyltransferases represent promising targets for the identification of epigenetic underpinnings of differential PTSD risk and resiliency.

Studies of epigenetic variation have provided important insights into PTSD risk, but have been largely limited by cross-sectional analyses of post-trauma samples. Most notably, epidemiological cohorts from Detroit (Uddin et al., 2010) and Atlanta (Smith et al., 2011a) have been the basis of research that has demonstrated cross-sectional differential DNAm that distinguishes between trauma-exposed individuals with vs. without PTSD. *DNMT3B* and *DNMT3L* were among the differentially methylated loci identified in the Detroit study (Uddin et al., 2010). More recently, longitudinal DNAm data among PTSD cases and controls have been reported, including studies using samples from a cohort of U.S. military personnel deployed to Iraq and Afghanistan (Rusiecki et al., 2012; Rusiecki et al., 2013). To further elucidate whether differential DNAm between trauma exposed controls and PTSD cases represent pre-existing susceptibility/resiliency factors or downstream biomarkers of PTSD, additional longitudinal analyses are required. Finally, while the identification of epigenetic variation associated with mental health outcomes is important, work must begin to test the putative functionality of mental health-associated differential DNAm. For example, the identification of transcription factor binding sites (TFBS) that overlap with differentially methylated CpG sites and to which transcription factor binding may be disrupted offer one possibility of supporting DNAm functionality (Weaver et al., 2004).

Here, we analyze DNAm from individuals pre- and post-trauma to identify differences that characterize individuals who are susceptible vs. resilient to PTSD following trauma. To assess potential functional consequences of examined DNAm differences, we then performed a bioinformatic search for the presence of putative transcription factor binding sites (Weaver et al., 2004). Results from this work suggest

that PTSD-relevant DNAm differences in DNMT loci may exist both prior to and following trauma, with implications for future targeted interventions.

Methods and Materials

Subjects

Samples are from a subset of participants from the Detroit Neighborhood Health Study (DNHS), a longitudinal, community-representative cohort of adult residents in Detroit, MI. The current study draws on peripheral blood samples and survey data obtained at two time points from 60 DNHS participants. Forty-six were female and fourteen male; forty-six were African-American, twelve were Caucasian, and two were Hispanic. The average age was 55.1 years. PTSD diagnosis was assessed via structured interview administered via telephone (Breslau et al., 1998). PTSD symptoms were assessed in reference to both the traumatic event the participant regarded as their worst and one randomly selected traumatic event from the remaining traumas the participant experienced. Lifetime PTSD cases met all six DSM-IV criteria in reference to either the worst or random traumatic event. The diagnostic interview showed good validity against the Clinician Administered PTSD Scale (Blake et al., 1995) as described elsewhere (Uddin et al., 2010). The Institutional Review Board of the University of Michigan reviewed and approved the study protocol. Incident cases (n=30) of PTSD were identified in either waves 2, 3, or 4 of DNHS data collection among individuals for whom blood samples were available at both the wave of first PTSD diagnosis and the immediately previous, pre-incident trauma wave. Non-PTSD controls (n=30) were matched to cases on the basis of age, sex, and number of traumatic event types. DNA

samples were isolated from both pre- and post-trauma time points for both cases and controls. The time between pre- and post-trauma time points was approximately 1 year. Cases and controls had no history of PTSD prior to the post-trauma wave.

DNA Isolation:

DNA was isolated from whole blood acquired via venipuncture when available from DNHS participants selected for inclusion in this study. Blood spots were used as an alternate source of whole blood-derived DNA when venipuncture samples were unavailable. The exact tissue type was shared between matched case-control pairs in all instances. Venipuncture- and bloodspot-derived whole blood represent the same tissue and therefore should not differ with respect to DNAm, as confirmed by numerous studies to date (Wong et al., 2008; Aberg et al., 2013; Hollegaard et al., 2013).

Whole blood:

DNA was isolated from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) and the QuickGene DNA Whole blood Kit S (Lifesciences, FujiFilm, Tokyo, Japan) using manufacturers' recommended protocols.

Blood spots:

DNA was isolated using the QIAamp DNA Micro Kit (Qiagen) using the manufacturer's recommended protocol. For each sample, one 6mm punch was taken from dried blood spots using a disposable, sterile biopsy punch (Miltex, York, PA) within a sterile field and placed immediately into a sterile 1.7ml microcentrifuge tube. New

gloves, biopsy punches, and sterile fields were utilized for each sample. Negative controls in the form of blank extractions were included with all DNA isolations.

Bisulfite conversion:

For each sample, ~750ng of DNA was bisulfite converted using the EpiTect Bisulfite Kit (Qiagen) using the manufacturer's recommended protocol. Negative controls in the form of bisulfite conversion of water were included with each bisulfite conversion.

Pyrosequencing:

Assays to assess the methylation levels of CpG sites found in the *DNMT1, DNMT3A*, and *DNMT3L* and *DNMT3B* (see below for assay-specific details) were custom designed using the Pyromark Q24 Assay Design Software 2.0 (Qiagen). Targeted CpG sites were selected based on prior evidence(Uddin *et al.*, 2010) of involvement in epigenetic regulation of PTSD risk (*DNMT3B, DNMT3L*) and to investigate whether longitudinal, PTSD-associated DNAm differences exist across DNA methyltransferase genes more broadly (*DNMT1*, *DNMT3A*, *DNMT3B,* and *DNMT3L*). Because the *DNMT3B* target CpG is located in a CpG island, our designed assay captures DNAm at 12 CpG sites in an approximately 70 base pair region of exon 1 (see *DNMT3B* assay section below for details). Single CpG sites were assessed at *DNMT1*, *DNMT3A*, and *DNMT3L* loci (see individual assay section below for details); these CpG sites did not fall into CpG islands. *DNMT1*, *DNMT3A*, and *DNMT3L* CpG sites and 2 *DNMT3B* CpG sites assessed are also found on the HM27 and HM450K methylation

bead chips from Illumina (see below for actual HG19 nucleotide location). The capacity for each assay to capture DNAm levels ranging from 0-100% was validated using commercially available demethylated and highly methylated DNA at dilutions of 1:0 (unmethylated), 3:1, 1:1, 1:3, and 0:1 (highly methylated). PCR amplification of target sequences was performed on 20ng of bisulfite-converted DNA samples using the PyroMark PCR kit (Qiagen). Bisulfite-converted, PCR-amplified DNA was pyrosequenced on the Pyromark Q24 Pyrosequencer (Qiagen) using the manufacturer's recommended protocol and default settings. All methylation analyses were conducted in triplicate with appropriate negative controls included at each of the following steps: DNA isolation, bisulfite conversion, PCR amplification, and pyrosequencing reaction.

Details of each custom assay are listed below.

DNMT1

PCR forward primer: TTTTTTTAGGTGTGATGGGGATAAAG

PCR reverse primer (biotinylated): CAAAAACTCTCACAAACCCTTAAA

PCR program (50 cycles):

Initial 15 minutes at 95°C

Denaturation 30 seconds at 94°C

Annealing 30 seconds at 58°C

Extension 30 seconds at 72°C

Final 10 minutes at 72°C

Hold 4°C

Sequencing primer: GTGATGGGGATAAAGT

Target sequence: AG**CG**AGAAGCCCCCAAGGGTTTGTGAGA (**CpG target in bold**; hg19: chr19:10,305,909-10,305,936)

DNMT3A

PCR forward primer: GGTGGGAGGTTGAATGAAATGA

PCR reverse primer (biotinylated): AATACCCAACCCCAAATCCTAC

PCR program (50 cycles):

Initial 15 minutes at 95°C

Denaturation 30 seconds at 94°C

Annealing 30 seconds at 58°C

Extension 30 seconds at 72°C

Final 243 10 minutes at 72°C

Hold 4°C

Sequencing primer: AGTTGGAAGATTTTGTG

Target sequence: TGTGCCTACACAC**CG**CCCTCACCCCTTCACYGTGG GGGCTGTTCTCCTTCCCCATGGAGYGCTCAGGGCTCTAGGTTCCTGACTTG GGGCACCTCTGTCTAATTCCACCAGCACAGCCACTCACTATGTGCTCATCTC ACTCCTCCAGCAGCYGCTGTAGGACTTGGGGCTGGGCACC (**CpG target in bold**; hg19: chr2:25,565,782-25,565,959)

DNMT3B

PCR forward primer: GGGGTTAAGTGGTTTAAGTAAAT

PCR reverse primer (biotinylated): CCTCCAAAAATCCCTAAAAAAAATCT CTCC

PCR program (45 cycles):

Initial 15 minutes at 95°C

Denaturation 30 seconds at 94°C

Annealing 30 seconds at 52°C

Extension 30 seconds at 72°C

Final 10 minutes at 72°C

Hold 4°C

Sequencing primer: GTTAAGTGGTTTAAGTAAATTTAG

Target sequence: CT**CG**G**CG**AT**CG**G**CG**C**CG**GAGATT**CGCG**AGCCCAG **CG**CCCTGCA**CG**GC**CG**CCAGC**CG**GCCTCC**CG**CCAGCCAGCCCCGACCCGC GGCTCCGCCGCCCAGCCGCGCCCCAGCCAGCCCTGCGGCAGGTGAGCGC CCCGGGGCCC 266 (**CpG targets in bold**; hg19: chr20:31,350,382-31,350,523)

DNMT3L

PCR forward primer: AGTTTTTTTTATTGGGGTAGTTAGG

PCR reverse primer (biotinylated): CTTAAAACCAAAAAACCACATTTTAT TCA

PCR program (45 cycles):

Initial 15 minutes at 95°C

Denaturation 30 seconds at 94°C

Annealing 30 seconds at 50°C

Extension 30 seconds at 72°C

Final 10 minutes at 72°C

Hold 4°C

Sequencing primer: GATTTAGGGATAGAGAGGG

Target sequence: G**CG**GTAGGGAGTGGGAAATCTGAATAA (**CpG target in bold**; hg19: chr21:45,683,527-45,683,553)

To demonstrate the ability of our assays to resolve DNAm differences as small as reported, we computed intraclass correlation coefficients (ICC) between triplicate replicates for each assay. Average within-sample coefficient of variation was computed using a two-way mixed model, using an absolute agreement definition (Shrout, 1979), as implemented in SPSS (IBM, USA). ICCs for the 15 total CpG sites assayed ranged from 0.703 to 0.937, with a mean ICC of 0.855 (standard deviation: 0.066). This strongly supports the conclusion that these assays are capable of consistently resolving small DNAm differences.

TFBS prediction

Putative TFBS were identified that overlap target CpG sites using the MatInspector (Cartharius et al., 2005) tool from Genomatix, with default parameters. Input sequence included 200bp up- and downstream of the CpG site. Only putative TFBS that directly overlapped CpG sites of interest were retained.

Statistical analyses

Statistical testing was performed using SPSS Statistics for Windows, Version 21.0 (IBM Corp., USA). DNAm at *DNMT3B* CpG sites was treated on a regional and an individual CpG site basis, similar to previous work (Rusiecki et al., 2013). Regional

values were calculated as the mean of 12 CpG sites. Paired-sample t-tests were used to test for differences in pre-trauma DNAm between cases and controls and to test for differences between pre- and post-trauma time points within cases and controls. Linear regression was used to test whether pre-trauma DNAm levels are predictive of post trauma symptom severity (PTSS) changes. PTSS change was calculated as the difference between PTSS and pre-trauma symptom severity. Analyses included severity scores of individual symptom criteria (hyperarousal, avoidance, or intrusion symptoms) as well as a total severity score that is inclusive of each symptom subdomain. Regression models were adjusted for age, gender, and pre-trauma symptom severity. The contribution of pre-trauma DNAm to post-trauma PTSS change was tested via the change in R square values comparing full to reduced models. We present primary results uncorrected for multiple testing as is consistent with the current state of the science of DNAm variation in association with psychiatric endpoints (Perroud et al., 2011; Unternaehrer et al., 2012; Perroud et al., 2013; Rusiecki et al., 2013). In addition, to assess the extent to which our results may be attenuated by multiple hypothesis testing correction, we calculated stringent Bonferroni-corrected significance values (Dunn, 1961) as well as false discovery rate (FDR) Q values (Benjamini, 1995). FDR has recently been utilized to correct multiple hypothesis testing in studies utilizing DNAm data, with user-defined Q-values ranging from 0.05 to 0.2 (Provencal et al., 2013; Zhao et al., 2013).

Results

PTSD cases and controls do not differ in age, gender, ethnicity, or pre-trauma symptom severity, including individual symptoms of intrusion, avoidance, and hyperarousal (Table 1).

Table 1. Demographic and pre-trauma characteristics of 30 posttraumatic stress disorder (PTSD) case-control pairs.

	Controls (N = 30)		PTSD (N = 30)		t(d.f.)	p-value
	Mean/#	s.d./%	Mean/#	s.d./%		
Age (years)	55.37	12.97	53.71	12.94	-0.47 (29)	0.638
Female	23	76.7%	23	76.7%	NA (1)	1.000
African-American	22	73.3%	24	80%	0.01 (1)	0.938
Lifetime traumas	3.80	3.83	4.43	3.70	-0.84 (29)	0.407
Assaultive Violence	0.87	1.33	1.07	1.55	-0.55 (29)	0.589
Other Injury or Shccking Experience	0.83	1.32	1.27	1.48	-1.51 (29)	0.141
Learning about traumas to others	1.20	1.50	1.17	1.37	0.12 (29)	0.909
Sudden Death	0.77	0.43	0.70	0.47	0.63 (29)	0.536
Other Event	0.13	0.35	0.23	0.43	-1.36 (29)	0.184
Pre-trauma Symptom Severity	26.90	10.96	39.33	16.20	-1.12 (29)	0.275
Intrusion	9.58	7.90	11.65	6.08	-0.99 (29)	0.333
Avoidance	11.65	11.04	14.69	6.97	-1.14 (29)	0.267
Hyperarousal	8.60	7.98	11.24	5.11	-1.24 (29)	0.226
Post-trauma Symptom Severity	25.41	7.01	54.20	11.59	11.4 (29)	<0.001

Pre-trauma DNAm variation is associated with PTSD

PTSD-associated DNAm variation may both pre-exist trauma and be associated with post-trauma PTSD outcome. To test for pre-existing protective/risk factors, pre-trauma DNAm at *DNMT1*, *DNMT3A*, *DNMT3B*, and *DNMT3L* loci was compared between trauma exposed individuals with vs. without PTSD. Pre-trauma DNAm was higher in controls compared with cases at a single *DNMT3B* CpG site (CpG 9) (Figure 3; t=2.250, 29 df, p=0.032); no difference in pre-trauma *DNMT3B* regional DNAm mean was observed (t=1.538, 29 df, p=0.135). We observed no pre-trauma differences between cases and controls at *DNMT1* (t=0.582, 29 df, p=335 0.565), *DNMT3A* (t=0.579, 29df, p=0.567), and *DNMT3L* (t=1.386, 29df, p=0.176) loci.

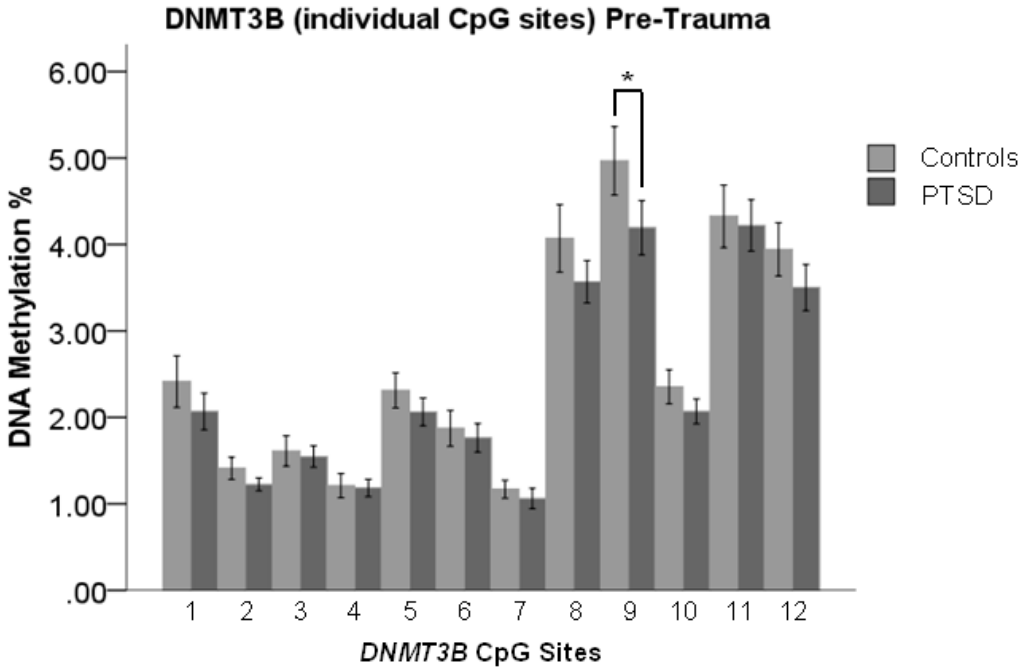

Figure 3. Pre-trauma DNA methyltransferase 3B (*DNMT3B*) DNA methylation (DNAm) is significantly higher in trauma-exposed controls compared to posttraumatic stress disorder (PTSD) cases at CpG 9. Pre-trauma DNAm did not differ between cases and controls at the other 11 *DNMT3B* CpG sites assessed. Light gray bars indicate mean DNAm of controls. Dark gray bars indicate mean DNAm of PTSD cases. Error bars represent standard error of the mean. Difference between controls and cases was tested by paired-sample t-tests (N = 60; 30 cases and 30 matched controls). *: p<0.05.

Pre-trauma DNAm variation predicts post-trauma changes in trauma symptom severity

To explore whether this PTSD-associated pre-trauma DNAm is predictive of trauma response, we performed linear regression analyses with pre-trauma DNAm of *DNMT3B* at CpG 9 and PTSS change as predictor and outcome variables, respectively. Controlling for age, gender, and pre-trauma symptom severity, pre-trauma DNAm of CpG 9 (Figure 4; unstandardized B=-2.318, SE=1.25 p=0.034) predicted post-trauma symptom severity change. In this model, only pre-trauma symptom severity and pre-trauma DNAm were significant predictor variables. *DNMT3B* CpG 9 DNAm explained approximately 6.8% of the variance in PTS severity change, as revealed by a comparison of the full and reduced models. The full model that included *DNMT3B* CpG 9 DNAm, age, gender, and pre-trauma symptom severity explained approximately 24% of the variance in post-trauma PTSS change (Adjusted R Square=0.242, p=0.005).

Because the relationship between pre-trauma DNAm and post-trauma changes in PTS symptom severity may be driven by distinct symptom subdomains (hyperarousal, avoidance, and intrusion), we regressed separately each subdomain symptom severity change onto pre-trauma DNAm, controlling for age, gender, and pre-trauma symptom severity of the relevant subdomain. Pre-trauma DNAm of *DNMT3B* CpG 9 (hyperarousal: p=0.249; avoidance: p=0.137; intrusion: p=0.071) did not predict change in subdomain symptom severity.

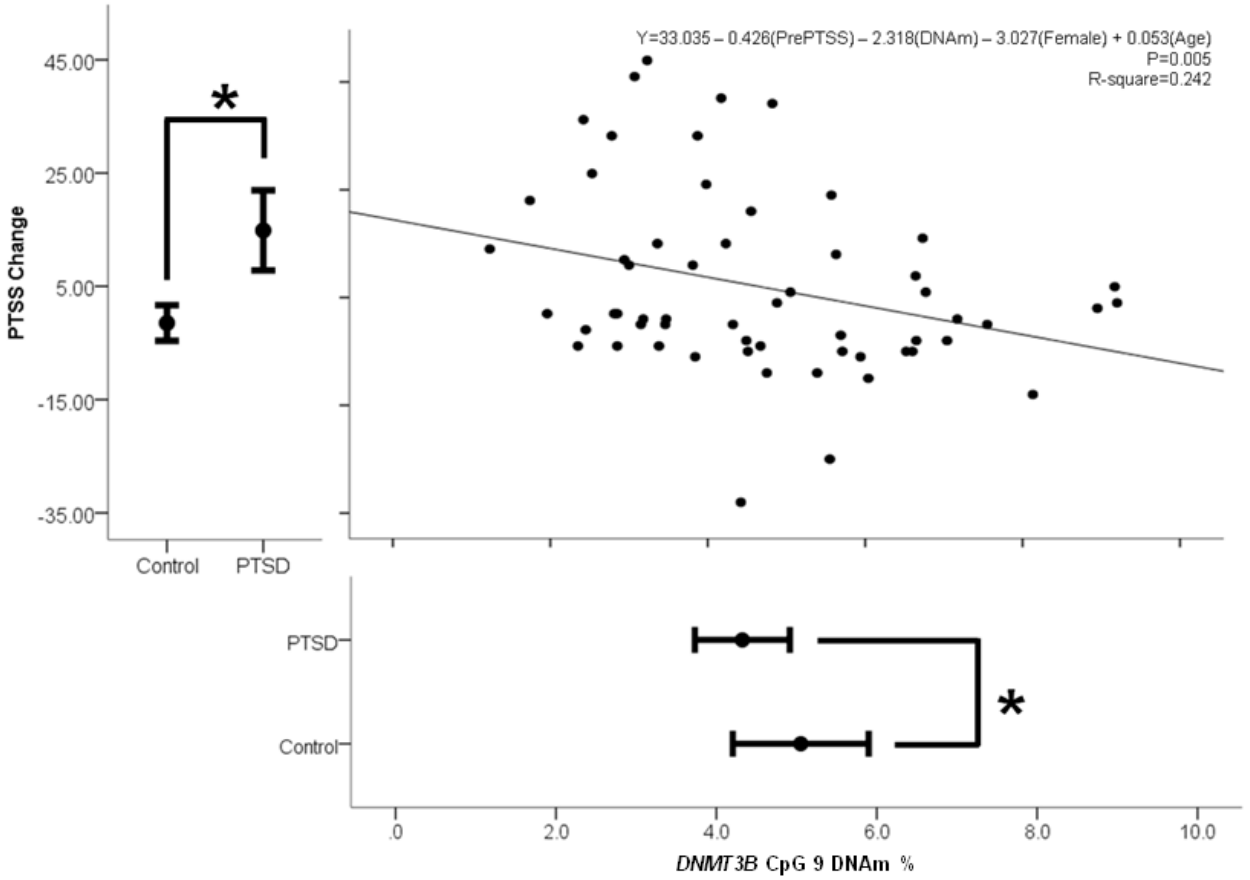

Figure 4. Linear regression model of symptom severity (PTSS) change post-trauma and pre-trauma DNA methyltransferase 3B (*DNMT3B*) CpG 9 DNA methylation (DNAm), adjusting for age, gender, and pre-trauma symptom severity (N=60). Only pre-trauma PTSS and DNAm were significant variables in this model. Error bar plots represent the mean plus/minus the 95% confidence intervals. Differences between posttraumatic stress disorder cases and trauma-exposed controls were tested by paired-sample t- tests (N = 60; 30 PTSD cases and 30 matched controls). *: $p<0.05$.

Trauma induces PTSD-associated DNAm modifications

DNAm differences may arise following trauma and be associated with PTSD development. To test this, we compared pre-trauma DNAm with post-trauma DNAm within PTSD cases and within trauma-exposed, healthy controls. Both PTSD-associated and PTSD-independent changes in DNAm following trauma were observed at *DNMT* loci (Figure 5). *DNMT1* DNAm increased (Figure 5A; t=3.887, 29 df, p=0.001) following

trauma in the PTSD group, but not the control group (t=1.903, 29 df, p=0.067). At *DNMT3A* (Figure 5B) and *DNMT3B* (Figure 5C) loci, DNAm increased following trauma in both PTSD case (*DNMT3A*: t=2.806, 29 df, p=0.009; *DNMT3B*: t=4.286, 29 df, p<0.001) and control (*DNMT3A*: t=3.421, 29 df, p=0.002; *DNMT3B*: t=3.938, 29 df, p<0.001) groups. No change was observed in *DNMT3L* (Figure 5D) DNAm in either cases (t=1.551, 29 df, p=0.132) or controls (t=1.146, 29 df, p=0.261). Table 2 presents a summary including uncorrected p values, Bonferroni-corrected p values, and FDR values, as well as accompanying effect sizes, of our results described above.

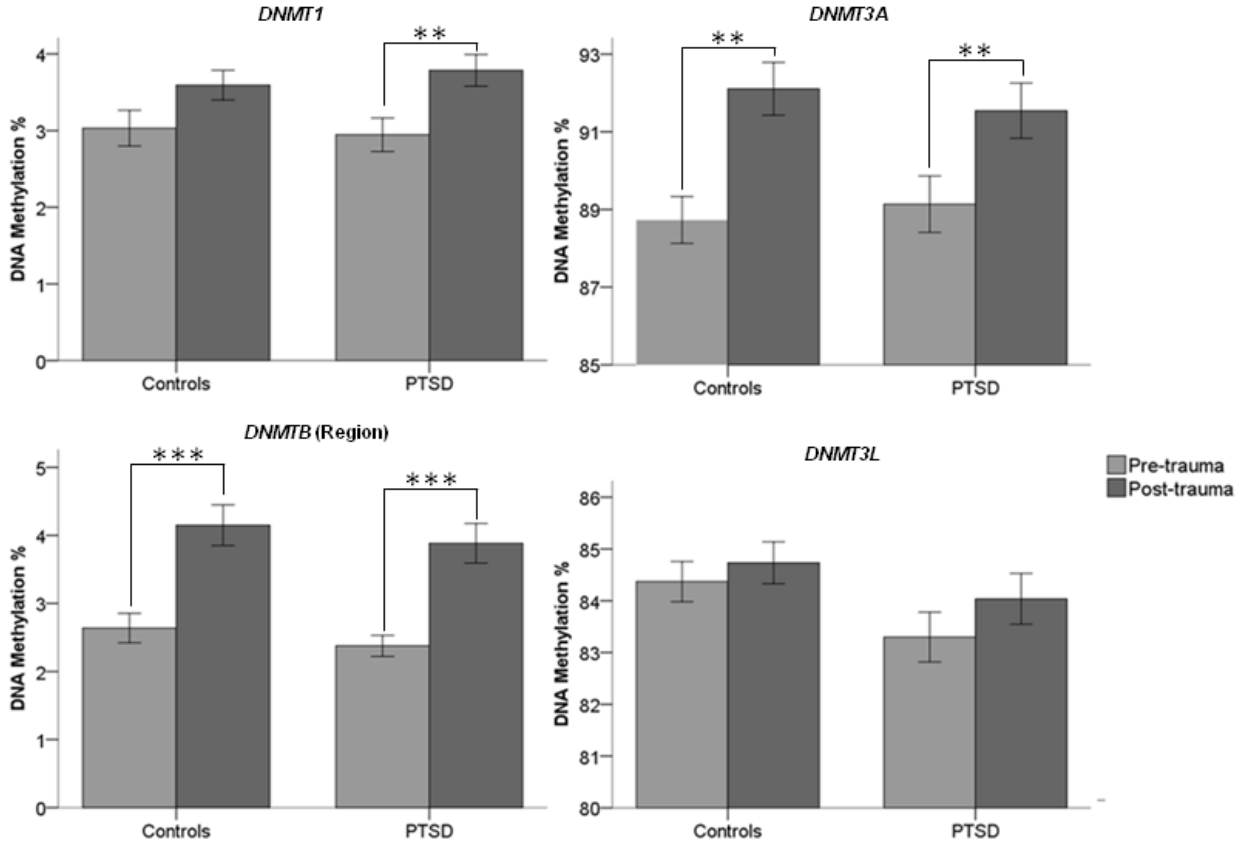

Figure 5. Longitudinal DNA methylation (DNAm) modifications of DNA methyltransferase (*DNMT*) loci in response to trauma in posttraumatic stress disorder (PTSD) cases and trauma-exposed controls. *DNMT3B* (region) represents the mean of 12 CpG sites. Differences between PTSD cases and trauma-exposed controls were tested by paired-sample t-tests (N = 60; 30 PTSD cases and 30 matched controls). Error bars represent standard error of the mean. *: p<0.05; **:p<0.01; ***:p<0.001.

Table 2. Observed and corrected significance values of tests[a]

Test	Mean Difference[b]	SE	Observed P[c]	Rank	Bonferroni threshold	FDR (BH) thresholds
Pre- vs. post-trauma DNMT3B in Cases	1.51	0.35	0.000*	1	0.002*	0.002*
Pre- vs. post-trauma DNMT3B in Controls	1.51	0.38	0.000*	2	0.002*	0.003*
Pre- vs. post-trauma DNMT1 in Cases	0.84	0.22	0.001*	3	0.002*	0.005*
Pre- vs. post-trauma DNMT3A in Controls	3.38	0.99	0.002*	4	0.002*	0.006*
Pre- vs. post-trauma DNMT3A in Cases	2.41	0.86	0.009*	5	0.002	0.008
Cases vs. Controls DNMT3B (CpG 9) pre-trauma	0.77	0.34	0.032*	6	0.002	0.009
CpG 9 Regression analysis (all symptoms)	B: -2.318		0.034*	7	0.002	0.011
Cases vs. Controls DNMT3B (CpG 2) pre-trauma	0.19	0.11	0.057	8	0.002	0.013
Pre- vs. post-trauma DNMT1 in Controls	0.56	0.30	0.067	9	0.002	0.014
CpG 9 Regression analysis (Intrusion symptoms)	B: -0.849		0.071	10	0.002	0.016
Cases vs. Controls DNMT3B (CpG 12) pre-trauma	0.44	0.24	0.122	11	0.002	0.017
Cases vs. Controls DNMT3B (CpG 10) pre-trauma	0.28	0.19	0.127	12	0.002	0.019
Pre- vs. post-trauma DNMT3L in Cases	0.74	0.47	0.132	13	0.002	0.020
Cases vs. Controls DNMT3B pre-trauma	0.27	0.17	0.135	14	0.002	0.022
CpG 9 Regression analysis (Avoidance symptoms)	B: -0.821		0.137	15	0.002	0.023
Cases vs. Controls DNMT3B (CpG 1) pre-trauma	0.35	0.23	0.151	16	0.002	0.025
Cases vs. Controls DNMT3B (CpG 8) pre-trauma	0.50	0.33	0.158	17	0.002	0.027
Cases vs. Controls DNMT3L pre-trauma	0.91	0.65	0.176	18	0.002	0.028

Cases vs. Controls DNMT3B (CpG 5) pre-trauma	0.25	0.19	0.221	19	0.002	0.030
CpG 9 Regression analysis (Hyperarousal symptoms)	B: -0.466		0.249	20	0.002	0.031
Pre- vs. post-trauma DNMT3L in Controls	0.36	0.32	0.261	21	0.002	0.033
Cases vs. Controls DNMT3L post-trauma	0.58	0.56	0.304	22	0.002	0.034
Cases vs. Controls DNMT1 post-trauma	-0..19	0.19	0.323	23	0.002	0.036
Cases vs. Controls DNMT3B post-trauma	0.18	0.22	0.351	24	0.002	0.038
Cases vs. Controls DNMT3A post-trauma	0.56	0.66	0.356	25	0.002	0.039
Cases vs. Controls DNMT3B (CpG 7) pre-trauma	0.11	0.12	0.487	26	0.002	0.041
Cases vs. Controls DNMT1 pre-trauma	0.09	0.20	0.565	27	0.002	0.042
Cases vs. Controls DNMT3A pre-trauma	-0.41	0.73	0.567	28	0.002	0.044
Cases vs. Controls DNMT3B (CpG 6) pre-trauma	0.11	0.17	0.603	29	0.002	0.045
Cases vs. Controls DNMT3B (CpG 3) pre-trauma	0.06	0.15	0.711	30	0.002	0.047
Cases vs. Controls DNMT3B (CpG 11) pre-trauma	0.11	0.39	0.773	31	0.002	0.048
Cases vs. Controls DNMT3B (CpG 4) pre-trauma	0.03	0.14	0.881	32	0.002	0.050

S.E., standard error; FDR, false discovery rate; DNMT, DNA methyltransferase.

[a] Corrected significance thresholds at $p<0.05$ are listed using two controlling procedures: Bonferroni and FDR using the procedure of Benjamini & Hochberg (Benjamini, 1995).

[b] For regression analyses, 'B' represents unstandardized B values.

[c] The list of observed p values is sorted from smallest to largest (indicated by rank column).

* Values meet significance at $p<0.05$ for the various correction procedures.

Transcription factor binding site prediction

DNAm is associated with gene expression. One mechanism by which increased DNAm can lead to decreased gene expression is by affecting the binding of trans-activating factors to *cis*-regulatory elements. To contextualize our DNAm findings, we used bioinformatic methods to identify putative TFBS that overlap CpG sites showing PTSD associated DNAm differences. In total, we identified 24 putative TFBS, including 2, 3, 14, and 5 that overlap *DNMT1*, *DNMT3A*, *DNMT3B*, and *DNMT3L* CpG target sites, respectively (Table 3). Notable among these 24 TFBS are those that overlap with CpG sites at which we identified PTSD-associated differential methylation (2 overlap the *DNMT1* CpG; 3 overlap *DNMT3B* CpG 9). Binding sites for heat shock factor 1 and E2F-4/DP-2 heterodimeric complex were identified to overlap with the *DNMT1* CpG site at which an increase in DNAm was observed in PTSD cases, but not controls. Overlapping with *DNMT3B* CpG site 9, at which lower pre-trauma DNAm was associated with PTSD development and predictive of worsening of PTSS, we identified binding sites for Human motif ten element, ZF5 POZ domain zinc finger, and the insulator protein CTCF.

Table 3. Putative transcription factor binding sites overlap DNA methyltransferase (*DNMT*) CpG sites of interest. V$ matrix families indicate Genomatix-annotated transcription factor binding site matrix families. *DNMT3B* CpG sites are described in the Methods.

Gene	Matrix Family	Matrix Information	Core similarity	*DNMT3B* CpG overlap
DNMT1	V$HEAT	Heat shock factor 1	1.000	-
	V$E2FF	E2F-4/DP-2 heterodimeric complex	0.847	-
DNMT3A	V$SP1F	TGFbeta-inducible early gene (TIEG)/Early growth response gene alpha (EGRalpha)	0.750	-
	V$SP1F	Stimulating protein 1, ubiquitous zinc finger transcription factor	1.000	-
	V$EGRF	EGR1, early growth response 1	0.802	-
	V$GCMF	Glial cells missing homolog 1, chorion-specific transcription factor GCMa	1.000	-
	V$KLFS	Kidney-enriched kruppel-like factor, KLF15	1.000	-
DNMT3B	O$MTEN	Human motif ten element	0.839	1, 2, 3, 4, 5
	V$PAX5	PAX5 paired domain protein	0.789	1, 2, 3, 4, 5, 6, 7
	V$E2FF	E2F transcription factor 3 (secondary DNA binding preference)	1.000	2, 3, 4, 5
	V$E2FF	E2F transcription factor 3 (secondary DNA binding preference)	1.000	2, 3, 4, 5, 6, 7
	V$ETSF	Ets variant 4	1.000	3, 4, 5, 6, 7
	O$MTEN	Human motif ten element	0.961	7, 8, 9
	V$ZF5F	ZF5 POZ domain zinc finger, zinc finger protein 161 (secondary DNA binding preference)	0.775	8, 9, 10
	V$CTCF	Insulator protein CTCF (CCCTC-binding factor)	0.818	9, 10, 11, 12
	V$HDBP	Huntington's disease gene regulatory region-binding protein 1 and 2 (SLC2A4 regulator and papillomavirus binding factor)	1.000	10, 11, 12
	V$EGRF	Collagen krox protein (zinc finger protein 67-zfp67)	1.000	11, 12
	V$PLAG	Pleomorphic adenoma gene (PLAG) 1, a developmentally regulated C2H2 zinc finger protein	0.958	12
	V$ZF02	Transcriptional repressor, binds to elements found predominantly in genes that participate in lipid metabolism	0.776	12
DNMT3L	V$KLFS	Basic transcription element (BTE) binding protein, BTEB3, FKLF-2	1.000	-
	V$SP1F	Stimulating protein 1, ubiquitous zinc finger transcription factor	1.000	-
	V$MYBL	C-Myb, important in hematopoesis, cellular equivalent to avian myoblastosis virus oncogene v-myb	0.797	-
	V$GLIF	Zinc finger transcription factor, Zic family member 2 (odd-paired homolog, Drosophila)	1.000	-
	V$CP2F	LBP-1c (leader-binding protein-1c), LSF (late SV40 factor, CP2, SEF (SAA3 enhancer factor)	0.875	-

Discussion

Our data represent preliminary findings suggesting that pre-trauma DNAm states and post-trauma DNAm modifications differ between those who develop PTSD following trauma and those who display resiliency. While baseline PTS symptoms did not differ between cases and controls, baseline DNAm at a *DNMT3B* CpG site was higher in resilient individuals compared to those who eventually developed PTSD. Additionally, longitudinal change in DNAm at a *DNMT1* CpG site was associated with PTSD, with an increase in DNAm being observed in those with PTSD but not controls. Finally, increases in DNAm were observed following trauma at *DNMT3A* and *DNMT3B* loci that were independent of PTSD outcome, being observed in both PTSD cases and trauma exposed controls. Although some of these results were attenuated following correction for multiple hypothesis testing, our findings suggest that epigenetic variation plays a complex regulatory role in PTSD risk and etiology.

One way in which DNAm may regulate gene transcription is by altering the strength and occupancy of transcription factor binding (Weaver et al., 2004). To provide insight into potential functional consequences of the observed PTSD-associated differences, we conducted a secondary analysis of TFBS overlapping the distinguishing CpG sites. Among the sites identified was a binding site for CTCF, a transcription factor known to be involved in chromatin remodeling (Barkess and West, 2012). We identified this binding site overlapping with *DNMT3B* CpG site 9, at which higher DNAm was identified as a protective/risk factor for PTSD and symptom severity change following trauma exposure. Differential methylation at this site is particularly compelling as a determinant of PTSD risk, given that DNAm at CTCF binding sites has been shown to

significantly affect CTCF occupancy (Wang et al., 2012) and downstream levels of gene transcription (Renaud et al., 2007). Due to the nature of our samples, we are unable to test directly whether DNAm at these identified TFBS influences gene expression. Where available, we have utilized ENCODE data (Consortium, 2011) to provide evidence for or against transcription factor binding at the PTSD-associated sites in blood-derived cell types. Among the TFBS identified that overlap PTSD-associated CpG sites (*DNMT1* and *DNMT3B* CpG 9), ENCODE data includes binding of CTCF and E2F4. ENCODE data supports the binding of CTCF to *DNMT3B* in blood tissue (specifically b-lymphocyte cell lines: GM12864 and GM12874), but does not support the binding of E2F4 to *DNMT1*. This supports the potential functionality of observed DNAm differences at *DNMT3B* CpG 9 in pre-trauma samples in cases vs. controls.

*DNMT*s have been previously implicated in PTSD, anxiety, and fear conditioning. In suicide completers relative to controls, *DNMT3B* was upregulated in the frontopolar cortex, hypothalamus, and dorsal vagal complex and down regulated, along with *DNMT1*, in the hippocampus (Poulter et al., 2008). Additionally, de novo methyltransferases have been shown to be upregulated during contextual fear conditioning, also in the hippocampus (Miller and Sweatt, 2007); DNMTs are required for fear conditioning and memory consolidation as demonstrated, respectively, by administration of DNMT inhibitors (Miller and Sweatt, 2007) and the creation of mice with the combined knockout of *DNMT1* and *DNMT3A (Feng et al., 2010)*. Our results thus add to the growing evidence implicating DNMTs in phenotypes of relevance to PTSD, and of psychiatric phenotypes more broadly.

The expression of *DNMT*s at the mRNA (Goto et al., 1994; Veldic et al., 2004; Kang et al., 2011; Sterner et al., 2012) and protein (Inano et al., 2000; Feng et al., 2005; Veldic et al., 2005) levels in post-mitotic neurons of the central nervous system suggests that they are involved in methyltransferase activity that persists into adulthood and that is unrelated to DNA replication (Goto et al., 1994). Indeed, previous work has identified DNMT1 protein expression in multiple brain regions in rodents (e.g. cortex, cerebellum (Inano et al., 2000)), as well as in specific cortical regions in adult humans (e.g. Broadmann's Area 9 (Veldic et al., 2005)). Furthermore, recent work suggests that our epigenetic findings in peripheral blood may be relevant to brain tissue: environmental exposures such as trauma have been shown to induce parallel epigenetic modifications in peripheral blood and brain (McGowan et al., 2011; Klengel et al., 2013). Although the current study, based on 450 living participants drawn from a population-based cohort, precludes such work, future research is needed to address whether the epigenetic determinants of risk observed here in peripheral blood-derived DNA is also found in brain-derived DNA.

Importantly, this study adds to emerging work utilizing a longitudinal study design capable of measuring biological markers prior to disease onset as well as change between pre-disease and post-disease time points (Nieratschker et al., 2012; Rusiecki et al., 2012; Perroud et al., 2013; Rusiecki et al., 2013). Existing longitudinal studies have documented the importance of DNAm to mental health disorder risk, including differential change in DNAm of *BDNF* among individuals with vs. without borderline personality disorder (Perroud et al., 2013), increased *DAT* (*SLC6A3*) DNAm with age that may be driven by alcohol dependence (Nieratschker et al., 2012), and increasing

SERT DNAm associated with bullying (Ouellet-Morin et al., 2013). Most relevant to the present study is work by Rusiecki et al. (Rusiecki et al., 2012) which provides evidence for increased global DNAm in controls, but not cases following trauma exposure, suggesting that resiliency is associated with increased global DNAm, potentially mediated by increased activity and expression of DNMTs. Indeed, our data presented here is consistent with this scenario, as DNAm of *DNMT1* was observed to increase following trauma in cases, but not controls. In contrast, however, we observed an increase in *DNMT3B* DNAm following trauma in both cases and controls, and a pre-trauma association between higher DNAm pre-trauma and resiliency post-trauma. The presence of a CTCF binding site opens the possibility that increased DNAm at this locus is associated with increased gene expression 473 because CTCF can act as either a transcriptional activator or repressor (Phillips and Corces, 2009), with strength of DNA binding inversely correlated with local DNAm (Barkess and West, 2012). If binding of CTCF to the *DNMT3B* locus results in transcriptional repression, then increased DNAm, and concurrent decreased CTCF binding, would be associated with increased, not decreased, gene expression. If true, this would put these findings in line with the previously published, longitudinal, trauma-associated epigenetic data: decreased DNAm in pre-trauma PTSD cases would result in tighter CTCF binding and reduced *DNMT3B* transcription and lower global DNAm levels, as reported by Ruisecki and colleagues (Rusiecki et al., 2012). Although DNMT1 is typically thought to maintain DNAm in adult tissues, evidence suggests that *DNMT1* and *DNMT3B* cooperatively maintain DNAm, with one or the other, but not both, required for global DNAm (Rhee et al., 2002). More broadly, our data adds to the emerging evidence that longitudinal DNAm changes may

contribute to the etiology of mental illness and can be taken as a proof of principle that locus-specific epigenetic variability both pre-exist and arise following disease-onset in biologically meaningful ways.

While our study is one of the first of its kind to compare pre- and post-trauma DNAm levels with regard to the development of PTSD, there is a minimum of four study limitations that should be kept in mind when interpreting our results. First, it is important to recognize that the epidemiological nature of our cohort precludes sample collection with a well-controlled experimental time course; times between pre-trauma data collection, trauma exposure, and post-trauma data collection differed between each test subject. As such, we are unable to resolve whether observed PTSD-associated post-trauma DNAm changes precede PTSD-development (i.e. occurred within the first four weeks following trauma). As DNMTs are involved in the global regulation of DNAm, it is tempting to conclude from our data that observed changes in *DNMT* DNAm are an upstream process of PTSD development, thereby having the potential to help explain differences in DNAm epigenome-wide reported elsewhere (Uddin et al., 2010; Smith et al., 2011a; Rusiecki et al., 2012). However, it is also possible that the observed PTSD associated DNAm changes are downstream effects of PTSD development, with no or little involvement in epigenetic modifications across the epigenome. Second, the nature of the epidemiological samples collected precluded the assessment of pre- and post-trauma gene expression differences and changes, as well as any analysis of blood cell composition. Third, the DNAm differences and effect sizes reported here are small; however, they are consistent with published work showing functional effects of DNAm variation (Tyrka et al., 2012). High intraclass correlation coefficients between

experimental replicates for each of our assays increases confidence of the validity of observed DNAm differences. Indeed, our sample size and observed effect sizes are consistent with published work in the field (Perroud et al., 2011; Byrne et al., 2013). Fourth, our results are not corrected for multiple testing. Although this is consistent with the current state of the science of DNAm variation in association with psychiatric endpoints (Perroud et al., 2011; Unternaehrer et al., 2012; Perroud et al., 2013; Rusiecki et al., 2013), we do report corrected results (Table 2) to assess the degree to which our findings might be attenuated by multiple hypothesis test correction. Accepting a stringent FDR of 0.05 requires that we reject several findings reported as significant in our study, notably pre-trauma DNAm differences between cases and controls at *DNMT3B* CpG 9. However, it also means that a significant association between DNAm and PTSD emerges as a result of correction, as a significant change in DNAm at *DNMT3A* following trauma is only seen in controls at this stringent FDR cutoff and would therefore be suggestive of a resiliency-associated change in DNAm (Table 2). While we have chosen to utilize a stringent FDR cut-off of 0.05, other DNAm analyses have accepted a cut-off as high as 0.20 (Provencal et al., 2013). Overall, we stress the preliminary nature of these findings—both uncorrected and corrected for multiple hypothesis testing—and the importance of replication in an independent cohort.

Individuals exposed to trauma differ in their risk for subsequent PTSD. Our data suggest that variation in pre-trauma DNAm and post-trauma DNAm change may be part of the molecular underpinnings of PTSD risk and resiliency. Future research is needed to determine if the DNAm variation observed here is associated with functional changes that affect the long-term biology of individuals exposed to trauma. The identification of

risk markers, including epigenetic markers, is an important step to understanding the biological underpinnings of PTSD risk and may lead to the development of tools to identify those individuals most at risk of developing PTSD as well as to develop evidence-based interventions.

Acknowledgements

We thank Dr. Chet Sherwood and Amy Bauernfeind from The George Washington University for contributions to this work. We thank the many Detroit residents who chose to participate in the DNHS.

This research was funded by NIH grants: 3R01DA022720-05, 3R01DA022720, and 1RC1MH088283-01 and NSF grant BCS-0827546. Levent Sipahi was funded by a Graduate Research Assistantship from the Wayne State University Office of the Vice President for Research, and a Grant-in-Aid of Research from Sigma Xi. Karestan Koenen is funded by R01MH093612, RC4MH092707, P51RR000165, U01OH010416, U01OH010407.

Chapter 3. Ancient evolutionary origins of epigenetic regulation associated with posttraumatic stress disorder

This chapter published as:
Sipahi L, Uddin M, Hou Z, Aiello AE, Koenen KC, Galea S, and Wildman DE (2014) Ancient evolutionary origins of epigenetic regulation associated with posttraumatic stress disorder. *Front. Hum. Neurosci.* **8:284.** doi: 10.3389/fnhum.2014.00284.

Abstract

Epigenetic marks, including DNA methylation, are modifiable molecular factors that may underlie mental disorders, especially responses to trauma, including the development of and resilience to posttraumatic stress disorder (PTSD). Previous work has identified differential DNA methylation at CpG dinucleotide sites genomewide between trauma exposed individuals with and without PTSD, suggesting a role for epigenetic potential – the capacity to epigenetically regulate behavior and physiology in response to lived experiences. The human species is characterized by an increased period of adaptive plasticity during brain development. The evolutionary history of epigenetic potential in relation to adaptive plasticity is currently unknown. Using phylogenetic methods and functional annotation analyses, we trace the evolution of over 7,000 CpG dinucleotides, including 203 associated with PTSD, during the descent of humans in during mammalian evolution and characterize the biological significance of this evolution. We demonstrate that few (7%) PTSD-associated CpG sites are unique to humans, while the vast majority of sites have deep evolutionary origins: 73% and 93% were unambiguously present in the last common ancestor of humans/orangutans and humans/chimpanzees, respectively. Genes proximal to evolved PTSD-associated CpG sites revealed significant enrichment for immune function during recent human evolution and regulation of gene expression during more ancient periods of human evolution.

Additionally, 765 putative transcription factor binding motifs (TFBMs) were identified that overlap with PTSD-associated CpG sites. Elucidation of the evolutionary history of PTSD-associated CpG sites may provide insights into the function and origin of epigenetic potential in trauma responses, generally, and PTSD, specifically. The human capacity to respond to trauma with stable physiologic and behavioral changes may be due to epigenetic potentials that are shared among many mammalian species.

Introduction

The human species is characterized by an increased period of adaptive plasticity during brain development (Chugani, 1998; Sterner et al., 2012). This phenotypic plasticity enables individuals to respond in unique ways to environmental stimuli. Among other mechanisms, DNA methylation regulates phenotypic plasticity through epigenetic marks that are stable yet capable of experience-mediated dynamic change (Moore et al., 2013). Although DNA methylation may underlie mechanisms of phenotypic plasticity, the functional evolution of DNA methylation-mediated plasticity requires the presence of a genetic nucleotide substrate – the CpG dinucleotide. We thus refer to the presence of CpG dinucleotides required for phenotypic plasticity, “epigenetic potential”. The evolutionary history of this epigenetic potential is crucial to understanding various forms of phenotypic plasticity and related mental health outcomes.

Posttraumatic stress disorder (PTSD) and resiliency comprise two phenotypes that may arise subsequent to exposure to a traumatic event. Given that trauma is unpredictable yet probable within a lifetime, mechanisms of phenotypic plasticity in response to trauma are expected to exist, despite the prediction that such mechanisms

will be costly (West-Eberhard, 2003). As such, we expect the epigenetic potential that underlies the capacity for trauma-induced phenotypic plasticity is possibly highly conserved. In humans, PTSD and trauma resiliency are associated with differential DNA methylation genomewide (Uddin et al., 2010; Smith et al., 2011b) and, therefore, may be regulated by epigenetic potential.

Diagnostically, PTSD is a mental health disorder characterized by symptoms of intrusion, avoidance, and alterations in cognition and mood that cause distress or social impairment, last for more than one month, and are associated with a traumatic event consisting of direct or indirect exposure to actual or threatened death, injury, or sexual violation (Association, 2013). Although PTSD diagnoses have historically been confined to humans, the capacity to express traumatic symptoms likely exists on other primate, and possibly non-primate mammalian, lineages. For example, abused chimpanzees have been reported to exhibit symptoms that in humans are considered characteristic of PTSD, including symptoms of avoidance, arousal, and dissociation (Bradshaw et al., 2008; Ferdowsian et al., 2011); elephants exposed to human violence have been documented exhibiting behaviors characterized by hyperaggression, abnormal startle response, depression, and asocial behaviors (Bradshaw et al., 2005); and rodents have been used as a model of PTSD, given their responses to trauma of social withdrawal and hyperarousal (Siegmund and Wotjak, 2006). These observations suggest that the capacity to express traumatic symptoms in response to extreme stress exists on non-human lineages within primate or mammalian clades and that this capacity may be an ancestral state (Horwitz AV, 2012).

Emerging evidence suggests that epigenetic variation may help explain observed differential susceptibility and resilience to PTSD in humans (Breslau et al., 1998). Indeed, recent studies (Uddin et al., 2010; Smith et al., 2011b; Mehta et al., 2013), have demonstrated differential DNA methylation genomewide between individuals with PTSD and trauma exposed individuals without PTSD. Each of these studies demonstrated epigenetic dysregulation of immune system genes (Uddin et al., 2010; Smith et al., 2011b) – a finding that is consistent with the knowledge that the hypothalamic-pituitary-adrenal axis modulates the immune system (Wong, 2002; Irwin and Cole, 2011) and findings from previous publications that differential gene expression patterns in genes involved in immune activation exist between PTSD-affected and -unaffected individuals (Segman et al., 2005; Zieker et al., 2007). PTSD, therefore, appears to be epigenetically regulated.

The evolutionary history of human epigenetic potential in relation to trauma-induced phenotypic plasticity would likely be informative of human phenotypic plasticity responses, generally, and PTSD, specifically. We hypothesized that the capacity to regulate behavioral, physiological, and psychological processes in response to traumatic experiences is mediated by epigenetic regulation at genetically inherited CpG loci that are largely conserved across mammalian species. Specifically, we expect that most of the CpG sites associated with the epigenetic regulation of PTSD will be largely conserved (that is, not unique to humans), but instead will have much more ancient origins. Here we test this hypothesis by tracing and characterizing the evolution of human CpG dinucleotides previously associated with DNA methylation patterns in whole blood that differentiate trauma exposed individuals with PTSD from those without

PTSD. Doing so, we reveal the phylogenetic history of genetic CpG sites previously shown to be associated with the capacity to epigenetically regulate PTSD in humans and characterize different periods of the evolution of humans and other mammals.

Materials and Methods

Emergence of CpG Sites during Human Descent

Human CpG site annotation data was obtained from the Infinium HumanMethylation27 (HM27) DNA Analysis BeadChip by Illumina, which targets 27,578 CpG sites in more than fourteen-thousand genes. Multiple sequence alignments were constructed using both Ensembl (v54, PECAN(Paten et al., 2008) for global multiple sequence alignments) and UCSC (Feb. 2009 assembly; Z-blast (Rosenbloom et al., 2010) for local multiple sequence alignments) using publicly available genomes of the following species: human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), orangutan (*Pongo abelii*), macaque (*Macaca mulatta*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), cow (*Bos taurus*), horse (*Equus caballus*), dog (*Canis lupus familiaris*), opossum (*Monodelphis domestica*), platypus (*Ornithorhynchus anatinus*), and chicken (*Gallus gallus*). The accelerated transformation maximum parsimony (ACCTRAN) algorithm (JS, 1970), implemented in PAUP*4.0, was used to infer ancestral CpG site statuses for 19,711 alignable CpG sites. Only CpG sites for which alignments could be constructed using both UCSC and Ensembl and for which ancestral states were inferred unambiguously by parsimony (CI=1) were utilized in downstream analyses. Using this process, the evolutionary history of 7202 CpG sites were unambiguously inferred along human descent within the context of a 12 species mammalian phylogenetic tree.

A note on human descent branch labels

Throughout this paper we refer to branches on human descent in the following way, with divergence times estimates from (Meredith et al., 2011) where applicable, and (Jameson et al., 2011) for primate nodes.

Human terminal: 7.2 million year period from the LCA of humans and chimpanzees until present.

Human/chimpanzee stem: 10.8 million year period from the LCA of humans and orangutans (18.0mya) to the LCA of humans and chimpanzees (7.2mya).

Ape stem: 7.4 million year period from the LCA of humans and macaques (24.5mya) to the LCA of humans and orangutans (18.0mya).

Primate stem: 57.9 million year period from the LCA of humans and rodents (83.3mya; here represented by rat and mouse) to the LCA of humans and macaques (25.4mya).

Euarchontoglires stem: 8.7 million year period from the LCA of humans and Laurasiatherians (92.0mya; here represented by dog, horse, and cow) to the LCA of humans and rodents (83.3mya).

Placental stem: 98.0 million year period from the LCA of humans and opposums (190.0mya) to the LCA of humans and Laurasiatherians (92.0mya).

Theria stem: 27.8 million year period from the LCA of humans and platypus (217.8mya) to the LCA of humans and opossum (190.0mya).

Mammal stem: 106.7 million year period from the LCA of humans and chickens (324.5mya) to the LCA of humans and platypus (217.8mya).

Evolution of PTSD-associated CpG sites during Human Descent

To gain insights into the evolutionary history and functional adaptive nature of PTSD, we inferred the evolutionary history of 203 PTSD-associated CpG sites identified by Uddin et al (Uddin et al., 2010). Smith et al. (Smith et al., 2011b) and Mehta et al. ((Mehta et al., 2013)) have also published associations between PTSD and differential DNA methylation. Because samples utilized by these studies were chosen based on substantially different selection criteria – confined to PTSD in Uddin et al (Uddin et al., 2010), whereas including selection for total life stress and childhood abuse in Smith et al. (Smith et al., 2011a) as well as Mehta et al. (Mehta et al., 2013) – we have chosen to analyze here only those PTSD-associated CpG sites identified by Uddin et al (Uddin et al., 2010).

The following was completed on the 203 PTSD-associated CpG sites from Uddin and colleagues (Uddin et al., 2010) that mapped unambiguously onto our 12 species phylogenetic tree. Branch-specific rates of evolution during human descent were calculated using divergence dates estimates detailed above. Chi-square tests were utilized to analyze branch specific rates of evolution and are described in following:

1) a Chi-square test was used to test whether statistically significant differences exist between the branch distribution observed in the whole dataset (7202 sites) and the PTSD-associated subset (203 sites). For this test, expected and observed values represent, respectively, the distribution of changes of the whole data set and the PTSD-associated dataset.

2: a Chi-square test was used to test whether the number of PTSD-associated changes was branch specific (all branches considered). Respectively, expected and observed values represent an even distribution of changes and the actual distribution of changes in the dataset.

3: given that there was a statistically significant difference according to branch, we used Chi-square tests to determine which branches differed from one-another (all individual branches compared to all other individual branches).

Putative transcription factor binding motifs (TFBMs) were identified that overlap with CpG sites of interest using the Genomatix MatInspector (Quandt et al., 1995; Cartharius et al., 2005) tool, with default settings. Input sequences included 60 base pairs up- and downstream of PTSD-associated CpG dinucleotide sites, as obtained from the Infinium HumanMethylation27 (HM27) DNA Analysis BeadChip by Illumina. The Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da et al., 2009) was used with default settings to assess branch-specific enrichment of gene ontology terms and functional annotation clustering (FAC) among genes proximal to PTSD-associated CpG sites. Genes proximal to the 7202 total CpG sites for which evolution was unambiguously inferred were used as background for enrichment computations. Functional annotation clusters with Enrichment Scores greater than 1.3 were considered significant (Cartharius et al., 2005; Huang da et al., 2009).

Results

Inference of PTSD-associated CpG dinucleotide phylogenetic history

The phylogenetic histories of 7202 CpG dinucleotide sites from across the genome and assessed by the DNA methylation microarray were unambiguously mapped onto a mammalian phylogenetic tree (Figure 6A) by inferring ancestral states using a maximum parsimony method (Supplemental Table 1). Of these, 203 were previously identified to be differentially methylated in association with PTSD (Uddin et al., 2010).

Of the 7202 human CpG sites examined in extant mammals, 10.5%, 52.0%, 75.0%, and 91.4% were present in the last common ancestor (LCA) of humans and rodents, Old World monkeys, orangutans, and chimpanzees, respectively. 8.6% of the CpG sites assessed evolved on the human terminal branch. The percentage of of 203 PTSD-associated CpG sites evolved prior to the LCA of humans and rodents (7%), Old World monkeys (48%), orangutans (73%), and chimpanzees (93%) showed a branch distribution that did not differ statistically from that observed among the total 7202 assessed CpG sites (chi-square=0.044, df=7, $p>0.99$) (Figure 6B). Among all CpG sites assessed as well as the subset of those associated with PTSD, there was a statistically significant association between branch of human descent and number of evolved CpG sites (Table 1; Total: chi-square=7936.26, df=7, $p<0.0001$; PTSD subset: chi-square=247.56 df=7, $p<0.0001$), such that the number of evolved PTSD-associated CpG sites was lowest on the mammal, Theria, placental, and Euarchontoglires stems, rose sharply beginning with the primate stem, peaked on the ape stem, and then decreased slightly on the human/chimpanzee stem and human terminal branch. The

number of PTSD-associated CpG sites that evolved on the primate stem was significantly greater than any other branch of human descent assessed. (Table 1, Figure 6B).

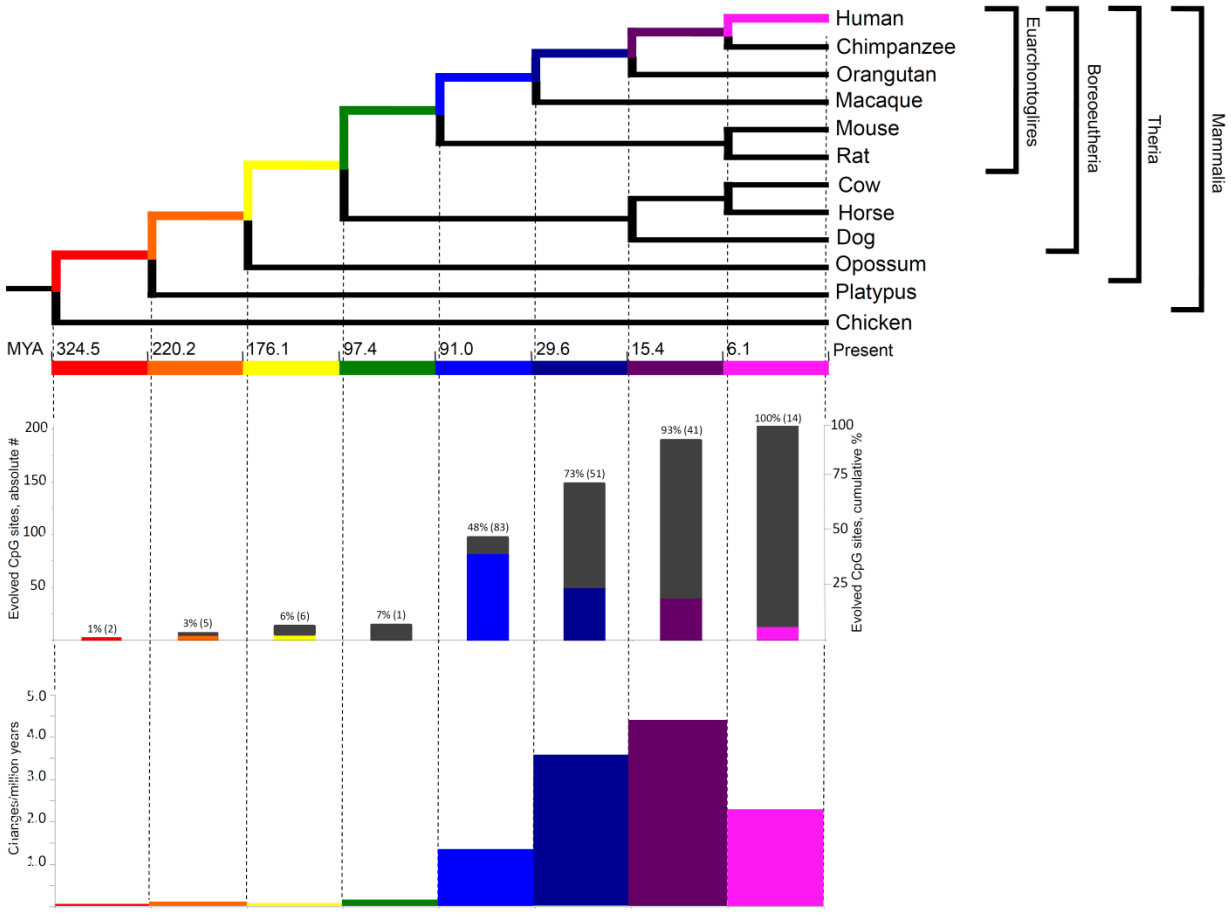

Figure 6. Evolutionary history of PTSD-associated CpG dinucleotides. Bars, tree branches, and time scales in panels A-C are color coordinated and vertically aligned. Red: mammal stem; Orange: Theria stem; Yellow: placental stem; Green: Euarchontoglires stem; Light Blue: primate stem; Dark Blue: ape stem; Purple: human/chimpanzee stem; Pink: human stem A) Mammalian phylogenetic tree with species genomes utilized for the inference of molecular evolution here. Divergence dates at internal nodes along human descent, in millions of years ago. B) The absolute number (colored bars) and cumulative percentage (gray bars) of PTSD-associated CpG sites evolved on branches of human descent, as inferred by parsimony. C) Branch-specific rates of evolution of PTSD-associated CpG sites through human descent.

Table 1. Chi-square tests for branch-specific number and rate of evolved PTSD-associated CpG sites. Values are (Chi-square/p-value) and represent the result of a chi-square test with 1 degree of freedom. Cells above and below the diagonal represent evolutionary rate and raw number, respectively. Cells highlighted in gray represent tests that failed to reject the null hypothesis.

	Human	Human/ Chimpanzee	Ape	Primate	Euarchontoglires	Placental	Theria	Mammal
Human		8.86/ 0.003	50.7/ <0.001	0.907/ 0.341	18.0/ <0.001	18.0/ <0.001	15.4/ <0.001	21.0/ <0.001
Human/ Chimpanzee	15.3/ <0.001		19.3/ <0.001	15.0/ <0.001	43.6/ <0.001	43.6/ <0.001	40.5/ <0.001	46.9/ <0.001
Ape	25.1/ <0.001	1.41/ 0.236		62.3/ <0.001	102.0/ <0.001	102.0/ <0.001	98.0/ <0.001	105.0/ <0.001
Primate	64.5/ <0.001	20.5/ <0.001	11.4/ 0.001		12.2/ <0.001	12.2/ <0.001	9.78/ 0.002	15.1/ <0.001
Euarchontoglires	11.7/ 0.001	42.5/ <0.001	55.1/ <0.001	101.0/ <0.001		0.00/ 1.000	0.338/ 0.561	1.01/ 0.316
Placental	3.37/ 0.067	29.5/ <0.001	41.3/ <0.001	85.3/ <0.001	3.63/ 0.057		0.338/ 0.561	1.01/ 0.316
Theria	4.47/ 0.034	31.8/ <0.001	43.8/ <0.001	88.3/ <0.001	2.71/ 0.100	0.09/ 0.760		2.02/ 0.155
Mammal	9.37/ 0.002	39.6/ <0.001	52.1/ <0.001	97.6/ <0.001	0.336/ 0.562	2.04/ 0.153	1.31/ 0.253	

Because there is high variability among the evolutionary time that spanned each of the branches assessed (Figure 6A), we computed branch-specific rates of evolution of total and PTSD-associated CpG sites (Figure 6C). The evolutionary rate of PTSD-associated CpG sites was statistically greater on the primate, ape, human/chimpanzee, and human terminal branches compared to that on the mammal, Theria, placental, and Euarchontoglires branches (Table 1, Figure 6C). PTSD-associated CpG sites evolved at the highest rate (6.9 changes/million years) on the ape stem (Table 1, $p<0.001$ vs. all other branches). The rate of PTSD-associated CpG site evolution was lowest (Table 1) during human descent from the last common ancestor of all extant mammals 324.5 million years ago until the LCA of humans and rodents 92 million years ago (combined mammal+Theria+placental+Euarchontoglires stem lineages), during which time the evolutionary rate of PTSD-associated CpG sites never exceeded 0.2 changes/million years; the rate of PTSD-associated CpG evolution did not significantly vary between these branches of more ancient human descent (Table 1). Additionally, we observed a strong association between branch-specific evolution of all CpG sites and PTSD-associated CpG sites (Figure 7; $R^2=0.98338$). That is, the proportion of PTSD-associated CpGs among all evolved CpGs was found to scale linearly through human descent.

Gene functional enrichment analyses of PTSD-associated CpG dinucleotides

In order to shed light on selection pressures and biological significance of the evolution of PTSD-associated CpG sites, we tested for enrichment of gene ontology terms among genes located proximal (as predicted by beadchip annotation) to evolved PTSD-associated CpG sites. Background for this enrichment analysis was genes proximally located to the 7202 total CpG sites for which evolutionary history was

unambiguously inferred (Supplemental Table 1). Enrichment analyses were completed for all 203 PTSD-associated CpG sites identified by Uddin et al (Uddin et al., 2010) (Supplemental Tables 2, 3, and 4) as well as on a branch-by-branch basis (Table 2). Annotation clusters with the five highest enrichment scores on each branch are listed in Table 2. The complete results of functional annotation analyses, including enriched GO terms (Supplemental Table 2) and significantly enriched Functional Annotation Clusters (Supplemental Table 3) can be found in Supplemental Tables; Significant FAC results (considered here to be those clusters with enrichment scores greater than 1.3 (Huang da et al., 2009)) can be found in Supplemental Table 4. We detail several noteworthy findings below.

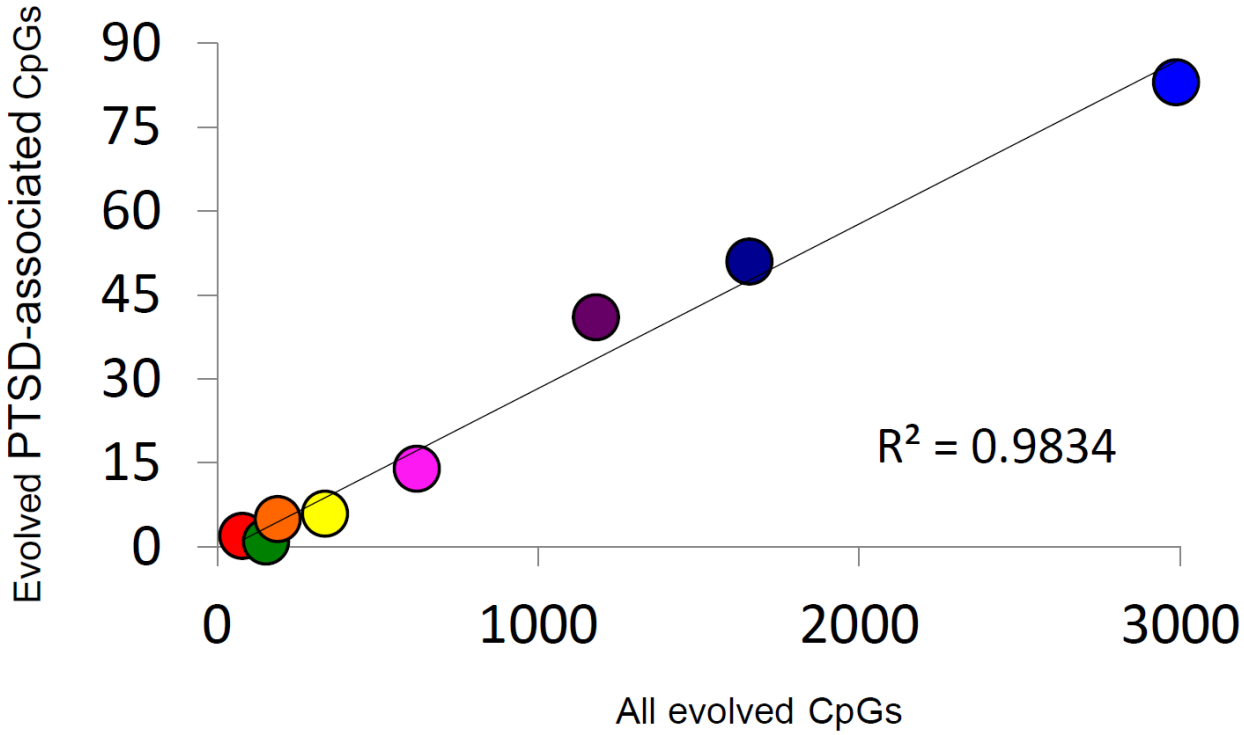

Figure 7. Proportion of PTSD-associated CpG dinucleotides among all evolved CpG dinucleotides scales linearly through human descent. Color of circles corresponds to branches of human descent, as indicated in Figure 6. Red: Mammal stem; Orange: Theria stem; Yellow: placental stem; Green: Euarchontoglires stem; Light Blue: primate stem; Dark Blue: ape stem; Purple: human/chimpanzee stem; Pink: human stem.

In general, enriched annotation clusters involved transcriptional regulation throughout human descent, with the addition of immune-related annotations during more recent periods of human descent (Table 2). The human/chimpanzee stem lineage, during which the rate of PTSD-associated CpG evolution was greatest, was significantly enriched in annotation clusters involved in immune function, including adaptive (b cell receptor signaling pathway) and innate immune response (natural killer cell-mediated cytotoxicity) (Table 2). Likewise, the human terminal and ape stem lineages were enriched for immune response and B cell signaling annotation clusters, respectively (Table 2). Previous studies have found differential expression of immune function genes between trauma exposed persons with and without PTSD (Segman et al., 2005; Zieker et al., 2007). It is noteworthy, therefore, that the human terminal branch showed a FAC enriched for a number of immune functions including among others: immune response, immune response-activating cell surface receptor signaling pathway, T cell activation, lymphocyte activation, and leukocyte activation (Table 2).

In contrast, the primate stem lineage, on which the greatest number of PTSD-associated CpG sites evolved, had a FAC heavily associated with transcriptional regulation, including genes involved in epigenetic regulation: *HDAC1* and *HDAC11* (Table 2). Additionally, the annoation clusters with the three highest enrichment scores were found on the primate stem; each involved in transcriptional regulation and heavily enriched for zinc finger transcription factors (Table 2). It is noteworthy also that, with the exception of the human terminal, human/chimpanzee branch, and Euarchontoglires branch, each branch contained among its five most enriched clusters at least one involved in transcriptional regulation (Table 2).

Table 5. Top 5 functional annotation clusters of genes proximal to PTSD-associated CpG sites evolved on different branches of human descent.

Stem Branch	Annotation Cluster	Cluster Rank	Enrichment Score
Human	Plasma membrane	1, 2, 3, 4	3.30, 2.98, 1.47,
	Immune Response	5	1.30
Human/chimpanzee	Pathways in cancer	1	3.20
	B cell receptor signaling pathway	2	3.17
	MAPK/GnRH/Fc epsilon RI signaling	3, 4	2.85, 2.38
	Intestinal immune network (IgA)	5	2.09
Ape	zinc finger/ion binding	1	3.75
	Plasma membrane	2	3.51
	Transcription/Zinc finger regions	3	3.29
	B cell/Fc epsilon RI/VEGF/GnRH/ Natural killer cell Signaling	4	2.68
	Long-term potentiation	5	2.46
Primate	zinc finger region:C2H2	1, 2	7.74, 4.09
	Transcription regulation	3	4.01
	Plasma membrane	4	3.52
	Intracellular organelle lumen	5	3.24
Euarchontoglires	Src homology-3 domain	1	1.32
Placental	transcription regulation	1, 2	2.88, 2.13
	Protein modification	3	1.84
	Cell fraction	4	1.35
	Regulation of apoptosis	5	1.31
Theria	intracellular, non-membrane bound	1	2.80
	Transcription regulation	2	2.39
	DNA-binding/helix-loop-helix	3	1.98
	Organelle lumen	4	1.94
	Sensory perception	5	1.42
Mammal	negative regulation of transcription	1	1.71

Transcription factor binding motif identification and characterization

As DNA methylation can regulate gene expression by structurally blocking transcription factor binding within gene promoter regions(Curradi et al., 2002), we sought to identify putative transcription factor binding sites that overlap with the 203 PTSD-associated CpG sites. Using a motif prediction algorithm, 765 total transcription factor binding motifs (TFBMs) were identified that overlap 196 PTSD-associated CpG sites. Of these, 765, 407, 188, and 22 putative TFBMs were identified that overlap with 196, 163, 107, and 21 unique PTSD-associated CpG sites at detection stringencies (mat_sim) of >0.80, >0.90, >0.95, and =1, respectively (Supplemental Table 5). A stringency score of 1 indicates that the candidate sequence corresponds to the most conserved nucleotide at each position of the reference matrix. The 22 TFBMs with the highest stringency score (i.e. 1) spanned 21 different PTSD-associated CpG sites (Table 3). Two TFBMs with a stringency score of 1 spanned the CpG site cg13316424 of the gene *CIZ1*. Further, these 22 TFBMs were distributed among 9 different matrix families (Table 3). Notably, 4 of these TFBMs represent binding sites for transcription factor II B, which makes up the RNA polymerase II pre-initiation complex, while 2 are putative bindings sites of the transcription factor Beta2/NeuroD, which is responsible for neuron- and endocrine cell-specific gene expression. PTSD-associated CpG sites that overlap the RNA polymerase II pre-initiation complex binding motifs evolved on the ape (cg10498097/*MGC50811)*, primate (cg04033774/*GPSM2* and cg20318748*/NANP*), and placental (cg24673765/*HSPB6*) stem lineages, while those that overlap with Beta2/NeuroD binding motifs evolved on the ape (cg04587829/*FN3K*) and primate (cg00427635/*TBC1D21*) stem lineages. The branch-specific breakdown of the evolution

of the 22 high stringency TFBMs is as follows: human (2), human/chimpanzee (5), ape (6), primate (5), placental (2), and therian (2).

Table 6. Matrix Families of TFBMs (mat_sim=1) that overlap PTSD-associated CpG dinucleotides.

Matrix Family	CpG	Gene	Stem Branch
C2H2 zinc finger transcription factors 2	cg27318281	*C18ORF37*	Human
	cg19047670	*CCND1*	Human/chimpanzee
	cg00962459	*PROKR1*	Ape
	cg03570766	*CATSPER1*	Ape
	cg12439773	*SLC22A6*	Theria
RNA Polymerase II transcription factor II B	cg10498097	*MGC50811*	Ape
	cg04033774	*GPSM2*	Primate
	cg20318748	*NANP*	Primate
	cg24673765	*HSPB6*	Placental
Pleomorphic adenoma gene	cg06445611	*GABRR2*	Human/chimpanzee
	cg24505375	*AMAC1L2*	Human/chimpanzee
	cg13316424	*CIZ1*	Primate
	cg21835643	*RBPSUHL*	Theria
NeuroD, Beta2, HLH domain	cg04587829	*FN3K*	Ape
	cg00427635	*TBC1D21*	Primate
TALE homeodomain class recognizingTG motifs	cg19531130	*ANGPTL5*	Human/chimpanzee
	cg01813965	*C16orf50*	Ape
Cart-1 (cartilage homeoprotein 1)	cg13316424	*CIZ1*	Primate
Human and murine ETS1 factors	cg06084117	*PLXNA4B*	Human/chimpanzee
	cg20792833	*PTPRCAP*	Placental
Vertebrate SNAD family of transcription factors	cg13471990	*ENTPD1*	Ape
Par/bZIP family	cg25293251	*GOLGA5*	Human

Discussion

Epigenetic modifications provide a mechanism by which lived experiences can reprogram gene expression patterns and affect biological processes broadly. The epigenetic potential to respond to stress and trauma is likely conserved across mammalian species. As such, we predicted that the genetic elements (CpG dinucleotide sites) required for this epigenetic potential would be conserved. We have previously demonstrated that differential DNA methylation of CpG dinucleotides genomewide distinguish those with PTSD from trauma exposed individuals without PTSD (Uddin et al., 2010). Here, by tracing and characterizing the evolutionary history of 203 of these PTSD-associated CpG dinucleotide sites using functional annotation clustering and transcription factor binding motif identification, we provide evidence that the genetic substrate associated with divergent epigenetic responses to trauma is largely conserved across mammalian species. Among those PTSD-associated CpG sites for which we could unambiguously infer ancestral states, we demonstrate that 1) the majority evolved prior to the LCA of humans and Old World monkeys, 2) there is an enrichment among genes found proximal to evolved PTSD-associated CpG dinucleotides on all branches of human descent for annotation clusters involved in transcriptional regulation, while more recent branches of human descent (ape and human/chimpanzee stem branches, and human terminal branch) are enriched also for immune function-related annotation clusters; and 3) there is overlap with more than 800 putative TFBMs, the 22 most stringently selected of which fall into 9 TFBM families and evolved overwhelmingly on primate, ape, human/chimpanzee, and human stem terminal. Taken together, our data demonstrate that the human potential to epigenetically regulate traumatic responses

may be shared with non-human primates and that the evolution of this capacity likely involved the targeting of TFBMs of many genes, including those involved in immune response and transcriptional regulation.

Functional annotation clustering and enrichment analysis of gene ontology terms identified enrichment of FACs involved in immune functioning that emerged throughout human descent generally, with over representation primarily concentrated during the most recent 15 million years of human evolution. It is notable that immune function dysregulation is a common finding among individuals with PTSD (Uddin et al., 2010; Smith et al., 2011b) and evidence suggests that the immune system may play an important role in PTSD phenomenology (Segman et al., 2005; Zieker et al., 2007). Although not surprising, it is important to note that functional annotation enrichment among the 203 PTSD-associated sites tested here do not markedly differ from that observed in the larger dataset of 624 differentially methylated sites from Uddin and colleagues (Uddin et al., 2010). Indeed, the immune system interfaces closely with the HPA axis, a key regulator of the stress response. Glucocorticoids from the HPA axis trigger changes in expression of cytokines and inflammatory genes in leukocytes, while cytokine receptors in the hypothalamus trigger the release of glucocorticoids from the HPA axis in response to immune activation (Irwin and Cole, 2011). This complex interplay between the HPA axis and the immune system supports the evidence of immune involvement presented here. The identification of enriched immune function FACs may suggest recent evolutionary innovations or selection pressures on the ability of the immune system to respond to environmental exposures, possibly including trauma. Moreover, there is now clear evidence that the immune response is associated

with a variety of mood disorders, and that cytokine activation in peripheral blood as well as brain cells, particularly microglia, underlie this association (Jones and Thomsen, 2013).

DNA methylation is thought to regulate gene expression, in part, by blocking the binding of transcription factor to binding sites (Iguchi-Ariga and Schaffner, 1989). Indeed, while several studies have identified differential methylation between trauma exposed individuals affected and unaffected by PTSD (Uddin et al., 2010; Smith et al., 2011b), it is unclear what functional connection explains this association. Here, we identified approximately 800 putative transcription factor binding motifs that overlap PTSD-associated CpG sites, 22 of which met the highest detection stringency. Interestingly, 13 of these 22 putative binding sites overlapped with PTSD-associated CpG sites that evolved during relatively recent human evolution (human terminal = 2, human/chimpanzee stem = 5, ape stem = 6).

Our approach is novel in that it combines empirical insights of epigenetic variation to inform a genetic comparative analysis for the purpose of understanding human evolution. Classically, comparative molecular studies have compared genetic sequence or expression variation across species. However, recent work has compared DNAm variation across species (Zeng et al., 2012; Hernando-Herraez et al., 2013), with the hypothesis that species differences are due in part to gene regulatory differences created by species-specific promoter methylation. In contrast, we explore here a trait thought to be undergirded by epigenetic malleability and conserved across species.

A speculative interpretation of this data is that, in light of the strong conservation of PTSD-associated CpG sites, the potential to epigenetically regulate responses to

extreme traumatic stress may well be adaptive. Depending on environmental circumstances, PTSD or anxiety states more generally, can increase or decrease evolutionary fitness. Increased states of anxiety, although perhaps not ideal in many situations, can theoretically increase evolutionary fitness (Nesse, 2001; 2005). For example, genetic variants of catechol-O-methyltransferase (*COMT*) have been associated with a tradeoff between cognitive ability and behavioral measures of anxiety and stress resilience, giving rise to the so-called Worrier/Warrior selectionist model (Stein et al., 2002; Goldman et al., 2005; Mier et al., 2010). An epigenetically regulated developmental program that facilitates the plasticity required to assume an appropriate phenotype in response to environmental conditions may be similarly adaptive(Meaney, 2010). Given that there is strong conservation of PTSD-associated CpG sites among non-human primates, it may be the case that selective pressures have maintained the capacity to increase or decrease PTSD-like responses to trauma, including re-experiencing-, avoidance-, emotional numbing-, social withdrawal-, and hyperarousal-type symptoms in response to lived experiences and environmental exposures. We propose that the capacity to develop and epigenetically regulate a PTSD-like syndrome is potentially present in many mammalian species. Indeed, symptoms resembling PTSD and other mood disorders have been observed in chimpanzees (Bradshaw et al., 2008; Ferdowsian et al., 2011). We do not, however, expect that all species will respond to trauma exposure in the same way. Just as only a subset of trauma exposed humans develop PTSD symptoms, it is likely that individual variation within and among species is common in response to trauma exposure. Finally, the evolutionary history of the capacity to epigenetically regulate trauma responsivity is significant in light of the finding

that environmentally induced epigenetic inheritance interacts with lifetime stress to alter brain development and genome activity (Crews et al., 2012).

Our study is limited by the number of CpG dinucleotides assessed by the methylation microarray and the number of publically available genomes utilized. While inclusion of additional genomes would have allowed the inference of phylogenetic histories with greater resolution, doing so would have limited the number of CpG sites analyzed in downstream analyses due to a larger number failing to meet our requirement of an unambiguous multiple sequence alignment. Inclusion of more species will provide valuable information and warrants further research. Indeed, because a large number of PTSD-associated CpG sites are shared among primates, the replication of this study with a more detailed primate phylogeny would be insightful. Additionally, characterization of the evolutionary history of the human capacity to epigenetically regulate trauma responses is limited by the incompleteness of PTSD-associated CpG sites assessed. Specifically, only 203 of 624 PTSD-associated CpG sites from Uddin et al (Uddin et al., 2010) were both alignable and perfectly parsimonious, and therefore characterized here. That a majority of PTSD-associated CpG sites were unalignable or perfectly parsimonious is evidence of the dynamic nature of CpG evolution. Future studies are also needed to address the results here with regard to rapid deamination of methylated cytosines. Because of the high deamination mutation rate of methylated cytosines to thymines, there is an underrepresentation of CpG dinucleotides in the human genome. This may largely explain the observation that the vast majority of CpG evolution, including that of PTSD-associated CpGs, occurred more recently during human descent. Given this, it may be reasonable to examine the functional significance

of those PTSD-associated CpG sites that evolved more anciently, assuming that they have been conserved because of a strong selection pressure to maintain them. The observation that immune system genes are enriched among the analyzed genes on more recent branches of human evolution does not necessarily imply that immune genes are not important in the evolution of nonhuman lineages. Finally, it should be noted that the identification of the PTSD-associated CpG sites analyzed here was performed using DNA methylation obtained from peripheral blood samples. While brain-derived DNA methylation data would be ideal to identify PTSD-associated biological markers, this is not feasible given a desire to use samples provided by living humans. However, individuals with PTSD have been shown to have reduced hippocampal area and hyperactivated amygdala relative to trauma exposed controls (reviewed in (Pitman et al., 2012)). Moreover, recent work suggests that there can be concordance between DNA methylation patterns in the blood and brain for stress-relevant genes (Klengel et al., 2013).

Interestingly, while we have traced and characterized the evolutionary history of PTSD-associated CpG sites, we also now have data on the evolution of non-PTSD-associated CpG sites. Comparing the branch-specific rates of evolution demonstrates that the rate of evolution of PTSD-associated CpG sites seems to scale linearly with human descent – suggesting that the evolutionary pattern observed, at least in terms of evolutionary rate, is a function of CpG evolution generally.

The vast majority of people experience a potentially traumatic event at some point in their lifetime, yet relatively few subsequently develop the diagnosable mental disorder PTSD. Elucidating the molecular and evolutionary underpinnings of severe

trauma responses, such as PTSD, are required for the prevention and treatment of the disorder and for identifying factors involved in discrepancies in risk and resilience. Here, we contribute to the ongoing study of epigenetic influences on PTSD etiology and differential risk by having traced and characterized the evolution of genomic sites associated with the development of PTSD. Our data suggest that PTSD-associated CpG sites are found at highly predicted transcription factor binding sites, that the majority of such sites are shared by all primates and that the overrepresentation of PTSD-associated CpGs proximal to immune system-related genes may have disproportionately evolved during our more recent evolutionary history. Taken together, this data supports the hypothesis that the DNA sequences necessary for the epigenetic potential to develop a range of phenotypes in response to trauma (e.g. PTSD or resiliency) in humans have deep evolutionary origins and are widely conserved among mammalian species. It will be exciting to discover whether these epigenetic signals contributed to the evolution of human brain function and/or dysfunction.

Acknowledgements

This work was funded by grants from the NIH (3R01DA022720, 1RC1MH088283-01) and NSF (BCS-0827546). LS was also funded by a Graduate Research Assistantship from the Wayne State University Vice President for Research. The funding sources were not involved in the design, nor collection, analysis, and interpretation of data.

Supplemental Data; available online with published article.

Supplemental Table 1. All evolved CpG dinucleotides (7202)

Supplemental Table 2. Gene Enrichment: Gene Ontology (all)

Supplemental Table 3. Gene Enrichment: Functional Annotation Clustering (all)

Supplemental Table 4. Gene Enrichment: Functional Annotation Clustering (Significant)

Supplemental Table 5. Putative TFBMs at 4 stringencies

Chapter 4. Genetic roles of the HPA axis in PTSD etiology

Abstract

Posttraumatic stress disorder (PTSD) is characterized by variable risk and resiliency, with a minority of individuals exposed to trauma subsequently developing diagnosable PTSD. Genetic variation may undergird this variable risk. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is commonly observed among individuals with PTSD and may underlie the etiology or symptoms of PTSD. As such, genetic variants of genes involved in the HPA axis constitute candidates for association with PTSD risk. The association between PTSD and 411 SNPs covering 21 HPA axis genes was tested using set-based association tests. We utilized genomic SNP data from a subset of the Detroit Neighborhood Health Study (DNHS), constituting 778 (trauma-exposed individuals 140 cases, 638 controls). Our analyses failed to provide evidence in support of association between HPA axis SNPs and PTSD risk. Although eight SNPs were nominally statistically significantly associated with PTSD, none passed correction for multiple hypothesis testing. Set-based tests were employed to test the significance of sets of HPA axis SNPs, by gene, however no evidence of association with PTSD was found at the set level. This study represents preliminary data on the association between PTSD risk and HPA axis genetic variation. Numerous study design issues may account for the negative results of this study. We discuss these explanations and provide recommendations for future studies.

Introduction

Post Traumatic Stress Disorder (PTSD) is an important medical issue (Kessler, 2000; Druss et al., 2009). In the United States, lifetime prevalence is estimated to be 6.8% in the general population (Kessler and Wang, 2008) and 15-40% in inner city and combat-veteran populations (Villagomez et al., 1995; Breslau et al., 2004; Horowitz et al., 2005; Schwartz et al., 2005). PTSD is associated with numerous comorbidities, including an increased risk of major depression (Breslau et al., 2000), substance dependence (Breslau et al., 2003), reduced life course opportunities (Kessler, 2000), and physical health problems (Farley and Patsalides, 2001; Simpson, 2002; Zayfert et al., 2002) including incident cardiovascular disease (Kessler, 2000; Kubzansky et al., 2007; Kubzansky and Koenen, 2009; Kubzansky et al., 2009). The total cost to the nation is an estimated $3.8 billion annually (Kessler, 2000). By definition, PTSD develops following exposure to a Potentially Traumatic Event (PTE) (Shalev, 2001), witnessed or experienced by the individual; this PTE must involve the threat or actuality of death, injury, or physical integrity; and must cause clinically significant distress or impairment in the individual's social interactions, capacity to work or other important areas of functioning(APA, 2013). Although a majority (50-90%) (Kessler et al., 1995) of Americans experience a PTE in their lifetime, only a minority develop PTSD (Kessler et al., 1995; Breslau et al., 1998; Acierno et al., 2007). Environmental factors at both individual and community levels, such as gender, race, educational attainment (Kulka RA, 1990; Brewin et al., 2000; Koenen et al., 2002; DiGrande et al., 2008; Galea et al., 2008; Kun et al., 2009), socioeconomic position (Kulka RA, 1990; Brewin et al., 2000; Koenen et al., 2002; Breslau et al., 2004; Koenen, 2007; DiGrande et al., 2008; Galea

et al., 2008; Kun et al., 2009), and growing up in a low-income neighborhood (Breslau et al., 1991; Koenen, 2007) have been associated with increased risk of PTSD. Whereas some of this increased risk may be accounted for by individuals' increased exposure to assaultive violence (Breslau et al., 1998; Breslau et al., 2004; Coulton et al., 2007; Melzer-Lange et al., 2007; Reyes et al., 2008; Gillespie et al., 2009; Obasaju et al., 2009) (the type of PTE with highest conditional risk of PTSD), genetic variables offer plausible biologic mechanisms to explain variable PTSD risk. Indeed, biological factors may account in part for variable PTSD risk (Galea et al., 2006). Risk factor models supported by meta-analytic studies explain only approximately 30-35% of the variance in PTSD (Yehuda, 2001).

As the primary neuroendocrine system controlling the mammalian stress response (Tsigos and Chrousos, 2002), genes involved in the regulation of the HPA axis offer strong candidates for loci involved in PTSD etiology. Perception of stressful stimuli causes the hypothalamic paraventricular nucleus to release corticotrophin-releasing hormone (CRH) and arginine vasopression (AVP) into the median eminence to synergistically trigger the secretion of adrenocorticotropic hormone (ACTH) by binding, respectively, the anterior pituitary corticotroph receptors CRH-R1 and V1B. ACTH in turn triggers the production and secretion of glucocorticoid stress hormones (GCs) – chiefly cortisol – from the adrenal cortex by binding melanocortin type2 receptors (MC2R) (Amweg et al., 2011). This interaction is facilitated by MC2R accessory protein (MRAP). By binding GC-receptors (NR3C1) expressed in tissues throughout the body, GCs mediate both the effects and, via negative feedback to the hypothalamus, the termination of the stress response. Also known as the "fight or flight"

mechanism, the stress response is initially adaptive; it mobilizes energy, increases vigilance and focus, facilitates memory formation, and depresses the immune response (Charney, 2004). However, failure to terminate the signal leads to the stress response becoming pathological.

Downstream effects of chronically elevated HPA axis activity can lead to detrimental consequences, including immune suppression and hippocampal atrophy (Pruessner et al., 2010; Golub et al., 2011). Particularly relevant here, stress hormones are implicated in the over-consolidation of memory in PTSD (Yehuda, 2001; Rasmusson et al., 2003; Claes, 2004; de Kloet et al., 2006), thus suggesting that variation in HPA axis genes may mediate a highly stress reactive phenotype vulnerable to HPA axis dysregulation in response to trauma and therefore to PTSD development (Kalin and Shelton, 2003). Indeed, aberrant functioning of the stress response is a characteristic of PTSD phenomenology, frequently marked by increased levels of CRH in CSF and decreased levels of plasma cortisol (Bremner et al., 1997; Baker et al., 1999; Martin et al., 2010). This observation, namely the disagreement between central and peripheral indicators of HPA axis activity, has led to the prediction that endocrine glands downstream of the hypothalamus are either downregulated, inhibited by GC negative feedback, or insensitive to the increased activity of stress response systems in the brain (Pace and Heim, 2011). At the level of the hypothalamus, GC sensitivity varies positively with GC and GC receptor concentration, and negatively with FKBP5 concentration. FKBP5 is a co-chaperone of the NR3C1 heterocomplex that regulates hormone binding affinity and translocation of the NR3C1 heterocomplex to the nucleus via an intracellular negative feedback loop. Several studies using dexamethasone

suppression tests (Heim et al., 2008; Yehuda et al., 2009) and measurements within circulating immune cells (de Kloet et al., 2007; Rohleder et al., 2010) have supported the hypothesis that PTSD patients may exhibit enhanced GC-sensitivity. At the level of the pituitary, decreased sensitivity may be due to either decreased concentration of CRH, decreased expression of CRH receptors, and/or increased concentrations of CRH-binding protein (CRH-BP). CRH-BP concentration is negatively correlated with HPA-axis activity due to its ability to bind and inactivate free CRH. Furthermore, studies have provided evidence that early life stress interacts with *FKBP5* SNPs (Binder et al., 2008) and CRH haplotype (Bradley et al., 2008) to modify risk of PTSD and depression, respectively. Finally, at the level of the adrenal cortex, responsivity to the stress signal is proportional to expression of melanocortin receptor type 2 (MC2R; receptor of ACTH) and MC2R accessory protein (MRAP). MRAP regulates trafficking and function of MC2R. As such, mutations of MRAP cause glucocorticoid deficiency (type 2).

It is well appreciated that PTSD risk is determined in part by genetic factors (Slater and Slater, 1944; Radant et al., 2001; Segman et al., 2005; Koenen, 2007; Koenen et al., 2008). Twin studies demonstrate that there is greater concordance of risk in monozygotic vs. dizygotic twins (True et al., 1993; Stein et al., 2002; Koenen et al., 2003), that PTSD is approximately 30% heritable (True et al., 1993; Stein et al., 2002), and that children of PTSD-affected parents are more likely to develop PTSD (True et al., 1993; Stein et al., 2002). Two studies have provided evidence for PTSD-related gene-by-environment interactions at four SNPs at the *FKBP5* locus (Binder et al., 2008; Xie et al., 2010). Studying an almost exclusively low-income African-American sample, Binder et al. provided evidence for an interaction between four *FKBP5* SNPs and child abuse,

such that genotype and early life trauma is predictive of PTSD symptom severity in later life (Binder et al., 2008). Likewise, Xie et al. provided evidence of a gene-by-environment interaction between a *FKBP5* SNP and childhood adversity on PTSD risk (Xie et al., 2010). This interaction was not significant for European-Americans (Xie et al., 2010), suggesting putative ethnic/race-specific gene-by-environment effects. Moreover, Bachmann et al. found that a negative correlation between basal cortisol levels and PTSD severity existed in a subset of PTSD-affected combat veterans with a specific genotype at a SNP at the NR3C1 locus (Bachmann et al., 2005). Finally, Lu et al. identified correlations between four *CNR1* SNPs and PTSD among white parents of youth with attention hyperactivity disorder (Lu et al., 2008).

Based on the above discussions and additional evidence (e.g. (Newport et al., 2003; Clark et al., 2008; de Kloet et al., 2008; Surget and Belzung, 2008; Carpenter et al., 2009; Kellner et al., 2010)) **we propose 21 HPA axis regulatory genes as candidate genes** for the study of association with PTSD risk (Table 7). Here, we examined the association of PTSD with individual SNPs as well as sets of SNPs, defined by gene.

Table 7. PTSD candidate genes, by level of HPA axis regulatory activity

Hypothalamus			Pituitary			Adrenal Cortex	Periphery	
AVP	*CRHR1*	*MC4R*	*CRH*	*CRHR2*	*POU1F1*	*MC2R*	*UCN2*	*NR3C2*
AVPR1A	*CRHR2*	*POMC*	*CRHBP*	*FKBP5*	*PROP1*	*MRAP*	*UCN3*	*CRHR1*
AVPR1B	*LEP*	*UCN2*	*CRHR1*	*NR3C1*	*TBX19*		*NR3C1*	*CRHR2*
CRH	*LEPR*	*UCN3*						

Materials and Methods

Sample Selection

The sample includes genotype data from a 778 individual subset of the Detroit Neighborhood Health Study (DNHS), an epidemiological, community-representative cohort of adult residents in Detroit, Michigan. The current study draws on survey data and genotype data derived from DNA isolated from peripheral blood samples. Among the samples, 140 have a history of PTSD and 638 have no history of PTSD. PTSD diagnosis was assessed via structured interview administered via telephone (Breslau et al., 1998). Lifetime PTSD cases met all six DSM-IV criteria in reference to either the worst or random traumatic event. The diagnostic interview showed good validity against the Clinician Administered PTSD Scale (Blake et al., 1995) as described elsewhere (Uddin et al., 2010). The Institutional Review Board of the University of Michigan reviewed and approved the study protocol. All individuals in this study had a history of trauma exposure. Thus, comparisons reported are between trauma-exposed individuals with and without PTSD. Participant demographic and PTSD status data is detailed in Table 8.

Genotype data

Genotype data were obtained for the 778 participants at approximately 733,000 loci using the HumanOmniExpress platform (Illumina). Annotation of the genotype data comes from the HumanOmniExpress_12v1_H dataset (Illumina), which utilized human genome build hg19. Imputed data was not utilized. Sequenced DNA was isolated from whole blood obtained via venipuncture from all study participants.

Candidate gene selection

21 genes (Table 7) involved in the regulation and signaling of the HPA axis were selected on the basis of evidence of involvement in the HPA axis as determined from a literature search and use of the gene network database, Pharmacogenomics Knowledge Base (PharmcoGKB) (Whirl-Carrillo et al., 2012) (Figure 8). The genes represent a diverse range of functions within the HPA axis, including hormones, hormone receptors, chaperones, targets and initiators of signaling. Using annotation data for the genotyping beadchip supplied by Illumina (HumanOmniExpress_12v1_H), 411 SNPs were identified that were annotated to the 21 HPA axis candidate genes. A breakdown of these SNPs, including SNP ID, refseq gene name, genomic coordinates, and in which part of the gene each SNP lies (e.g. intron, exon, promoter region), can be found in Supplemental Table S1.

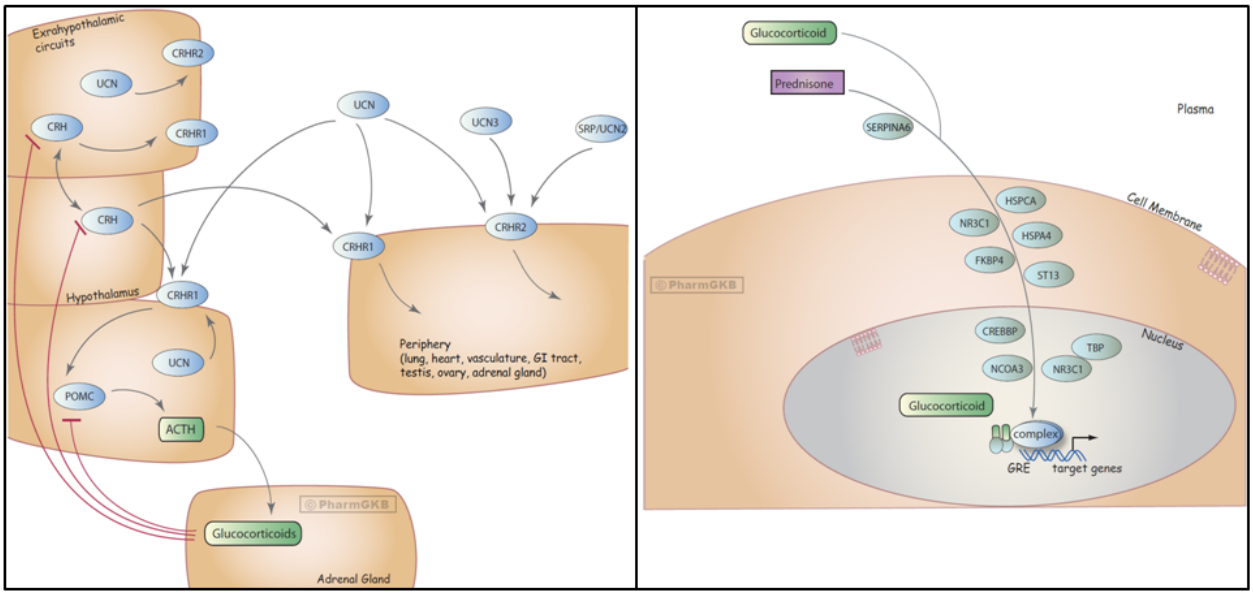

Figure 8. HPA axis candidate gene activity within the HPA axis (left panel) and the periphery (right panel). Figures used with permission from PharmaGKB (Whirl-Carrillo et al., 2012) and Stanford University. Copyright to PharmGKB.

Linkage disequilibrium, haplotype blocks, and tagging SNPs

For SNPs included in the study, Haploview (Broad Institute) (Barrett et al., 2005) was used to estimate linkage disequilibrium and identify haplotype blocks and tagging SNPs using population genetic data from the HapMap Project. Data representing Yoruba from Ibadan, Nigeria (YRI) was utilized. Haploview was employed using default settings.

Association tests

Association was tested between PTSD status and SNP genotype using a self-contained set-test, employed in PLINK. We tested for associations between PTSD status and individual SNPs as well as between PTSD status and sets of SNPs. Sets of SNPs tested were defined by gene. Detailed lists of each set tested can be found in Supplemental Table S2. A significance value of $p<0.05$ was utilized, with correction for multiple hypothesis testing being completed using a Bonferroni correction.

A secondary analysis was completed by which gene sets were parsed by genic context. Sets were parsed into a promoter/first exon set and a non-promoter/non-first exon set. Promoter regions were defined as 1kb 5' to the transcriptional start site.

Results

PTSD cases and controls differed with regard to age and gender (Table 8). PTSD cases have significantly higher post-trauma symptom severity than do controls (Table 8).

Table 8. Demographic and pre-trauma characteristics of 140 PTSD cases and 638 trauma-exposed controls.

	PTSD (N=140)	Control (N=638)	Chi-square (df)	p-value

	Mean/#	se/%	Mean/#	se/%		
Age (years)	51.1	1.18	54.6	0.67	2.28 (776)	0.023
Female	94	67%	353	55%	3.90(1)	0.048
Post-trauma symptom severity	57.41	1.17	28.10	0.62	20.46 (776)	<0.0001

HPA axis SNPs are not associated with PTSD risk

411 HPA axis SNPs were tested individually for association with PTSD status between trauma-exposed individuals with and without PTSD. 8 SNPs were nominally significant (Table 9), but did not withstand correction for multiple hypothesis testing.

Table 9. HPA axis SNPs nominally significantly associated with PTSD risk

Gene	SNP	Genomic Coordinates	Affected Frequency	Unaffected Frequency	CHISQ	P	OR
LEPR	rs17415296	1:66099013	0.040	0.080	5.505	0.01897	0.4744
FKBP5	rs16878806	6:35569119	0.207	0.151	5.469	0.01936	1.4750
NR3C2	rs17484063	4:149164905	0.022	0.056	5.428	0.01981	0.3799
NR3C2	rs17024437	4:149081808	0.182	0.134	4.419	0.03553	1.4440
NR3C2	rs11099681	4:149103454	0.072	0.114	4.211	0.04016	0.6036
MC2R	rs877128	18:13911628	0.300	0.243	3.953	0.04680	1.3350
LEP	rs17151922	7:127895216	0.179	0.233	3.891	0.04855	0.7166
NR3C1	rs6861962	5:142750301	0.096	0.141	3.878	0.04893	0.6528

Because no individual SNPs demonstrated a significant association with PTSD following correction for multiple hypothesis testing, set-based tests were employed to test for the significance of sets of SNPs. Neither a set composed of all HPA axis SNPs, nor individual gene based sets were significantly associated with PTSD risk (Table 10).

Table 10. HPA axis set-based association tests with PTSD risk.

SET	NSNP	NSIG	ISIG	EMP1	SNPS
AVP	3	0	0	1	NA
AVPR1A	8	0	0	1	NA
AVPR1B	13	0	0	1	NA
CRH	4	0	0	1	NA
CRHBP	8	0	0	1	NA
CRHR1	12	0	0	1	NA
CRHR2	9	0	0	1	NA
FKBP5	15	1	1	0.1379	rs16878806
LEP	18	1	1	0.5222	rs17151922
LEPR	57	1	1	0.317	rs17415296
MC2R	14	1	1	0.367	rs877128
MC4R	5	0	0	1	NA
MRAP	12	0	0	1	NA
NR3C1	97	1	1	0.6962	rs6861962
NR3C2	99	3	3	0.7594	rs17484063|rs17024437|rs11099681
POMC	5	0	0	1	NA
POU1F1	2	0	0	1	NA
PROP1	5	0	0	1	NA
TBX19	16	0	0	1	NA
UCN2	2	0	0	1	NA
UCN3	7	0	0	1	NA

NSNP, number of SNPs in gene-based set; NSIG, number of SNPs from set that are nominally significantly associated with PTSD risk; ISIG, number of SNPs from set that are nominally significantly associated with PTSD risk correcting for linkage disequilibrium-based independence; EMP1, empirically significance value; SNPS, identificantion of independent (no evidence of genetic linkage) SNPs from gene-based set that are nominally significantly associated with PTSD risk.

Three NR3C2 SNPs were nominally significant for association with PTSD (Table 10). Linkage disequilibrium between any or all of these SNPs may affect any set-based association tests which include them. Linkage disequilibrium analysis was thus employed to rule out this potential confounder. rs17024437 (chr4:149,081,808) and rs11099681 (chr4:149,103,454) are separated by approximately 20kb and thus were considered candidates for being affected by genetic linkage. However, upon

investigation, no evidence of linkage disequilibrium between these loci was found (Figure 9).

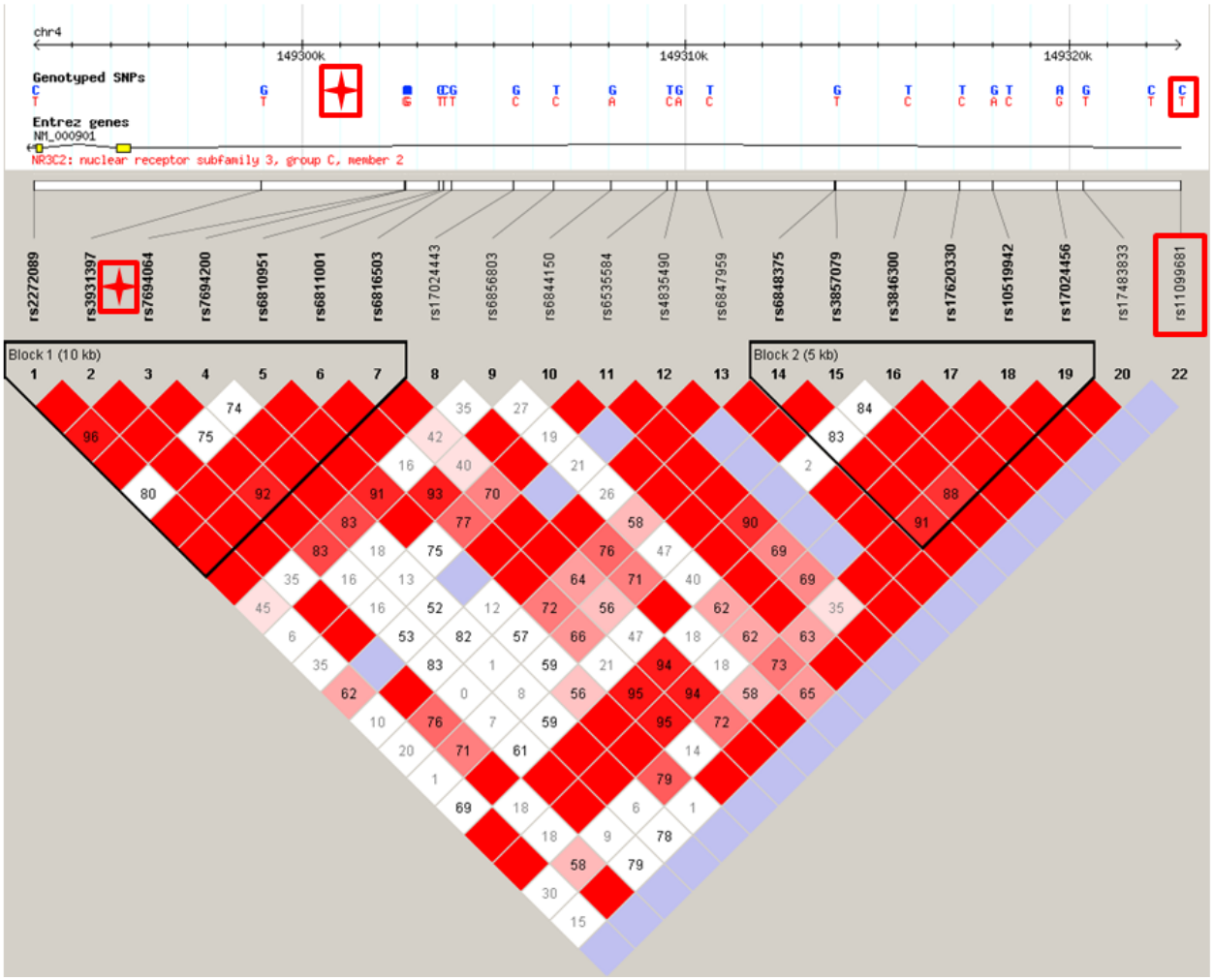

Figure 9. No linkage disequilibrium between *NR3C2* SNPs rs17024437 (chr4:149,081,808) and rs11099681 (chr4:149,103,454). rs11099681 is located in the red box to the right of the figure. rs17024437 was not included in the Haploview population data; it's relative genomic location is marked in the figure above by a boxed in star to the left of the figure. Figure created in Haploview 4.2, from the Broad Institute (Barrett et al., 2005).

Because evidence suggests that DNAm is associated with gene expression in ways that are contingent upon the context within which the CpG site of methylation is

located, because DNAm is highly associated with PTSD risk, and because DNAm patterns can be influenced by proximal genetic variation, we conducted a secondary analysis to explore whether genetic context influenced SNP association with PTSD risk. SNP sets were still defined by gene, but were parsed into separate “Promoter/First Exon” and “Non-Promoter/Non-First Exon” sets. Despite this, no additional significant findings resulted. The “Promoter/First Exon” sets contained no significant individual SNPs and no significant sets. The “Non-Promoter/Non-First Exon” sets contained no significant sets, including no substantial improvements upon non-parsed sets.

Discussion

Our data represents preliminary evidence that fails to support an association between genetic variation within HPA axis genes and risk for or resiliency to PTSD following exposure to trauma. Although eight SNPs were nominally significantly associated with PTSD, none withstood corrections for multiple hypothesis testing. Additionally, set-based tests that theoretically can demonstrate significance of sets even when the SNPs that compose them are not individually significant also failed to demonstrate significant associations with PTSD. While it is possible that there is indeed no association between PTSD risk and the SNPs tested, there are many reasons why this study design may have failed to detect a relationship between HPA axis genetic variation and PTSD risk, including: the effects of gender, trauma history, and childhood adversity; gene set selection that does not capture genetic diversity or fails to represent biologically significant processes.

Female gender (Kessler et al., 1995; Brewin et al., 2000; Tolin and Foa, 2006), increased trauma history (Kessler et al., 1995), and the presence of childhood adversity (Yehuda et al., 2010) are each associated with increased risk of PTSD. The primary analyses reported here did not include these potentially important covariates. Future work using this dataset may benefit from inclusion of these variables.

Composition of genes within sets would likely affect results of set-based tests, even if using identical data. The set-based tests employed here use data from multiple SNPs to estimate a set-based p-value. While the inclusion of more SNPs in this study increased the probability of identifying highly significant SNPs, doing so may have decreased the effective significance of any given set tested. The negative results from this study may be taken as evidence that more biologically nuanced sets should be tested. For example, limiting sets to SNPs found in regulatory regions or from genes directly involved in regulation may improve the power of these set-based tests. Indeed, many prior findings of significance between HPA axis genetic and epigenetic variants and PTSD risk have included those genes that have direct regulatory roles (e.g. FKBP5 and NR3C1).

A more nuanced definition of PTSD may be helpful in identifying effects of HPA axis gene variants. In this study we tested for the association between SNPs and PTSD risk, with PTSD risk defined as any history of PTSD. As PTSD diagnostic criteria is composed of three distinct categories of symptoms (hyperarousal, avoidance, and intrusive memories), it may be helpful to analyze the association between HPA axis genetic variants and individual symptoms types, separately. Indeed, it may make biological sense that dysregulation the HPA axis may be more associated with

symptoms of hyperarousal than of either of the other two symptom categories because of the general alignment between this category and the classically-understood fight or flight response. Another way in which PTSD definition may affect our results is the decision to analyze PTSD as a dichotomous outcome. Post-trauma symptom severity is a continuous measure of PTSD that has been alternatively utilized in the literature. Employment of this measure may give additional power to the association analyses, although an alternative method to set-based association tests would have to be employed.

Finally, one speculative interpretation of this data is that failure to achieve statistical significance may indicate a general conflation of chronic stress and acute stress. The HPA axis functions to regulate the stress response. As such, allostatic changes in the sensitivity of the system may be more increased following extended periods of stress and trauma opposed to a single traumatic event. It is possible that genetic variants affect the plasticity of the trauma response in such a way that a gene by environment effect may be at the heart of the association between the HPA axis and PTSD risk. In this way, certain genetic variants may be more susceptible to acute stress, but more resilient to chronic stress (or vice versa). Analyzing all cases of PTSD together, regardless of how chronic trauma is and number of traumatic exposures (e.g. inclusion of childhood adversity history) and ignoring other aspects of stress, may have prevented the elucidation of significant PTSD-HPA axis associations.

Chapter 5. Discussion

Outlook

In this project I have undertaken studies designed to elucidate the proximate and evolutionary underpinnings of PTSD, with an emphasis on the capacity for epigenetic regulation. I have utilized Tinbergen's Four Questions (Figure 2 from Chapter 1) guide the design of the research that makes up this dissertation, with the aim to provide proximate and evolutionary insights into the capacity to develop PTSD. In Chapter 2, I reported evidence that the risk of PTSD is associated with pre-trauma epigenetic variation and post-trauma epigenetic change at DNA methyltransferase genes in a study that examined DNA methylation change longitudinally in trauma exposed individuals with and without PTSD. That study shed light on the complex role of epigenetics on PTSD etiology and thus contributes to answering Tinbergen's Question #2: What is the ontogeny? In Chapter 3, I reported evidence for the deep evolutionary origins of PTSD epigenetic potential. That study demonstrated that the majority of genetic CpG sites associated with epigenetic regulation of PTSD (at which differential DNA methylation is associated with PTSD risk) are not unique to humans, but evolved prior to the divergence of humans and chimpanzees, with many having considerably more ancient origins. In that study I also characterized distinct periods of evolution throughout human descent by calculating evolutionary rates and identifying enriched functional annotation clusters of PTSD-associated CpG dinucleotide sites Chapter 3, therefore, contributes to answering Tinbergen's Question #4: What is the phylogeny? Finally, in Chapter 4, I reported preliminary data that failed to support involvement of genetic variation of HPA axis genes in differential risk and resiliency of PTSD. Despite

negative results, I noted in that chapter various reasons why they may not reflect the absence of a genetic involvement of HPA axis variation in PTSD risk and provided recommendations for future studies. Thus, Chapter 4 contributes to answering Tinbergen's Question #3: What is the mechanism? These three projects may seem to be conceptually very diverse, but in fact provide varying perspectives on the same process, that of epigenetic regulation of PTSD. In sum, I have presented evidence that it is important to look outside of genetics to identify biological risk factors for PTSD (Chapters 4, 2), that epigenetic variation is associated with PTSD risk in complex and dynamic ways both before and after trauma exposure (Chapter 2), and that the capacity to epigenetically regulate PTSD has deep evolutionary origins with functional implications (Chapter 3). These studies combine to paint a picture of PTSD as being mediated by an epigenetically-regulated developmental process the capacity for which is ancient and conserved. There are various ways in which this empirical evidence could be understood and synthesized. Here, I approach PTSD as the extreme end of a trauma response reaction norm that is the product of a capacity for developmental plasticity that is evolutionarily conserved and mediated by ongoing epigenetic modifications that respond to environmental stimuli. As such, this discussion will synthesize the research detailed in Chapters 2-4 in order to contribute to the answer to Tinbergen's Question #4: What is the adaptation? Understanding how this perspective is novel first requires a survey of the biological perspectives of PTSD that currently predominate.

PTSD's epistemic issues

Although important work has been done on the genealogy of the biological understanding of trauma(Young, 1995) and the ways in which this understanding structures nosology, research, and policy (Kendler KS, 2012), the epistemology of PTSD is often ignored by biologists working in fields related to traumatic stress studies. Instead, a common epistemological narrative has emerged both popularly and within the scientific literature that understands PTSD to be both wound and disorder. This is unique among mental health conditions defined by the DSM, as all other DSM codifications are understood to be disorders. The acceptance of PTSD as both disorder and wound is understandable given the lack of empirical data to resolve the nature of PTSD and a need for a simple model upon which biological hypotheses can be built and tested, but doing so may prevent the advancement of a more complete biological theory of PTSD, as it precludes thinking about PTSD as a potentially adaptive trait, presumes disorder and wound to be necessarily separate entities, and reduces PTSD etiology to a simple interaction that can be localized in space and time. Our interpretations of empirical data are shaped and constrained by the epistemology we accept and while demystifying the biological nature of PTSD at one turn, the acceptance of this narrative of PTSD potentially remystifies the problem anew at the next.

My intention here is not to resolve the question of the fundamental nature of PTSD. As stated earlier, there is not at present enough empirical data to resolve even the most basic of problems regarding whether PTSD is wound, disorder, both, or something else entirely. What I would like to propose, however, is that thinking about PTSD as an adaptive and evolutionarily conserved trait that is regulated by epigenetic

potential allows for a novel interpretation of PTSD molecular data that spans ontological scales from individual development to phylogenetic evolution and that has practical implications for both research and clinical practice.

PTSD as wound and disorder

What is the basic understanding of PTSD and how does it structure our understanding of empirical data? Diagnostically, PTSD is composed of two fundamental elements – exposure to a traumatic event and the subsequent development of post-trauma symptoms (hyperarousal, intrusive memories, and avoidance) (APA, 1994; Association, 2013). Criterion A of DSM-IV PTSD diagnostic criteria requires *"exposure to a traumatic event in which the person (1) experienced, witnessed, or was confronted by death or serious injury to self or others AND (2) responded with intense fear, helplessness, or horror"* (APA, 1994). As such, Criterion A establishes PTSD as a wound such that the condition of having PTSD is contingent upon first having an experience that exists outside of one's own biology. PTSD is caused, therefore, by something external. It should be noted that PTSD is unique among DSM psychopathologies in requiring a precipitating event (APA, 1994; Lopez-Ibor, 2002; Association, 2013). The upshot is that PTSD is understood to be a wound, roughly analogous to that caused by a gunshot (for example, see (Committee on the Assessment of Ongoing Effects in the Treatment of Posttraumatic Stress and Institute of, 2012).

But a gunshot injures all individuals in roughly the same way, whereas traumatic experiences affect people in dramatically different ways. Although a majority of

Americans experience a potentially traumatic event in their lifetime (Norris, 1992; Resnick et al., 1993; Kessler et al., 1995; Breslau et al., 1998), only a minority subsequently develop PTSD (Kessler et al., 1995; Breslau et al., 1998; Acierno et al., 2007). Additionally, those who experience even those traumatic events which carry the highest conditional risk for PTSD (among civilians, the conditional risk of PTSD following rape is 65% in males and 46% in females (Kessler et al., 1995)) are not guaranteed to develop PTSD. The development of PTSD, therefore, remains rare despite the high frequency of traumatic experiences. Is PTSD, then, more correctly seen as a disorder, analogous to all of the other psychopathologies codified within the DSM?

The disorder model understands PTSD to be the result of something gone wrong within an individual as a susceptibility only to be revealed upon exposure to a traumatic experience. As mentioned above, all other mental health diagnoses roughly take this form. Although we may point to environmental factors that influence the etiology of depression, schizophrenia, and autism, to name just a few, these conditions are ultimately understood to be caused by something innately different between those who suffer from them and those who do not. Hypotheses about the disordered nature of PTSD predict the ability to locate the disorder that confers PTSD risk in everything from culturally-determined psychology, such as perceived discrimination and religious involvement (Scrimin et al., 2014), to brain form and function (Li et al., 2014), to genetic variants (Guffanti et al., 2013; Logue et al., 2013; Solovieff et al., 2014).

Combining these models of PTSD we arrive at the typical understanding of PTSD today – PTSD is the result of the interaction between a trauma induced wound and a preexisting, risk-conferring disorder.

In the remainder of this chapter I will start by describing this general conception of PTSD – as depicted by the diathesis-stress model. I will describe the ways in which a focus on epigenetics has changed the thinking about PTSD in such a way as to biologically unify the "wounds" of traumatic experience and risk-conferring "disorder." I will relate how this has given rise to a more nuanced life course model of PTSD. Drawing from the developmental origins of health and disease literature, I will then propose an alternative model of PTSD – as a phenotypic range within a trauma response reaction norm that arises out of an epigenetically-regulated developmental plasticity that is conserved through evolutionary history. Having described this perspective, I will contrast it with other evolutionary perspectives on PTSD and attempt to incorporate into this model my own research, as described in detail in Chapters 2-4, to demonstrate how this model can incorporate thinking about PTSD on multiple levels. Finally, I will provide a brief synopsis on how this perspective may influence research, clinical practice, and policy.

From diathesis-stress to an epigenetic life course model

The diathesis stress model of PTSD argues that differential risk of PTSD is the result of the interaction between external trauma (stress) and pre-existing susceptibility factors (diathesis) (Yehuda et al., 2011; Mehta and Binder, 2012). This model has informed much of the important research on PTSD in recent years and has primarily led to the

attempt to identify risk and resiliency factors as biological correlates of the psychiatric symptoms of PTSD. Specifically, this research has led to the identification of numerous biological risk factors as well as gene by environment interactions that are associated with PTSD risk. For example, studying an almost exclusively low-income African-American sample, Binder and colleagues provided evidence for an interaction between four *FKBP5* SNPs and child abuse, such that genotype and early life trauma was found to be predictive of PTSD symptom severity in later life (Binder et al., 2008); Xie and colleagues also provided evidence of a gene-by-environment interaction between a *FKBP5* SNP and childhood adversity, although this interaction was not significant for European-Americans (Xie et al., 2010). Bachmann and colleagues reported a negative correlation between basal cortisol levels and PTSD severity in a subset of PTSD-affected combat veterans with a specific *NR3C1* SNP genotype (Bachmann et al., 2005). And Lu and colleagues identified correlations between four *CNR1* SNPs and PTSD among white parents of youth with attention hyperactivity disorder(Lu et al., 2008). Within the realm of neuroanatomy, morphological abnormalities have been linked to PTSD in various forms, including a volumetric reduction in cerebral gray matter and hippocampal volume (Li et al., 2014), and functional and connective differences in numerous central nervous system regions, including: the amygdala, insula, and prefrontal lobe (Jin et al., 2013).

While much of this research has centered on identifying genetic and neuroanatomical risk factors and the interaction between these risk factors and environmental trauma exposure, evidence of involvement of epigenetics has revolutionized the research program by providing a putative mechanism to unite stress

and diathesis (Meaney, 2010). In short, because epigenetic factors are stable yet modifiable in response to lived experience, they potentially can function as both disorder and wound, as both diathesis and stress.

Indeed, recent work in the field of epigenetic psychiatry has identified epigenetic modifications that affect an individual's response to trauma and others that are induced by trauma. The first indication of this role of epigenetic variation came from the foundational work by Michael Meaney and colleagues who demonstrated that epigenetic modifications are capable of being induced by early life experiences and persisting into adulthood to modify behavior and physiology. Most famously, rat pups that received high levels of maternal care during early postnatal life, as measured by the amount of received licking and grooming and arched-back nursing, as adults displayed more mild physiological responses to stress and higher maternal care behaviors themselves compared to pups that received low levels of maternal care (Weaver et al., 2004). These effects were found to be mediated by an epigenetic modification, induced by exposure to early childhood to licking, grooming and arched-back nursing, of the gene that codes for the glucocorticoid receptor *Nuclear Receptor Subfamily 3, Group C, Member 1* (*NR3C1)*. Pups who received high degrees of maternal care had reduced levels of hippocampal DNA methylation and increased gene expression of *NR3C1* glucocorticoid receptor (Weaver et al., 2004). Importantly, *NR3C1* is a modulator of the stress response and an integral component of the hypothalamic-pituitary-adrenal (HPA) axis. Indeed, these epigenetic changes were found to be concordant with differences in adult HPA axis responses to stressful stimuli, as measured by circulating corticosterone following restraint stress. This research demonstrated that lived experiences could lead

to epigenetic modifications capable of modulating behavior and biology and stable through time. Follow up work built on these findings by identifying similar epigenetic effects in other genes, including *BDNF* (Roth et al., 2009) and *estrogen receptor-alpha1b* (Champagne et al., 2006). Further research has yielded yet more evidence of the role of epigenetics in mental health, generally, and PTSD, specifically, by expanding this foundational work into humans.

As in mice, stress and trauma experienced early in life has epigenetic effects that last into adulthood in humans. Among suicide victims, increased *NR3C1* methylation was observed in the brains of suicide victims with a history of childhood abuse, compared to suicide victims without a history of childhood abuse. The association between increased DNA methylation at *NR3C1* loci and early life exposures to stress and trauma has been demonstrated in additional studies. Increased methylation of a *NGFI-A* binding site at a *NR3C1* locus while in cord blood was found to be associated with maternal mood, with more active stress responses measured via salivary cortisol at 3 months postnatally (Oberlander et al., 2008). Additionally, increased *NR3C1* DNA methylation was observed in whole blood samples from individuals with a history of childhood adversity (Tyrka et al., 2012). In addition to focused candidate gene studies, epigenetic differences associated with exposure to early life stress have been identified – Compared with adolescents whose mothers were unexposed to high stress levels during their first year of life, adolescents whose mothers reported high stress levels during their infancy showed higher DNAm levels at 139 CpG sites in buccal cell-derived DNA (Essex et al., 2013). Together, these data from animal and human studies suggest that early life experiences, and especially adverse early life experiences, have the

potential to alter epigenetic gene regulation and downstream gene function with lasting physiological, behavioural, and psychological implications (Meaney, 2010; Szyf, 2011).

By constituting both disorder and wound, epigenetic variation and modifications provides a core around which a coherent life course approach to PTSD can be constructed. A, perhaps oversimplified, description of such an approach, emphasizing the role of epigenetics, would locate both risk factors that pre-exist trauma ("disorders") and trauma-induced risk factors ("wound") in epigenetic variation. Doing so collapses false dichotomies of diathesis vs. stress, nature vs. nurture, gene vs. environment, and biology vs. society/culture on to the mediating effects of epigenetics; the effect of each seemingly disparate input affects the output of mental health via epigenetic states and modifications. In this way, an individual at greater risk of PTSD is "disordered" because of certain epigenetic variation and is "wounded" by trauma by the induction of further epigenetic variation, all of which leads to more global effects physiology and behavior via changes in gene expression and transcription factor binding.

Additionally, a life course approach creates a more nuanced understanding of the role of environmental trauma exposures on the etiology of PTSD. Despite diagnostic criteria (APA, 1994), research (Hong et al., 2014), and treatment (e.g. exposure therapy) that often centers on a single traumatic experience as the precipitating event of subsequent PTSD development, it is clear that the biological reality of PTSD is more complex; the etiology of PTSD is influenced by the entirety of lived experience. Indeed, evidence suggests that the number of traumatic experiences interacts with genetic variation to affect risk of PTSD (Koenen et al., 2011; Uddin et al., 2011a), risk of PTSD increases with each traumatic experience, and one of the greatest risk factors for the

development of adult PTSD is childhood adversity even when childhood adversity does not itself induce PTSD (LeardMann et al., 2010). Furthermore, many individuals develop PTSD following several traumatic event exposures, with symptoms that don't track cleanly onto any single experience (Cloitre et al., 2009). Finally, numerous social factors exist that increase the risk of PTSD, independent of any association with increased exposure to traumatic exposure, including: perceived discrimination, lower religious involvement, and less social support (Scrimin et al., 2014). These observations are putatively explained using this life course approach by the induction of epigenetic modifications of lived experiences that, in turn, increase the risk of PTSD in response to future traumatic exposures.

An epigenetic life course approach to PTSD considers the ongoing interaction between phenotype and environment, as opposed to a single interaction (Uddin and Sipahi, 2013). As such, PTSD as a phenotype arises out of a series of epigenetically-mediated phenotype-by-environment interactions (Figure 10; (Uddin and Sipahi, 2013)).

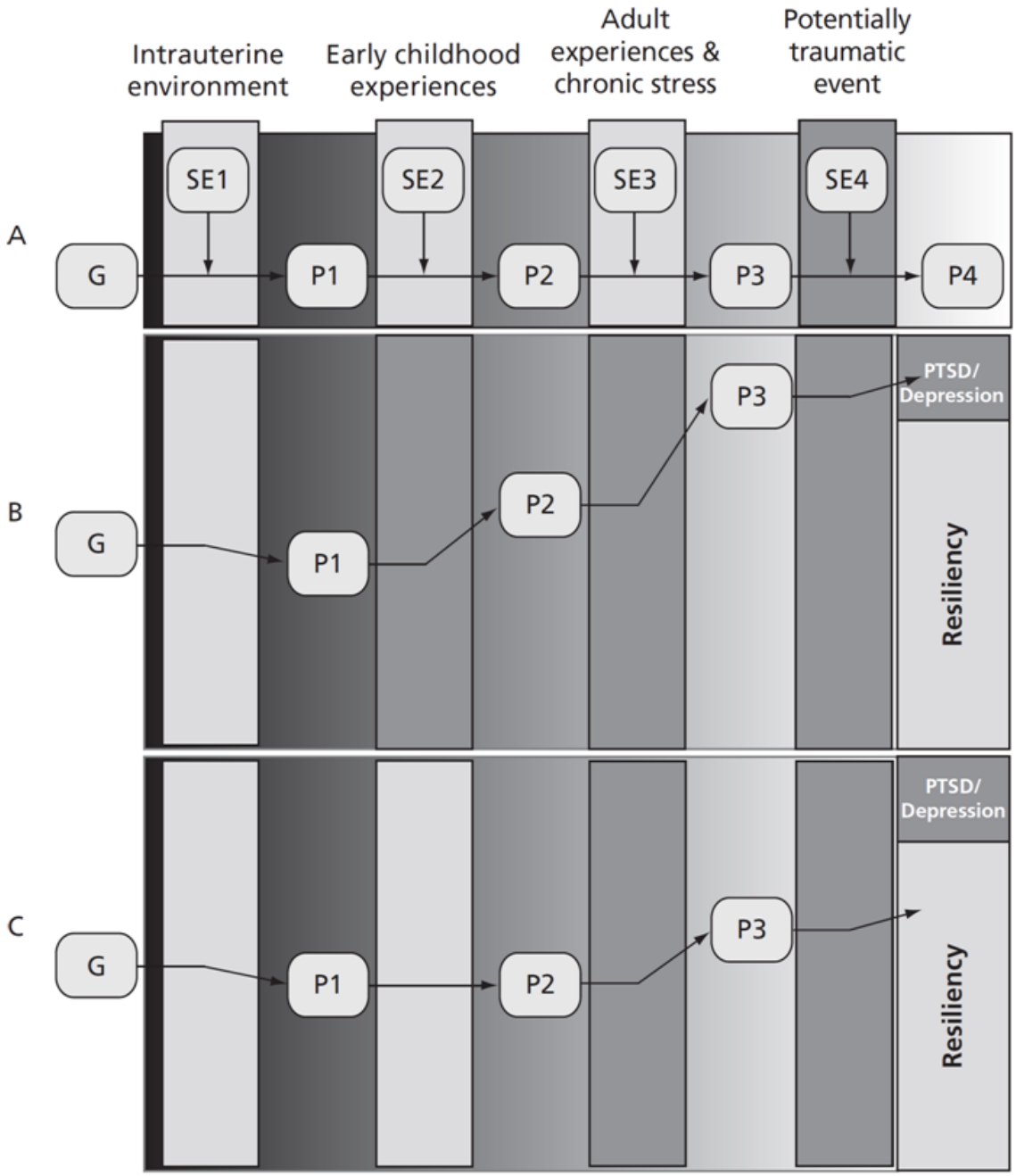

Figure 10. A life course model of PTSD. (A) The etiology of posttraumatic stress disorder (PTSD) is contingent upon a series of developmental responses, mediated by epigenetic modifications, to lifetime social experiences. Two potential scenarios are illustrated in panels B and C. (B) Adverse childhood and adult experiences compound to increase risk of mental illness. Arrows represent epigenetically mediated developmental responses to social exposures. (C) Absence of childhood adversity allows for resiliency in the face of traumatic experiences in adulthood. In the social exposure (SE) columns, background color gradient represents developmental plasticity, with darker background indicating greater developmental plasticity and thus likely greater influence over future health. G, underlying inherited genotype.

It is important to note that while both my description here and the accompanying figure each present the etiology of PTSD as arising out of a series of distinct events or timeframes, it is perhaps more accurate to consider PTSD as the product of a long developmental process that may be marked by events of greater or lesser significance, but as a whole is neither disjointed nor discrete. We can envision, for example, that experiences of everyday racism and sexism, chronic stress over a lack or loss of life course opportunities, and trouble integrating within stable social networks, to name but a few contributing factors, each are translated into increased risk of PTSD via subtle, ongoing epigenetic modifications.

Indeed, the research presented in Chapter 3 supports a life course approach to PTSD. In that study, risk-associated biological differences were observed in the form of pre-trauma epigenetic risk factors and post-trauma DNA methylation modifications. Importantly, we observed some changes in DNA methylation from pre- to post-trauma time points that were unique to individuals who developed PTSD and others which were shared by cases and controls alike (Sipahi et al., 2014). This suggests that trauma induces epigenetic changes in a predictable way regardless of whether PTSD subsequently develops. This may represent an empirical example of the type of events displayed in Figure 10. While that research controlled for the number of trauma exposure types, future research that took prior traumatic experiences into account in more nuanced ways may provide insight into the etiology of PTSD as the product of a lifelong developmental process. Additionally, while it is possible that the pre-trauma epigenetic risk factors identified in Chapter 3 may have been inherited or otherwise independent of lived experience, this observation is also consistent with the prediction

that these are the products of previous lived experiences, previous epigenetically regulated phenotype by environment interactions. Finally, while we only studied a limited number of loci – 15 total CpG dinucleotide sites over 4 DNMT genes – we predict that similar dynamic effects are at play at other epigenetic loci. Uddin and colleagues previously identified over 600 differentially methylated CpG sites across the genome in association with PTSD risk (Uddin et al., 2010). How many of these are dynamically regulated in response to traumatic experiences, how many occupy biologically relevant roles and thus confer risk or resiliency, and how many are reliable biomarkers of PTSD risk or state are questions that remain to be elucidated via future research, but that may be informed by a life course approach.

Developmental plasticity and developmental origins of health and disease

We have seen that stressful and traumatic life experiences are translated into biological consequences by epigenetic modifications. That this happens predictably and in ways that in turn modulate future responses to stressful and traumatic experiences in a form that approximates a norm of reaction suggests that these changes may be functional. It is difficult, yet important, to distinguish between 1) the phenotypic results of a developmental program that is passively shaped by environmental inputs and 2) the phenotypic results of a developmental program that actively and functionally responds to environment inputs. This is a subtle difference, but one that carries tremendous implications for understanding PTSD and thus worth teasing apart. The distinction essentially can be reduced to whether a given example of phenotypic plasticity is adaptive or nonadaptive. In the case of PTSD, if we take for granted that the biological

trauma response is plastic in response to lived traumatic exposures, the question becomes whether this represents an active or passive form of plasticity.

A clear articulation of this distinction comes from the examination of the effect of mechanical forces on the developing organism by Moore (Moore, 2003), who has identified four functions of the mechanical environment on the developing organism: 1) the mechanical environment can alter development via the inherent developmental plasticity of the organism; 2) the mechanical environment can provide information used by the developmental process itself; 3) the mechanical environment can provide a selection pressure; and 4) the mechanical environment can be itself changed by the developing organism. Items (3) and (4) relate to broader evolutionary questions, so I will focus, for the time being, on (1) and (2), which correspond to the numbered distinctions on the previous page. Function (1), passive plasticity, can be seen in many examples: enzymatic responses to pH and temperature changes (Pavasovic et al., 2004), decreased growth rate in response to low nutrient availability (Larque et al., 2013), and growth shape of coral molded by wave forces (Dollar, 1982). In contrast, classic examples of scenario (2), active plasticity, include plasticity that results in distinct polyphenisms, such as solitary and gregarious locust phenotypes (Applebaum and Heifetz, 1999), heterophylly in semiaquatic plants (Wells and Pigliucci, 2000), and morphology, reproductive strategy, and behavior of horned beetles (Valena and Moczek, 2012), to name but a few. Although most easily understood as plasticity that is responsible for polyphenic traits, active plasticity can also result in less distinct phenotypic forms that approximate norms of reactions, such as the development of the adaptive immune system (Galli et al., 2011). In addition, more complex syndromes,

composed of expansive complements of traits, have been predicted to represent the result of an adaptive form of phenotypic plasticity – for example, the thrifty phenotype and thrifty epigenotype theories of metabolic syndrome (Hales and Barker, 1992; Bateson and Gluckman, 2011). From this perspective, metabolic syndrome is the result of a plastic developmental process that responds to cues from the environment. Low nutrient availability in utero is hypothesized to initiate a developmental trajectory, mediated by epigenetic modifications, towards a thriftier phenotype (Barker et al., 1989; Waterland and Michels, 2007; Wadhwa et al., 2009). Such a phenotype is thought to be evolutionarily advantageous if the in utero cues accurately represent and predict the future environmental reality – namely, nutrient availability.

Can we conceive of a similar process leading to the etiology of PTSD? Similar to metabolic disorder, PTSD is characterized by phenotypic plasticity in response to environmental cues – past trauma may predict future trauma (Tyler and Johnson, 2006; Klest, 2012). Also, similar to metabolic syndrome, PTSD seems to be a disorder in so far as the phenotype does not appropriately match the environment. However, in contrast to metabolic disorder, which is defined in relation to a largely stable environment (in the western world, high nutrient availability is a fairly consistent reality), PTSD is defined in relation to a much more unstable environment.

It is illustrative to consider when PTSD becomes a disorder. If we hypothesize that the symptoms of PTSD (hyperarousal, avoidance, emotional numbing, and intrusive memories) are effective in leading to decreased exposure to future traumatic events, then it becomes clear that there are no clear delimiting boundaries for disease onset. Take for instance, a soldier who develops PTSD following exposure to combat. Is PTSD

a disorder if it prevents her from future exposure to life-threatening trauma? Is it a disorder as soon as she leaves the combat zone and returns to the relative safety of home? If she is then redeployed does her PTSD again cease to be a disorder? The case of a military veteran with multiple deployments may be an extreme example of dynamic environmental change, but it is also true for many civilians who face changing interpersonal relationships and a more difficult time escaping from the types of situations that may relate to past traumatic exposures.

It is difficult to know when tradeoffs such as developmental plasticity are evolutionarily adaptive. Likewise, it is unclear if the capacity to develop symptoms of PTSD in the face of trauma is adaptive. But, by combining the life course model of PTSD etiology described earlier with the proximate ontological evidence from Chapter 2 and the phylogenetic evidence from Chapter 3, it becomes plausible that the capacity to develop PTSD via active plasticity is adaptive.

An evolutionary perspective

Interest in the relationship between evolution and epigenetics has recently seen a resurgence, although it is not a new field. Theoreticians are revisiting and reinterpreting work by Waddington, Baldwin, and others in order to elucidate the role epigenetics can play in driving evolution (West-Eberhard, 2003; Pigliucci, 2010). The relationship between evolution and epigenetics is bidirectional – epigenetics influences and, perhaps, drives evolution and evolution shapes epigenetic processes (Baldwin, 1896; 1897; Badyaev, 2009; Feinberg and Irizarry, 2010). My own research has focused on the latter of this relationship.

Evolutionary theories about PTSD tend to occupy three camps. In the first, PTSD is seen to be the result of normal biological processes gone awry. The stress-diathesis model, when considering evolution, largely falls into this camp. In addition to the basic premises of the stress-diathesis model, the language utilized is revealing of its evolutionary theoretical underpinnings; as a representative example, (Ehlert, 2013) refers to certain responses to traumatic experiences, including PTSD, as *"severe maladjustment with co-occurring psychiatric and physical pathologies."*. In the second, PTSD is seen as an unfortunate evolutionary trade-off; for example, the biological sensitivity to context theory hypothesizes that individuals who are more susceptible to negative health effects following adversity (e.g. increased risk of PTSD) also have more potential to benefit from positive conditions (Boyce and Ellis, 2005; Ellis et al., 2011). As such, this theory hypothesizes that the development of PTSD in response to trauma is evolutionarily disadvantageous, but the very neurodevelopmental processes increase fitness in more "positive" social conditions. Finally, in the third, PTSD is seen as a mismatch between phenotype and environment such that the development of PTSD in response to traumas may have once been evolutionarily advantageous, but not longer makes sense (Silove, 1998)

Implications for research, clinical practice, and policy

What is most restricting about the prevailing model of PTSD is the assumption of negativity. Wounds are bad and disorders are bad, necessarily, and the assumption of negativity precludes thinking about PTSD as adaptive or beneficial in any way. The stress-diathesis model attempts to be valueless, yet what constitutes stress is largely

informed by assumptions that arise primarily from cultural norms. Our theories about PTSD, specifically, and mental health, generally, tend to conflate cultural understandings of ordered vs. disordered thinking, biological understandings of function and dysfunction, and evolutionary understandings of adaptation and maladaptation. We take for granted that PTSD is a disorder and a wound at all levels, from molecular to evolutionary to societal. Theories of PTSD that are informed by an evolutionary perspective tend to recognize the need to differentiate between cultural norms, biological dysfunction, and mismatches between phenotype and environment. In this way they are improvements upon theories that ignore evolutionary insights. However, at their core, they still operate on the assumption that PTSD is "bad". Perhaps this is true. I in no way mean to suggest that PTSD is somehow a preferable state, nor that it shouldn't be treated. PTSD is real and it is a medical and social issue. However, I would like to suggest that PTSD is neither disorder nor wound, in that PTSD represents the product of a functional developmental program that responds to lived traumatic experiences. The upshot of this perspective is that the responsibility of PTSD falls upon a society that creates the conditions for trauma, in the first instance. If PTSD arises in individuals that are responding appropriately, in an evolutionary sense, to traumatic exposures, then it becomes difficult to rationalize the focus of treatment, prevention, and policy being placed on individuals with PTSD. There are few examples of targeting a functional system for medical treatment. As an analogy, although we medically treat a fever if it is life threatening, we recognize that a fever is an evolved, adaptive response to infection. As such, we tend not to treat a fever, per se, but focus our intention on the

actual cause of medical concern. The infection is the real problem and, appropriately, we focus our efforts to prevent, treat, and limit the spread of the infectious agent.

We ought to take a similar approach with PTSD. While understanding that PTSD is rooted in biology, treatment and prevention of PTSD may best be accomplished by pursuing policy and social reforms designed to reduce the etiological agent of PTSD, which ultimately is trauma itself.

APPENDIX

Supplemental Table 1. All evolved PTSD-associated CpG dinucleotides (7202)

Name	Symbol	GeneID	Accession	node_change	PTSD-association
cg06648029	NR1I2	8856	NM_022002.1	node_13_human	Uniquely Methylated, Affected
cg10125195	LACRT	90070	NM_033277.1	node_13_human	Uniquely Methylated, Affected
cg13397379	OR2C3	81472	NM_198074.3	node_13_human	Uniquely Methylated, Affected
cg17108383	PCDHGC5	56097	NM_018929.2	node_13_human	Uniquely Methylated, Affected
cg18809535	LDHAL6B	92483	NM_033195.1	node_13_human	Uniquely Methylated, Affected
cg24172553	FLJ25660	148109	NM_152481.1	node_13_human	Uniquely Methylated, Affected
cg27303882	PAGE2	203569	NM_207339.1	node_13_human	Uniquely Methylated, Affected
cg27318281	C18orf37	125476	NM_194281.2	node_13_human	Uniquely Methylated, Affected
cg27412902	IL29	282618	NM_172140.1	node_13_human	Uniquely Methylated, Affected
cg01091565	MESP1	55897	NM_018670.1	node_14_node_13	Uniquely Methylated, Affected
cg01558777	C10orf99	387695	NM_207373.1	node_14_node_13	Uniquely Methylated, Affected
cg01770400	SERPINC1	462	NM_000488.2	node_14_node_13	Uniquely Methylated, Affected
cg06084117	PLXNA4B	91584	NM_181775.2	node_14_node_13	Uniquely Methylated, Affected
cg06445611	GABRR2	2570	NM_002043.1	node_14_node_13	Uniquely Methylated, Affected
cg08256781	ACSBG2	81616	NM_030924.2	node_14_node_13	Uniquely Methylated, Affected
cg09950370	GFRA2	2675	NM_001495.4	node_14_node_13	Uniquely Methylated, Affected
cg10134939	FLJ13391	84141	NM_032181.1	node_14_node_13	Uniquely Methylated, Affected
cg11204562	C10orf81	79949	NM_024889.3	node_14_node_13	Uniquely Methylated, Affected
cg12177001	IFI27	3429	NM_005532.3	node_14_node_13	Uniquely Methylated, Affected
cg18129786	ZNF445	353274	NM_181489.4	node_14_node_13	Uniquely Methylated, Affected
cg18330203	TNNT2	7139	NM_000364.2	node_14_node_13	Uniquely Methylated, Affected
cg18790143	OTOS	150677	NM_148961.3	node_14_node_13	Uniquely Methylated, Affected
cg20732137	GMEB2	26205	NM_012384.2	node_14_node_13	Uniquely Methylated, Affected
cg21695020	DPEP3	64180	NM_022357.1	node_14_node_13	Uniquely Methylated, Affected
cg23654219	TCF19	6941	NM_007109.1	node_14_node_13	Uniquely Methylated, Affected
cg24505375	AMAC1L2	83650	NM_054028.1	node_14_node_13	Uniquely Methylated, Affected
cg25729716	CD79B	974	NM_000626.1	node_14_node_13	Uniquely Methylated, Affected
cg02044879	PLA2G12B	84647	NM_032562.2	node_15_node_14	Uniquely Methylated, Affected
cg03570766	CATSPER1	117144	NM_053054.2	node_15_node_14	Uniquely Methylated, Affected
cg04587829	FN3K	64122	NM_022158.2	node_15_node_14	Uniquely Methylated, Affected
cg05244766	GSTP1	2950	NM_000852.2	node_15_node_14	Uniquely Methylated, Affected
cg10498097	MGC50811	375307	NM_198559.1	node_15_node_14	Uniquely Methylated, Affected
cg11148307	C10orf92	54777	NM_017609.2	node_15_node_14	Uniquely Methylated, Affected
cg18271969	HTR3C	170572	NM_130770.2	node_15_node_14	Uniquely Methylated, Affected
cg21596858	DCST2	127579	NM_144622.1	node_15_node_14	Uniquely Methylated, Affected
cg00962459	PROKR1	10887	NM_138964.2	node_15_node_14	Uniquely Methylated, Affected
cg02629257	EPPB9	27077	NM_015681.2	node_15_node_14	Uniquely Methylated, Affected

cg02854090	HIST1H2AA	221613	NM_170745.3	node_15_node_14	Uniquely Methylated, Affected
cg08025786	CLK3	1198	NM_003992.1	node_15_node_14	Uniquely Methylated, Affected
cg09174741	THSD1	55901	NM_018676.2	node_15_node_14	Uniquely Methylated, Affected
cg16242770	KRTAP17-1	83902	NM_031964.1	node_15_node_14	Uniquely Methylated, Affected
cg18034859	MYLK2	85366	NM_033118.2	node_15_node_14	Uniquely Methylated, Affected
cg18992201	DPPA2	151871	NM_138815.2	node_15_node_14	Uniquely Methylated, Affected
cg24751129	GNMT	27232	NM_018960.4	node_15_node_14	Uniquely Methylated, Affected
cg25833031	PAPD1	55149	NM_018109.2	node_15_node_14	Uniquely Methylated, Affected
cg26687173	LOC126248	126248	NM_173479.2	node_15_node_14	Uniquely Methylated, Affected
cg27077685	SLC7A6OS	84138	NM_032178.1	node_15_node_14	Uniquely Methylated, Affected
cg00512031	CYTL1	54360	NM_018659.2	node_17_node_15	Uniquely Methylated, Affected
cg02009694	C9orf132	399665	NM_203305.1	node_17_node_15	Uniquely Methylated, Affected
cg02254461	AXUD1	64651	NM_033027.2	node_17_node_15	Uniquely Methylated, Affected
cg03662459	IL11RA	3590	NM_004512.3	node_17_node_15	Uniquely Methylated, Affected
cg03870261	TIMM13	26517	NM_012458.2	node_17_node_15	Uniquely Methylated, Affected
cg03986640	MIP	4284	NM_012064.2	node_17_node_15	Uniquely Methylated, Affected
cg04033774	GPSM2	29899	NM_013296.3	node_17_node_15	Uniquely Methylated, Affected
cg05958352	RNASE1	6035	NM_198232.1	node_17_node_15	Uniquely Methylated, Affected
cg06308323	C9orf25	203259	NM_147202.1	node_17_node_15	Uniquely Methylated, Affected
cg07443748	CESK1	150160	NM_014406.4	node_17_node_15	Uniquely Methylated, Affected
cg07531356	INSL6	11172	NM_007179.2	node_17_node_15	Uniquely Methylated, Affected
cg09923671	GATA5	140628	NT_011362.9	node_17_node_15	Uniquely Methylated, Affected
cg13288195	FBXL22	283807	NM_203373.1	node_17_node_15	Uniquely Methylated, Affected
cg14845091	ADPRHL1	113622	NM_199162.1	node_17_node_15	Uniquely Methylated, Affected
cg17754680	NPC1L1	29881	NM_013389.1	node_17_node_15	Uniquely Methylated, Affected
cg17894008	NACAL	342538	NM_199290.2	node_17_node_15	Uniquely Methylated, Affected
cg19233472	FOXI1	2299	NM_012188.3	node_17_node_15	Uniquely Methylated, Affected
cg19812619	ITGB7	3695	NM_000889.1	node_17_node_15	Uniquely Methylated, Affected
cg20074593	GPR17	2840	NM_005291.1	node_17_node_15	Uniquely Methylated, Affected
cg20668607	DNAJC11	55735	NM_018198.1	node_17_node_15	Uniquely Methylated, Affected
cg20972495	SEC22L3	9117	NM_032970.2	node_17_node_15	Uniquely Methylated, Affected
cg22194129	CLEC4C	170482	NM_130441.2	node_17_node_15	Uniquely Methylated, Affected
cg24024214	BTNL8	79908	NM_024850.1	node_17_node_15	Uniquely Methylated, Affected
cg24073022	TAL2	6887	NM_005421.1	node_17_node_15	Uniquely Methylated, Affected
cg24661752	AP2M1	1173	NM_004068.3	node_17_node_15	Uniquely Methylated, Affected
cg24734575	SLC7A9	11136	NM_014270.3	node_17_node_15	Uniquely Methylated, Affected
cg24867501	MIOX	55586	NM_017584.5	node_17_node_15	Uniquely Methylated, Affected
cg25762706	STMN4	81551	NM_030795.2	node_17_node_15	Uniquely Methylated, Affected
cg26220985	DPT	1805	NM_001937.3	node_17_node_15	Uniquely Methylated, Affected
cg27182551	RB1	5925	NT_024524.13	node_17_node_15	Uniquely Methylated, Affected
cg07711515	BAG1	573	NM_004323.3	node_21_node_20	Uniquely Methylated, Affected
cg16772207	MYT1	4661	NM_004535.2	node_21_node_20	Uniquely Methylated, Affected
cg12439773	SLC22A6	9356	NM_004790.3	node_22_node_21	Uniquely Methylated, Affected

cg02838492	KIF12	113220	NM_138424.1	node_23_node_22	Uniquely Methylated, Affected
cg02774160	GGT1	2678	NM_005265.2	node_13_human	Uniquely Methylated, Unaffected
cg23106779	CXorf2	1527	NM_001586.1	node_13_human	Uniquely Methylated, Unaffected
cg01344518	RGS11	8786	NM_183337.1	node_14_node_13	Uniquely Methylated, Unaffected
cg05654164	C1orf52	148423	NM_198077.1	node_14_node_13	Uniquely Methylated, Unaffected
cg07196761	WDR71	80227	NM_025155.1	node_14_node_13	Uniquely Methylated, Unaffected
cg10618882	LRRC25	126364	NM_145256.2	node_14_node_13	Uniquely Methylated, Unaffected
cg14959707	ZC3H7A	29066	NM_014153.2	node_14_node_13	Uniquely Methylated, Unaffected
cg16087263	PLA2G2F	64600	NM_022819.2	node_14_node_13	Uniquely Methylated, Unaffected
cg16879596	CYP3A5	1577	NM_000777.2	node_14_node_13	Uniquely Methylated, Unaffected
cg19047670	CCND1	595	NT_078088.3	node_14_node_13	Uniquely Methylated, Unaffected
cg24950749	SDCBP2	27111	NM_015685.3	node_14_node_13	Uniquely Methylated, Unaffected
cg25685838	GDPD2	54857	NM_017711.2	node_14_node_13	Uniquely Methylated, Unaffected
cg03343942	SLC39A5	283375	NM_173596.1	node_15_node_14	Uniquely Methylated, Unaffected
cg05788638	SERPINA10	51156	NM_016186.1	node_15_node_14	Uniquely Methylated, Unaffected
cg14701962	C1orf111	284680	NM_182581.1	node_15_node_14	Uniquely Methylated, Unaffected
cg14784348	HCFC1	3054	NM_005334.1	node_15_node_14	Uniquely Methylated, Unaffected
cg18598959	PIK4CB	5298	NM_002651.1	node_15_node_14	Uniquely Methylated, Unaffected
cg20828084	KIAA1199	57214	NM_018689.1	node_15_node_14	Uniquely Methylated, Unaffected
cg03003745	UNQ473	284340	NM_198477.1	node_17_node_15	Uniquely Methylated, Unaffected
cg13316424	CIZ1	25792	NM_012127.2	node_17_node_15	Uniquely Methylated, Unaffected
cg20399252	EBPL	84650	NM_032565.1	node_17_node_15	Uniquely Methylated, Unaffected
cg20437604	ANXA9	8416	NM_003568.1	node_17_node_15	Uniquely Methylated, Unaffected
cg21142272	LOC283849	283849	NM_178516.2	node_17_node_15	Uniquely Methylated, Unaffected
cg23431988	PIWIL2	55124	NM_018068.2	node_17_node_15	Uniquely Methylated, Unaffected
cg14818279	SH3TC2	79628	NM_024577.2	node_20_node_17	Uniquely Methylated, Unaffected
cg25677709	NDST1	3340	NM_001543.3	node_21_node_20	Uniquely Methylated, Unaffected
cg08341924	TGM1	7051	NM_000359.1	node_22_node_21	Uniquely Methylated, Unaffected
cg01443452	GAPDHS	26330	NM_014364.3	node_23_node_22	Uniquely Methylated, Unaffected
cg11098259	AQP9	366	NM_020980.2	node_13_human	Uniquely Unmethylated, Affected
cg22956254	GDF3	9573	NM_020634.1	node_13_human	Uniquely Unmethylated, Affected
cg01155039	AMN	81693	NM_030943.1	node_14_node_13	Uniquely Unmethylated, Affected
cg01623438	CTSZ	1522	NM_001336.2	node_14_node_13	Uniquely Unmethylated, Affected
cg08123074	PHACTR4	65979	NM_023923.2	node_14_node_13	Uniquely Unmethylated, Affected
cg11952714	SNX7	51375	NM_015976.2	node_14_node_13	Uniquely Unmethylated, Affected
cg16296356	RAD51L3	5892	NM_002878.2	node_14_node_13	Uniquely Unmethylated, Affected
cg19531130	ANGPTL5	253935	NM_178127.2	node_14_node_13	Uniquely Unmethylated, Affected
cg20938359	SLC6A12	6539	NM_003044.2	node_14_node_13	Uniquely Unmethylated, Affected
cg21406461	IFI16	3428	NM_005531.1	node_14_node_13	Uniquely Unmethylated, Affected
cg22933847	MRGPRF	219928	NM_145015.2	node_14_node_13	Uniquely Unmethylated, Affected
cg27365426	ARHGAP15	55843	NM_018460.2	node_14_node_13	Uniquely Unmethylated, Affected
cg05091653	SP100	6672	NM_003113.2	node_15_node_14	Uniquely Unmethylated, Affected
cg06933965	CMKLR1	1240	NM_004072.1	node_15_node_14	Uniquely Unmethylated, Affected

cg08090640	IFI35	3430	NM_005533.2	node_15_node_14	Uniquely Unmethylated, Affected
cg09503974	RARRES1	5918	NM_002888.2	node_15_node_14	Uniquely Unmethylated, Affected
cg09735598	RGL1	23179	NM_015149.2	node_15_node_14	Uniquely Unmethylated, Affected
cg14165663	GALR2	8811	NM_003857.2	node_15_node_14	Uniquely Unmethylated, Affected
cg17749456	HSPBP1	23640	NM_012267.2	node_15_node_14	Uniquely Unmethylated, Affected
cg00540769	ACOT12	134526	NM_130767.1	node_15_node_14	Uniquely Unmethylated, Affected
cg01813965	C16orf50	84229	NM_032269.3	node_15_node_14	Uniquely Unmethylated, Affected
cg07612655	PTGIS	5740	NM_000961.3	node_15_node_14	Uniquely Unmethylated, Affected
cg13406950	GBP1	2633	NM_002053.1	node_15_node_14	Uniquely Unmethylated, Affected
cg13471990	ENTPD1	953	NM_001776.3	node_15_node_14	Uniquely Unmethylated, Affected
cg14324675	LST1	7940	NM_205838.1	node_15_node_14	Uniquely Unmethylated, Affected
cg17966192	SULT1C2	27233	NM_006588.2	node_15_node_14	Uniquely Unmethylated, Affected
cg18302652	IL8	3576	NM_000584.2	node_15_node_14	Uniquely Unmethylated, Affected
cg00427635	TBC1D21	161514	NM_153356.1	node_17_node_15	Uniquely Unmethylated, Affected
cg01860753	RASSF5	83593	NT_021877.18	node_17_node_15	Uniquely Unmethylated, Affected
cg02151301	HM13	81502	NM_178580.1	node_17_node_15	Uniquely Unmethylated, Affected
cg03017653	TTC13	79573	NM_024525.2	node_17_node_15	Uniquely Unmethylated, Affected
cg05163348	RPP30	10556	NM_006413.2	node_17_node_15	Uniquely Unmethylated, Affected
cg05829479	C6orf141	135398	NM_153344.1	node_17_node_15	Uniquely Unmethylated, Affected
cg06196379	TREM1	54210	NM_018643.2	node_17_node_15	Uniquely Unmethylated, Affected
cg06306751	F8	2157	NM_000132.2	node_17_node_15	Uniquely Unmethylated, Affected
cg06495347	SUOX	6821	NM_000456.2	node_17_node_15	Uniquely Unmethylated, Affected
cg08475827	RIF1	55183	NM_018151.3	node_17_node_15	Uniquely Unmethylated, Affected
cg09076077	FLJ33860	284756	NM_173644.1	node_17_node_15	Uniquely Unmethylated, Affected
cg09580336	ATP1A1	476	NM_000701.6	node_17_node_15	Uniquely Unmethylated, Affected
cg10213821	G10	8896	NM_003910.2	node_17_node_15	Uniquely Unmethylated, Affected
cg10521852	EDG4	9170	NM_004720.4	node_17_node_15	Uniquely Unmethylated, Affected
cg11299964	MAPKAP1	79109	NM_001006620.1	node_17_node_15	Uniquely Unmethylated, Affected
cg11368643	PCDHB15	56121	NM_018935.2	node_17_node_15	Uniquely Unmethylated, Affected
cg12127282	HOXD4	3233	NM_014621.2	node_17_node_15	Uniquely Unmethylated, Affected
cg12697789	TLR3	7098	NM_003265.2	node_17_node_15	Uniquely Unmethylated, Affected
cg15239579	TRAP1	10131	NM_016292.1	node_17_node_15	Uniquely Unmethylated, Affected
cg17173423	MS4A3	932	NM_006138.4	node_17_node_15	Uniquely Unmethylated, Affected
cg19342782	ANKRD13C	81573	NM_030816.2	node_17_node_15	Uniquely Unmethylated, Affected
cg20318748	NANP	140838	NM_152667.1	node_17_node_15	Uniquely Unmethylated, Affected
cg21042619	EED	8726	NM_003797.2	node_17_node_15	Uniquely Unmethylated, Affected
cg21092324	MMRN1	22915	NM_007351.2	node_17_node_15	Uniquely Unmethylated, Affected
cg22764341	ATP10D	57205	NM_020453.2	node_17_node_15	Uniquely Unmethylated, Affected
cg23398173	MTPN	136319	NM_145808.1	node_17_node_15	Uniquely Unmethylated, Affected
cg23591853	IFT172	26160	NM_015662.1	node_17_node_15	Uniquely Unmethylated, Affected
cg26815229	CYP2J2	1573	NM_000775.2	node_17_node_15	Uniquely Unmethylated, Affected
cg08899626	LDB2	9079	NM_001290.2	node_21_node_20	Uniquely Unmethylated, Affected
cg24673765	HSPB6	126393	NM_144617.1	node_21_node_20	Uniquely Unmethylated, Affected

cg25293251	GOLGA5	9950	NM_005113.2	node_13_human	Uniquely Unmethylated, Unaffected
cg02806777	PGLYRP1	8993	NM_005091.1	node_14_node_13	Uniquely Unmethylated, Unaffected
cg07455279	NDUFA3	4696	NM_004542.1	node_14_node_13	Uniquely Unmethylated, Unaffected
cg17421623	C3orf9	56983	NM_152305.1	node_14_node_13	Uniquely Unmethylated, Unaffected
cg01079126	MTMR1	8776	NM_003828.1	node_15_node_14	Uniquely Unmethylated, Unaffected
cg11320084	RNF2	6045	NM_007212.3	node_15_node_14	Uniquely Unmethylated, Unaffected
cg19535609	B3GNT4	79369	NM_030765.2	node_15_node_14	Uniquely Unmethylated, Unaffected
cg24127874	HES6	55502	NM_018645.3	node_15_node_14	Uniquely Unmethylated, Unaffected
cg00186701	TSPYL5	85453	NM_033512.2	node_15_node_14	Uniquely Unmethylated, Unaffected
cg11325578	GPR143	4935	NM_000273.1	node_15_node_14	Uniquely Unmethylated, Unaffected
cg19441691	MAOA	4128	NM_000240.2	node_15_node_14	Uniquely Unmethylated, Unaffected
cg19564367	AFAP	60312	NM_198595.1	node_15_node_14	Uniquely Unmethylated, Unaffected
cg20371650	PRAF2	11230	NM_007213.1	node_15_node_14	Uniquely Unmethylated, Unaffected
cg27389185	ZNF540	163255	NM_152606.2	node_15_node_14	Uniquely Unmethylated, Unaffected
cg02493771	KRTAP13-2	337959	NM_181621.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg06207804	ARTN	9048	NM_003976.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg08532057	NUPL1	9818	NM_014089.3	node_17_node_15	Uniquely Unmethylated, Unaffected
cg12335708	DPP4	1803	NM_001935.3	node_17_node_15	Uniquely Unmethylated, Unaffected
cg14132995	SLC35A2	7355	NM_005660.1	node_17_node_15	Uniquely Unmethylated, Unaffected
cg15768203	PPIG	9360	NM_004792.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg16227684	GDI1	2664	NM_001493.1	node_17_node_15	Uniquely Unmethylated, Unaffected
cg17552650	WDR45	11152	NM_007075.3	node_17_node_15	Uniquely Unmethylated, Unaffected
cg17860158	CNTN2	6900	NM_005076.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg17963840	ADRA1A	148	NM_033302.1	node_17_node_15	Uniquely Unmethylated, Unaffected
cg18486150	KIF17	57576	NM_020816.1	node_17_node_15	Uniquely Unmethylated, Unaffected
cg18910313	P2RY11	5032	NM_002566.4	node_17_node_15	Uniquely Unmethylated, Unaffected
cg19118077	AKR1C3	8644	NM_003739.4	node_17_node_15	Uniquely Unmethylated, Unaffected
cg20507276	OR2L13	284521	NM_175911.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg20622056	SLC7A3	84889	NM_032803.3	node_17_node_15	Uniquely Unmethylated, Unaffected
cg20825323	IGBP1	3476	NM_001551.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg22598563	P4HA2	8974	NM_004199.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg23066860	GPRASP2	114928	NM_138437.3	node_17_node_15	Uniquely Unmethylated, Unaffected
cg25713185	SLC19A3	80704	NM_025243.2	node_17_node_15	Uniquely Unmethylated, Unaffected
cg20792833	PTPRCAP	5790	NM_005608.2	node_21_node_20	Uniquely Unmethylated, Unaffected
cg10709021	WRN	7486	NM_000553.2	node_22_node_21	Uniquely Unmethylated, Unaffected
cg14473924	PDZRN3	23024	NM_015009.1	node_22_node_21	Uniquely Unmethylated, Unaffected
cg21835643	RBPSUHL	11317	NM_014276.2	node_22_node_21	Uniquely Unmethylated, Unaffected

REFERENCES

Aberg, K.A., Xie, L.Y., Nerella, S., Copeland, W.E., Costello, E.J., and Van Den Oord, E.J. (2013). High quality methylome-wide investigations through next-generation sequencing of DNA from a single archived dry blood spot. *Epigenetics* 8.

Acierno, R., Ruggiero, K.J., Galea, S., Resnick, H.S., Koenen, K., Roitzsch, J., De Arellano, M., Boyle, J., and Kilpatrick, D.G. (2007). Psychological sequelae resulting from the 2004 Florida hurricanes: implications for postdisaster intervention. *Am J Public Health* 97 Suppl 1**,** S103-108. doi: 10.2105/ajph.2006.087007.

Amweg, A.N., Paredes, A., Salvetti, N.R., Lara, H.E., and Ortega, H.H. (2011). Expression of melanocortin receptors mRNA, and direct effects of ACTH on steroid secretion in the bovine ovary. *Theriogenology* 75**,** 628-637. doi: 10.1016/j.theriogenology.2010.10.003.

Anderson, D.J., and Adolphs, R. (2014). A Framework for Studying Emotions across Species. *Cell* 157**,** 187-200. doi: 10.1016/j.cell.2014.03.003.

Andrews, J.A., and Neises, K.D. (2012). Cells, biomarkers, and post-traumatic stress disorder: evidence for peripheral involvement in a central disease. *J Neurochem* 120**,** 26-36. doi: 10.1111/j.1471-4159.2011.07545.x.

Apa (1980). *Diagnostic and Statistical Manual of Mental Disorders, 3rd ed.* Washington, DC: American Psychiatric Press.

Apa (1994). *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington, DC: American Psychiatric Press.

Apa (2013). *Diagnostic and statistical manual of mental disorders (5th ed.).* Arlington, VA: American Psychiatric Publishing.

Applebaum, S.W., and Heifetz, Y. (1999). Density-dependent physiological phase in insects. *Annual Review of Entomology* 44**,** 317-341.

Association, A.P. (2013). *Diagnostic and Statistical Manual of Mental Disorders: Fifth Edition (DSM-5).* Arlington, VA: American Psychiatric Publishing.

Bachmann, A.W., Sedgley, T.L., Jackson, R.V., Gibson, J.N., Young, R.M., and Torpy, D.J. (2005). Glucocorticoid receptor polymorphisms and post-traumatic stress disorder. *Psychoneuroendocrinology* 30**,** 297-306. doi: 10.1016/j.psyneuen.2004.08.006.

Badyaev, A.V. (2009). Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the Baldwin effect. *Philos Trans R Soc Lond B Biol Sci* 364**,** 1125-1141. doi: 10.1098/rstb.2008.0285.

Baker, D.G., West, S.A., Nicholson, W.E., Ekhator, N.N., Kasckow, J.W., Hill, K.K., Bruce, A.B., Orth, D.N., and Geracioti, T.D., Jr. (1999). Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry* 156**,** 585-588.

Baldwin, J. (1896). A New Factor in Evolution. *The American Naturalist* 30**,** 441-451.

Baldwin, J. (1897). Organic Selection. *Science* 5**,** 634-636. doi: 10.1126/science.5.121.634.

Barker, D.J., Osmond, C., Golding, J., Kuh, D., and Wadsworth, M.E. (1989). Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *Bmj* 298**,** 564-567.

Barkess, G., and West, A.G. (2012). Chromatin insulator elements: establishing barriers to set heterochromatin boundaries. *Epigenomics* 4**,** 67-80. doi: 10.2217/epi.11.112.

Barrett, J.C., Fry, B., Maller, J., and Daly, M.J. (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21**,** 263-265. doi: 10.1093/bioinformatics/bth457.

Bateson, P., and Gluckman, P. (2011). "Plasticity, Robustness, Development and Evolution." (New York: Cambridge University Press).

Benjamini, Y., Hochberg Y. (1995). Controlling the False Discovery Rate: a practical and powerful approach to multiple testing. *J Royal Stat Soc Ser B* 57**,** 289-300.

Bernstein, B.E., Meissner, A., and Lander, E.S. (2007). The mammalian epigenome. *Cell* 128**,** 669-681. doi: S0092-8674(07)00128-6 [pii]

10.1016/j.cell.2007.01.033.

Binder, E.B., Bradley, R.G., Liu, W., Epstein, M.P., Deveau, T.C., Mercer, K.B., Tang, Y., Gillespie, C.F., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F., and Ressler, K.J. (2008). Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *Jama* 299**,** 1291-1305. doi: 10.1001/jama.299.11.1291.

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev* 16**,** 6-21. doi: 10.1101/gad.947102.

Bjornsson, H.T., Sigurdsson, M.I., Fallin, M.D., Irizarry, R.A., Aspelund, T., Cui, H., Yu, W., Rongione, M.A., Ekstrom, T.J., Harris, T.B., Launer, L.J., Eiriksdottir, G., Leppert, M.F., Sapienza, C., Gudnason, V., and Feinberg, A.P. (2008). Intra-

individual change over time in DNA methylation with familial clustering. *JAMA* 299**,** 2877-2883. doi: 299/24/2877 [pii]

10.1001/jama.299.24.2877.

Blake, D.D., Weathers, F.W., Nagy, L.M., Kaloupek, D.G., Gusman, F.D., Charney, D.S., and Keane, T.M. (1995). The development of a Clinician-Administered PTSD Scale. *J Trauma Stress* 8**,** 75-90.

Bourc'his, D., and Bestor, T.H. (2004). Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* 431**,** 96-99. doi: 10.1038/nature02886.

Bourc'his, D., Xu, G.L., Lin, C.S., Bollman, B., and Bestor, T.H. (2001). Dnmt3L and the establishment of maternal genomic imprints. *Science* 294**,** 2536-2539. doi: 10.1126/science.1065848.

Boyce, W.T., and Ellis, B.J. (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev Psychopathol* 17**,** 271-301.

Bracha, H.S., Yoshioka, D.T., Masukawa, N.K., and Stockman, D.J. (2005). Evolution of the human fear-circuitry and acute sociogenic pseudoneurological symptoms: the Neolithic balanced-polymorphism hypothesis. *J Affect Disord* 88**,** 119-129. doi: 10.1016/j.jad.2005.05.013.

Bradley, R.G., Binder, E.B., Epstein, M.P., Tang, Y., Nair, H.P., Liu, W., Gillespie, C.F., Berg, T., Evces, M., Newport, D.J., Stowe, Z.N., Heim, C.M., Nemeroff, C.B., Schwartz, A., Cubells, J.F., and Ressler, K.J. (2008). Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor

gene. *Arch Gen Psychiatry* 65**,** 190-200. doi: 10.1001/archgenpsychiatry.2007.26.

Bradshaw, G.A., Capaldo, T., Lindner, L., and Grow, G. (2008). Building an inner sanctuary: complex PTSD in chimpanzees. *J Trauma Dissociation* 9**,** 9-34.

Bradshaw, G.A., Schore, A.N., Brown, J.L., Poole, J.H., and Moss, C.J. (2005). Elephant breakdown. *Nature* 433**,** 807. doi: 10.1038/433807a.

Bremner, J.D., Licinio, J., Darnell, A., Krystal, J.H., Owens, M.J., Southwick, S.M., Nemeroff, C.B., and Charney, D.S. (1997). Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. *Am J Psychiatry* 154**,** 624-629.

Breslau, N., Davis, G.C., Andreski, P., and Peterson, E. (1991). Traumatic events and posttraumatic stress disorder in an urban population of young adults. *Arch Gen Psychiatry* 48**,** 216-222.

Breslau, N., Davis, G.C., Peterson, E.L., and Schultz, L.R. (2000). A second look at comorbidity in victims of trauma: the posttraumatic stress disorder-major depression connection. *Biol Psychiatry* 48**,** 902-909.

Breslau, N., Davis, G.C., and Schultz, L.R. (2003). Posttraumatic stress disorder and the incidence of nicotine, alcohol, and other drug disorders in persons who have experienced trauma. *Arch Gen Psychiatry* 60**,** 289-294.

Breslau, N., Kessler, R.C., Chilcoat, H.D., Schultz, L.R., Davis, G.C., and Andreski, P. (1998). Trauma and posttraumatic stress disorder in the community: the 1996 Detroit Area Survey of Trauma. *Arch Gen Psychiatry* 55**,** 626-632.

Breslau, N., Wilcox, H.C., Storr, C.L., Lucia, V.C., and Anthony, J.C. (2004). Trauma exposure and posttraumatic stress disorder: a study of youths in urban America. *J Urban Health* 81**,** 530-544. doi: 10.1093/jurban/jth138.

Brewin, C.R., Andrews, B., and Valentine, J.D. (2000). Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. *J Consult Clin Psychol* 68**,** 748-766.

Byrne, E.M., Carrillo-Roa, T., Henders, A.K., Bowdler, L., Mcrae, A.F., Heath, A.C., Martin, N.G., Montgomery, G.W., Krause, L., and Wray, N.R. (2013). Monozygotic twins affected with major depressive disorder have greater variance in methylation than their unaffected co-twin. *Transl Psychiatry* 3**,** e269. doi: 10.1038/tp.2013.45.

Cantor, C. (2009). Post-traumatic stress disorder: evolutionary perspectives. *Aust N Z J Psychiatry* 43**,** 1038-1048. doi: 10.3109/00048670903270407.

Carpenter, L.L., Tyrka, A.R., Ross, N.S., Khoury, L., Anderson, G.M., and Price, L.H. (2009). Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biol Psychiatry* 66**,** 69-75. doi: 10.1016/j.biopsych.2009.02.030.

Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A., Frisch, M., Bayerlein, M., and Werner, T. (2005). MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* 21**,** 2933-2942. doi: 10.1093/bioinformatics/bti473.

Champagne, F.A. (2009). Nurturing nature: social experiences and the brain. *J Neuroendocrinol* 21**,** 867-868. doi: 10.1111/j.1365-2826.2009.01901.x.

Champagne, F.A., and Meaney, M.J. (2006). Stress during gestation alters postpartum maternal care and the development of the offspring in a rodent model. *Biol Psychiatry* 59**,** 1227-1235. doi: 10.1016/j.biopsych.2005.10.016.

Champagne, F.A., Weaver, I.C., Diorio, J., Dymov, S., Szyf, M., and Meaney, M.J. (2006). Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology* 147**,** 2909-2915. doi: 10.1210/en.2005-1119.

Chang, S.C., Koenen, K.C., Galea, S., Aiello, A.E., Soliven, R., Wildman, D.E., and Uddin, M. (2012). Molecular variation at the SLC6A3 locus predicts lifetime risk of PTSD in the Detroit Neighborhood Health Study. *PLoS One* 7**,** e39184. doi: 10.1371/journal.pone.0039184.

Charney, D.S. (2004). Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *Am J Psychiatry* 161**,** 195-216.

Chugani, H.T. (1998). A critical period of brain development: studies of cerebral glucose utilization with PET. *Prev Med* 27**,** 184-188. doi: S0091-7435(98)90274-2 [pii]

10.1006/pmed.1998.0274.

Claes, S.J. (2004). Corticotropin-releasing hormone (CRH) in psychiatry: from stress to psychopathology. *Ann Med* 36**,** 50-61.

Clark, K.A., Shin, A.C., Sirivelu, M.P., Mohankumar, S.M., and Mohankumar, P.S. (2008). Systemic administration of leptin decreases plasma corticosterone levels:

role of hypothalamic norepinephrine. *Brain Res* 1195**,** 89-95. doi: 10.1016/j.brainres.2007.12.009.

Cloitre, M., Stolbach, B.C., Herman, J.L., Van Der Kolk, B., Pynoos, R., Wang, J., and Petkova, E. (2009). A developmental approach to complex PTSD: childhood and adult cumulative trauma as predictors of symptom complexity. *J Trauma Stress* 22**,** 399-408. doi: 10.1002/jts.20444.

Cohen, H., and Zohar, J. (2004). An animal model of posttraumatic stress disorder: the use of cut-off behavioral criteria. *Ann N Y Acad Sci* 1032**,** 167-178. doi: 10.1196/annals.1314.014.

Committee on the Assessment of Ongoing Effects in the Treatment of Posttraumatic Stress, D., and Institute Of, M. (2012). in *Treatment for Posttraumatic Stress Disorder in Military and Veteran Populations: Initial Assessment*. (Washington (DC): National Academies Press (US)

Copyright 2012 by the National Academy of Sciences. All rights reserved.).

Consortium, E. (2011). A user's guide to the encyclopedia of DNA elements (ENCODE). *PLoS Biol* 9**,** e1001046. doi: 10.1371/journal.pbio.1001046.

Cornelis, M.C., Nugent, N.R., Amstadter, A.B., and Koenen, K.C. (2010). Genetics of post-traumatic stress disorder: review and recommendations for genome-wide association studies. *Curr Psychiatry Rep* 12**,** 313-326. doi: 10.1007/s11920-010-0126-6.

Coulton, C.J., Crampton, D.S., Irwin, M., Spilsbury, J.C., and Korbin, J.E. (2007). How neighborhoods influence child maltreatment: a review of the literature and

alternative pathways. *Child Abuse Negl* 31**,** 1117-1142. doi: 10.1016/j.chiabu.2007.03.023.

Crews, D., Gillette, R., Scarpino, S.V., Manikkam, M., Savenkova, M.I., and Skinner, M.K. (2012). Epigenetic transgenerational inheritance of altered stress responses. *Proc Natl Acad Sci U S A* 109**,** 9143-9148. doi: 10.1073/pnas.1118514109.

Curradi, M., Izzo, A., Badaracco, G., and Landsberger, N. (2002). Molecular mechanisms of gene silencing mediated by DNA methylation. *Mol Cell Biol* 22**,** 3157-3173.

De Kloet, C.S., Vermetten, E., Bikker, A., Meulman, E., Geuze, E., Kavelaars, A., Westenberg, H.G., and Heijnen, C.J. (2007). Leukocyte glucocorticoid receptor expression and immunoregulation in veterans with and without post-traumatic stress disorder. *Mol Psychiatry* 12**,** 443-453. doi: 10.1038/sj.mp.4001934.

De Kloet, C.S., Vermetten, E., Geuze, E., Kavelaars, A., Heijnen, C.J., and Westenberg, H.G. (2006). Assessment of HPA-axis function in posttraumatic stress disorder: pharmacological and non-pharmacological challenge tests, a review. *J Psychiatr Res* 40**,** 550-567. doi: 10.1016/j.jpsychires.2005.08.002.

De Kloet, C.S., Vermetten, E., Geuze, E., Wiegant, V.M., and Westenberg, H.G. (2008). Elevated plasma arginine vasopressin levels in veterans with posttraumatic stress disorder. *J Psychiatr Res* 42**,** 192-198. doi: 10.1016/j.jpsychires.2006.11.009.

Digangi, J., Guffanti, G., Mclaughlin, K.A., and Koenen, K.C. (2013). Considering trauma exposure in the context of genetics studies of posttraumatic stress

disorder: a systematic review. *Biol Mood Anxiety Disord* 3**,** 2. doi: 10.1186/2045-5380-3-2.

Digrande, L., Perrin, M.A., Thorpe, L.E., Thalji, L., Murphy, J., Wu, D., Farfel, M., and Brackbill, R.M. (2008). Posttraumatic stress symptoms, PTSD, and risk factors among lower Manhattan residents 2-3 years after the September 11, 2001 terrorist attacks. *J Trauma Stress* 21**,** 264-273. doi: 10.1002/jts.20345.

Dollar, S.J. (1982). Wave Stress and Coral Community Structure in Hawaii. *Coral Reefs* 1**,** 71-81.

Druss, B.G., Hwang, I., Petukhova, M., Sampson, N.A., Wang, P.S., and Kessler, R.C. (2009). Impairment in role functioning in mental and chronic medical disorders in the United States: results from the National Comorbidity Survey Replication. *Mol Psychiatry* 14**,** 728-737. doi: 10.1038/mp.2008.13.

Dunn, O.J. (1961). Multiple comparisons among means. *J Am Stat Assoc* 56**,** 52-64.

Eckhardt, F., Lewin, J., Cortese, R., Rakyan, V.K., Attwood, J., Burger, M., Burton, J., Cox, T.V., Davies, R., Down, T.A., Haefliger, C., Horton, R., Howe, K., Jackson, D.K., Kunde, J., Koenig, C., Liddle, J., Niblett, D., Otto, T., Pettett, R., Seemann, S., Thompson, C., West, T., Rogers, J., Olek, A., Berlin, K., and Beck, S. (2006). DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* 38**,** 1378-1385. doi: ng1909 [pii]

10.1038/ng1909.

Ehlert, U. (2013). Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology* 38**,** 1850-1857. doi: 10.1016/j.psyneuen.2013.06.007.

El-Sayed, A.M., Haloossim, M.R., Galea, S., and Koenen, K.C. (2012). Epigenetic modifications associated with suicide and common mood and anxiety disorders: a systematic review of the literature. *Biol Mood Anxiety Disord* 2**,** 10. doi: 10.1186/2045-5380-2-10.

Ellis, B.J., Boyce, W.T., Belsky, J., Bakermans-Kranenburg, M.J., and Van Ijzendoorn, M.H. (2011). Differential susceptibility to the environment: an evolutionary--neurodevelopmental theory. *Dev Psychopathol* 23**,** 7-28. doi: 10.1017/s0954579410000611.

Essex, M.J., Boyce, W.T., Hertzman, C., Lam, L.L., Armstrong, J.M., Neumann, S.M., and Kobor, M.S. (2013). Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Dev* 84**,** 58-75. doi: 10.1111/j.1467-8624.2011.01641.x.

Farley, M., and Patsalides, B.M. (2001). Physical symptoms, posttraumatic stress disorder, and healthcare utilization of women with and without childhood physical and sexual abuse. *Psychol Rep* 89**,** 595-606.

Feinberg, A.P. (2008). Epigenetics at the epicenter of modern medicine. *Jama* 299**,** 1345-1350. doi: 10.1001/jama.299.11.1345.

Feinberg, A.P., and Irizarry, R.A. (2010). Evolution in health and medicine Sackler colloquium: Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. *Proc Natl Acad Sci U S A* 107 Suppl 1**,** 1757-1764. doi: 10.1073/pnas.0906183107.

Feng, J., Chang, H., Li, E., and Fan, G. (2005). Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *J Neurosci Res* 79**,** 734-746. doi: 10.1002/jnr.20404.

Feng, J., and Fan, G. (2009). The role of DNA methylation in the central nervous system and neuropsychiatric disorders. *Int Rev Neurobiol* 89**,** 67-84. doi: 10.1016/s0074-7742(09)89004-1.

Feng, J., Zhou, Y., Campbell, S.L., Le, T., Li, E., Sweatt, J.D., Silva, A.J., and Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat Neurosci* 13**,** 423-430. doi: 10.1038/nn.2514.

Ferdowsian, H.R., Durham, D.L., Kimwele, C., Kranendonk, G., Otali, E., Akugizibwe, T., Mulcahy, J.B., Ajarova, L., and Johnson, C.M. (2011). Signs of mood and anxiety disorders in chimpanzees. *PLoS One* 6**,** e19855. doi: 10.1371/journal.pone.0019855.

Galea, S., Acierno, R., Ruggiero, K., Resnick, H., Tracy, M., and Kilpatrick, D. (2006). Social context and the psychobiology of posttraumatic stress. *Ann N Y Acad Sci* 1071**,** 231-241. doi: 10.1196/annals.1364.018.

Galea, S., Ahern, J., Tracy, M., Hubbard, A., Cerda, M., Goldmann, E., and Vlahov, D. (2008). Longitudinal determinants of posttraumatic stress in a population-based cohort study. *Epidemiology* 19**,** 47-54. doi: 10.1097/EDE.0b013e31815c1dbf.

Galli, S.J., Borregaard, N., and Wynn, T.A. (2011). Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat Immunol* 12**,** 1035-1044. doi: 10.1038/ni.2109.

Gillespie, C.F., Bradley, B., Mercer, K., Smith, A.K., Conneely, K., Gapen, M., Weiss, T., Schwartz, A.C., Cubells, J.F., and Ressler, K.J. (2009). Trauma exposure and stress-related disorders in inner city primary care patients. *Gen Hosp Psychiatry* 31**,** 505-514. doi: 10.1016/j.genhosppsych.2009.05.003.

Goldman, D., Oroszi, G., and Ducci, F. (2005). The genetics of addictions: uncovering the genes. *Nat Rev Genet* 6**,** 521-532. doi: 10.1038/nrg1635.

Golub, Y., Kaltwasser, S.F., Mauch, C.P., Herrmann, L., Schmidt, U., Holsboer, F., Czisch, M., and Wotjak, C.T. (2011). Reduced hippocampus volume in the mouse model of Posttraumatic Stress Disorder. *J Psychiatr Res* 45**,** 650-659. doi: 10.1016/j.jpsychires.2010.10.014.

Goto, K., Numata, M., Komura, J.I., Ono, T., Bestor, T.H., and Kondo, H. (1994). Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* 56**,** 39-44.

Guffanti, G., Galea, S., Yan, L., Roberts, A.L., Solovieff, N., Aiello, A.E., Smoller, J.W., De Vivo, I., Ranu, H., Uddin, M., Wildman, D.E., Purcell, S., and Koenen, K.C. (2013). Genome-wide association study implicates a novel RNA gene, the lincRNA AC068718.1, as a risk factor for post-traumatic stress disorder in women. *Psychoneuroendocrinology* 38**,** 3029-3038. doi: 10.1016/j.psyneuen.2013.08.014.

Hales, C.N., and Barker, D.J. (1992). Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 35**,** 595-601.

Harvey, A.G., and Bryant, R.A. (1999). Acute stress disorder across trauma populations. *J Nerv Ment Dis* 187**,** 443-446.

Heim, C., Mletzko, T., Purselle, D., Musselman, D.L., and Nemeroff, C.B. (2008). The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. *Biol Psychiatry* 63**,** 398-405. doi: 10.1016/j.biopsych.2007.07.002.

Hernando-Herraez, I., Prado-Martinez, J., Garg, P., Fernandez-Callejo, M., Heyn, H., Hvilsom, C., Navarro, A., Esteller, M., Sharp, A.J., and Marques-Bonet, T. (2013). Dynamics of DNA methylation in recent human and great ape evolution. *PLoS Genet* 9**,** e1003763. doi: 10.1371/journal.pgen.1003763.

Hollegaard, M.V., Grauholm, J., Norgaard-Pedersen, B., and Hougaard, D.M. (2013). DNA methylome profiling using neonatal dried blood spot samples: a proof-of-principle study. *Mol Genet Metab* 108**,** 225-231. doi: 10.1016/j.ymgme.2013.01.016.

Hong, S.B., Youssef, G.J., Song, S.H., Choi, N.H., Ryu, J., Mcdermott, B., Cobham, V., Park, S., Kim, J.W., Shin, M.S., Yoo, H.J., Cho, S.C., and Kim, B.N. (2014). Different clinical courses of children exposed to a single incident of psychological trauma: a 30-month prospective follow-up study. *J Child Psychol Psychiatry*. doi: 10.1111/jcpp.12241.

Horowitz, K., Mckay, M., and Marshall, R. (2005). Community violence and urban families: experiences, effects, and directions for intervention. *Am J Orthopsychiatry* 75**,** 356-368. doi: 10.1037/0002-9432.75.3.356.

Horwitz Av, W.J. (2012). *All We Have to Fear: Psychiaty's transformation of natural anxieties into mental disorders.* New York: Oxford University Press.

Houston, I., Peter, C.J., Mitchell, A., Straubhaar, J., Rogaev, E., and Akbarian, S. (2013). Epigenetics in the human brain. *Neuropsychopharmacology* 38**,** 183-197. doi: 10.1038/npp.2012.78.

Huang Da, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4**,** 44-57. doi: nprot.2008.211 [pii]

10.1038/nprot.2008.211.

Iguchi-Ariga, S.M., and Schaffner, W. (1989). CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. *Genes Dev* 3**,** 612-619.

Inano, K., Suetake, I., Ueda, T., Miyake, Y., Nakamura, M., Okada, M., and Tajima, S. (2000). Maintenance-type DNA methyltransferase is highly expressed in post-mitotic neurons and localized in the cytoplasmic compartment. *J Biochem* 128**,** 315-321.

Irwin, M.R., and Cole, S.W. (2011). Reciprocal regulation of the neural and innate immune systems. *Nat Rev Immunol* 11**,** 625-632. doi: 10.1038/nri3042.

Jameson, N.M., Hou, Z.C., Sterner, K.N., Weckle, A., Goodman, M., Steiper, M.E., and Wildman, D.E. (2011). Genomic data reject the hypothesis of a prosimian primate clade. *J Hum Evol* 61**,** 295-305. doi: 10.1016/j.jhevol.2011.04.004.

Jin, C., Qi, R., Yin, Y., Hu, X., Duan, L., Xu, Q., Zhang, Z., Zhong, Y., Feng, B., Xiang, H., Gong, Q., Liu, Y., Lu, G., and Li, L. (2013). Abnormalities in whole-brain functional connectivity observed in treatment-naive post-traumatic stress disorder

patients following an earthquake. *Psychol Med*, 1-10. doi: 10.1017/s003329171300250x.

Johns, L.E., Aiello, A.E., Cheng, C., Galea, S., Koenen, K.C., and Uddin, M. (2012). Neighborhood social cohesion and posttraumatic stress disorder in a community-based sample: findings from the Detroit Neighborhood Health Study. *Soc Psychiatry Psychiatr Epidemiol* 47, 1899-1906. doi: 10.1007/s00127-012-0506-9.

Jones, K.A., and Thomsen, C. (2013). The role of the innate immune system in psychiatric disorders. *Mol Cell Neurosci* 53, 52-62. doi: 10.1016/j.mcn.2012.10.002.

Js, F. (1970). Methods for computing Wagner trees. *Syst Zool* 19, 83-92.

Kalin, N.H., and Shelton, S.E. (2003). Nonhuman primate models to study anxiety, emotion regulation, and psychopathology. *Ann N Y Acad Sci* 1008, 189-200.

Kaneda, M., Okano, M., Hata, K., Sado, T., Tsujimoto, N., Li, E., and Sasaki, H. (2004). Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* 429, 900-903. doi: 10.1038/nature02633.

Kang, H.J., Kawasawa, Y.I., Cheng, F., Zhu, Y., Xu, X., Li, M., Sousa, A.M., Pletikos, M., Meyer, K.A., Sedmak, G., Guennel, T., Shin, Y., Johnson, M.B., Krsnik, Z., Mayer, S., Fertuzinhos, S., Umlauf, S., Lisgo, S.N., Vortmeyer, A., Weinberger, D.R., Mane, S., Hyde, T.M., Huttner, A., Reimers, M., Kleinman, J.E., and Sestan, N. (2011). Spatio-temporal transcriptome of the human brain. *Nature* 478, 483-489. doi: 10.1038/nature10523.

Kato, Y., Kaneda, M., Hata, K., Kumaki, K., Hisano, M., Kohara, Y., Okano, M., Li, E., Nozaki, M., and Sasaki, H. (2007). Role of the Dnmt3 family in de novo

methylation of imprinted and repetitive sequences during male germ cell development in the mouse. *Hum Mol Genet* 16**,** 2272-2280. doi: 10.1093/hmg/ddm179.

Kellner, M., Muhtz, C., Peter, F., Dunker, S., Wiedemann, K., and Yassouridis, A. (2010). Increased DHEA and DHEA-S plasma levels in patients with post-traumatic stress disorder and a history of childhood abuse. *J Psychiatr Res* 44**,** 215-219. doi: 10.1016/j.jpsychires.2009.08.009.

Kendler Ks, P.J. (2012). *Philosophical issues in psychiatry II: nosology.* Oxford: Oxford University Press.

Kessler, R.C. (2000). Posttraumatic stress disorder: the burden to the individual and to society. *J Clin Psychiatry* 61 Suppl 5**,** 4-12; discussion 13-14.

Kessler, R.C., Sonnega, A., Bromet, E., Hughes, M., and Nelson, C.B. (1995). Posttraumatic stress disorder in the National Comorbidity Survey. *Arch Gen Psychiatry* 52**,** 1048-1060.

Kessler, R.C., and Wang, P.S. (2008). The descriptive epidemiology of commonly occurring mental disorders in the United States. *Annu Rev Public Health* 29**,** 115-129. doi: 10.1146/annurev.publhealth.29.020907.090847.

Kim, J.K., Samaranayake, M., and Pradhan, S. (2009). Epigenetic mechanisms in mammals. *Cell Mol Life Sci* 66**,** 596-612. doi: 10.1007/s00018-008-8432-4.

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J.C., Pariante, C.M., Pace, T.W., Mercer, K.B., Mayberg, H.S., Bradley, B., Nemeroff, C.B., Holsboer, F., Heim, C.M., Ressler, K.J., Rein, T., and Binder, E.B. (2013). Allele-specific

FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci* 16**,** 33-41. doi: 10.1038/nn.3275.

Klest, B. (2012). Childhood trauma, poverty, and adult victimization. *Psychological Trauma: Theory, Research, Practice and Policy* 4**,** 245-251. doi: 10.1037/a0024468.

Koenen, K.C. (2007). Genetics of posttraumatic stress disorder: Review and recommendations for future studies. *J Trauma Stress* 20**,** 737-750. doi: 10.1002/jts.20205.

Koenen, K.C., Harley, R., Lyons, M.J., Wolfe, J., Simpson, J.C., Goldberg, J., Eisen, S.A., and Tsuang, M. (2002). A twin registry study of familial and individual risk factors for trauma exposure and posttraumatic stress disorder. *J Nerv Ment Dis* 190**,** 209-218.

Koenen, K.C., Nugent, N.R., and Amstadter, A.B. (2008). Gene-environment interaction in posttraumatic stress disorder: review, strategy and new directions for future research. *Eur Arch Psychiatry Clin Neurosci* 258**,** 82-96. doi: 10.1007/s00406-007-0787-2.

Koenen, K.C., Stellman, J.M., Stellman, S.D., and Sommer, J.F., Jr. (2003). Risk factors for course of posttraumatic stress disorder among Vietnam veterans: a 14-year follow-up of American Legionnaires. *J Consult Clin Psychol* 71**,** 980-986. doi: 10.1037/0022-006x.71.6.980.

Koenen, K.C., Uddin, M., Chang, S.C., Aiello, A.E., Wildman, D.E., Goldmann, E., and Galea, S. (2011). SLC6A4 methylation modifies the effect of the number of

traumatic events on risk for posttraumatic stress disorder. *Depress Anxiety* 28**,** 639-647. doi: 10.1002/da.20825.

Kubzansky, L.D., and Koenen, K.C. (2009). Is posttraumatic stress disorder related to development of heart disease? An update. *Cleve Clin J Med* 76 Suppl 2**,** S60-65. doi: 10.3949/ccjm.76.s2.12.

Kubzansky, L.D., Koenen, K.C., Jones, C., and Eaton, W.W. (2009). A prospective study of posttraumatic stress disorder symptoms and coronary heart disease in women. *Health Psychol* 28**,** 125-130. doi: 10.1037/0278-6133.28.1.125.

Kubzansky, L.D., Koenen, K.C., Spiro, A., 3rd, Vokonas, P.S., and Sparrow, D. (2007). Prospective study of posttraumatic stress disorder symptoms and coronary heart disease in the Normative Aging Study. *Arch Gen Psychiatry* 64**,** 109-116. doi: 10.1001/archpsyc.64.1.109.

Kulka Ra, S.W., Fairbank Ja, Hough Rl, Jordan Bk, Marmar Cr, Weiss Ds (1990). *Trauma and the Vietnam war generation: Report of findings from the National Vietnam Veterans Readjustment Study.* Philadelphia, PA, US: Brunner/Mazel.

Kun, P., Han, S., Chen, X., and Yao, L. (2009). Prevalence and risk factors for posttraumatic stress disorder: a cross-sectional study among survivors of the Wenchuan 2008 earthquake in China. *Depress Anxiety* 26**,** 1134-1140. doi: 10.1002/da.20612.

Larque, E., Ruiz-Palacios, M., and Koletzko, B. (2013). Placental regulation of fetal nutrient supply. *Curr Opin Clin Nutr Metab Care* 16**,** 292-297. doi: 10.1097/MCO.0b013e32835e3674.

Leardmann, C.A., Smith, B., and Ryan, M.A. (2010). Do adverse childhood experiences increase the risk of postdeployment posttraumatic stress disorder in US Marines? *BMC Public Health* 10**,** 437. doi: 10.1186/1471-2458-10-437.

Li, E., Bestor, T.H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69**,** 915-926.

Li, L., Wu, M., Liao, Y., Ouyang, L., Du, M., Lei, D., Chen, L., Yao, L., Huang, X., and Gong, Q. (2014). Gray matter reduction associated with posttraumatic stress disorder and traumatic stress. *Neurosci Biobehav Rev*. doi: 10.1016/j.neubiorev.2014.04.003.

Logue, M.W., Baldwin, C., Guffanti, G., Melista, E., Wolf, E.J., Reardon, A.F., Uddin, M., Wildman, D., Galea, S., Koenen, K.C., and Miller, M.W. (2013). A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha (RORA) gene as a significant risk locus. *Mol Psychiatry* 18**,** 937-942. doi: 10.1038/mp.2012.113.

Lopez-Ibor, J.J. (2002). The classification of stress-related disorders in ICD-10 and DSM-IV. *Psychopathology* 35**,** 107-111. doi: 65128.

Lopez-Leon, S., Janssens, A.C., Gonzalez-Zuloeta Ladd, A.M., Del-Favero, J., Claes, S.J., Oostra, B.A., and Van Duijn, C.M. (2008). Meta-analyses of genetic studies on major depressive disorder. *Mol Psychiatry* 13**,** 772-785. doi: 10.1038/sj.mp.4002088.

Lu, A.T., Ogdie, M.N., Jarvelin, M.R., Moilanen, I.K., Loo, S.K., Mccracken, J.T., Mcgough, J.J., Yang, M.H., Peltonen, L., Nelson, S.F., Cantor, R.M., and Smalley, S.L. (2008). Association of the cannabinoid receptor gene (CNR1) with

ADHD and post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 147b**,** 1488-1494. doi: 10.1002/ajmg.b.30693.

Martin, E.I., Ressler, K.J., Binder, E., and Nemeroff, C.B. (2010). The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. *Clin Lab Med* 30**,** 865-891. doi: 10.1016/j.cll.2010.07.006.

Maxwell, M. (2011). A study in contrasts: inscriptions of posttraumatic stress disorder (PTSD) in two works of fiction. *Work* 38**,** 19-32. doi: 10.3233/wor-2011-1101.

Mcgowan, P.O., Sasaki, A., D'alessio, A.C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., and Meaney, M.J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 12**,** 342-348. doi: 10.1038/nn.2270.

Mcgowan, P.O., Suderman, M., Sasaki, A., Huang, T.C., Hallett, M., Meaney, M.J., and Szyf, M. (2011). Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One* 6**,** e14739. doi: 10.1371/journal.pone.0014739.

Meaney, M.J. (2010). Epigenetics and the biological definition of gene x environment interactions. *Child Dev* 81**,** 41-79.

Mehta, D., and Binder, E.B. (2012). Gene x environment vulnerability factors for PTSD: the HPA-axis. *Neuropharmacology* 62**,** 654-662. doi: 10.1016/j.neuropharm.2011.03.009.

Mehta, D., Klengel, T., Conneely, K.N., Smith, A.K., Altmann, A., Pace, T.W., Rex-Haffner, M., Loeschner, A., Gonik, M., Mercer, K.B., Bradley, B., Muller-Myhsok, B., Ressler, K.J., and Binder, E.B. (2013). Childhood maltreatment is associated

with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci U S A* 110**,** 8302-8307. doi: 10.1073/pnas.1217750110.

Melzer-Lange, M.D., Van Thatcher, C.D., Liu, J., and Zhu, S. (2007). Urban community characteristics and adolescent assault victims. *Wmj* 106**,** 394-396.

Meredith, R.W., Janecka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simao, T.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.S., and Murphy, W.J. (2011). Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. *Science* 334**,** 521-524. doi: 10.1126/science.1211028.

Mier, D., Kirsch, P., and Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol Psychiatry* 15**,** 918-927. doi: 10.1038/mp.2009.36.

Miller, C.A., and Sweatt, J.D. (2007). Covalent modification of DNA regulates memory formation. *Neuron* 53**,** 857-869. doi: 10.1016/j.neuron.2007.02.022.

Mitchell, K.S., Aiello, A.E., Galea, S., Uddin, M., Wildman, D., and Koenen, K.C. (2013). PTSD and obesity in the Detroit neighborhood health study. *Gen Hosp Psychiatry* 35**,** 671-673. doi: 10.1016/j.genhosppsych.2013.07.015.

Moore, L.D., Le, T., and Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology* 38**,** 23-38. doi: 10.1038/npp.2012.112.

Moore, S.W. (2003). Scrambled eggs: mechanical forces as ecological factors in early development. *Evol Dev* 5**,** 61-66.

Navarro-Mateu, F., Escamez, T., Koenen, K.C., Alonso, J., and Sanchez-Meca, J. (2013). Meta-analyses of the 5-HTTLPR polymorphisms and post-traumatic stress disorder. *PLoS One* 8**,** e66227. doi: 10.1371/journal.pone.0066227.

Nesse, R.M. (2001). The smoke detector principle. Natural selection and the regulation of defensive responses. *Ann N Y Acad Sci* 935**,** 75-85.

Nesse, R.M. (2005). Natural Selection and the regulation of defenses: A signal detection analysis of the smoke detector principle. *Evolution and Human Behavior* 26**,** 88-105.

Nevell, L., Zhang, K., Aiello, A.E., Koenen, K., Galea, S., Soliven, R., Zhang, C., Wildman, D.E., and Uddin, M. (2014). Elevated systemic expression of ER stress related genes is associated with stress-related mental disorders in the Detroit Neighborhood Health Study. *Psychoneuroendocrinology* 43**,** 62-70. doi: 10.1016/j.psyneuen.2014.01.013.

Newport, D.J., Heim, C., Owens, M.J., Ritchie, J.C., Ramsey, C.H., Bonsall, R., Miller, A.H., and Nemeroff, C.B. (2003). Cerebrospinal fluid corticotropin-releasing factor (CRF) and vasopressin concentrations predict pituitary response in the CRF stimulation test: a multiple regression analysis. *Neuropsychopharmacology* 28**,** 569-576. doi: 10.1038/sj.npp.1300071.

Nieratschker, V., Grosshans, M., Frank, J., Strohmaier, J., Von Der Goltz, C., El-Maarri, O., Witt, S.H., Cichon, S., Nothen, M.M., Kiefer, F., and Rietschel, M. (2012). Epigenetic alteration of the dopamine transporter gene in alcohol-dependent patients is associated with age. *Addict Biol*. doi: 10.1111/j.1369-1600.2012.00459.x.

Norris, F.H. (1992). Epidemiology of trauma: frequency and impact of different potentially traumatic events on different demographic groups. *J Consult Clin Psychol* 60**,** 409-418.

Obasaju, M.A., Palin, F.L., Jacobs, C., Anderson, P., and Kaslow, N.J. (2009). Won't you be my neighbor? Using an ecological approach to examine the impact of community on revictimization. *J Interpers Violence* 24**,** 38-53. doi: 10.1177/0886260508314933.

Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., and Devlin, A.M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* 3**,** 97-106.

Ooi, S.K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z., Erdjument-Bromage, H., Tempst, P., Lin, S.P., Allis, C.D., Cheng, X., and Bestor, T.H. (2007). DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* 448**,** 714-717. doi: 10.1038/nature05987.

Ouellet-Morin, I., Wong, C.C., Danese, A., Pariante, C.M., Papadopoulos, A.S., Mill, J., and Arseneault, L. (2013). Increased serotonin transporter gene (SERT) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins. *Psychol Med* 43**,** 1813-1823. doi: 10.1017/s0033291712002784.

Pace, T.W., and Heim, C.M. (2011). A short review on the psychoneuroimmunology of posttraumatic stress disorder: from risk factors to medical comorbidities. *Brain Behav Immun* 25**,** 6-13. doi: 10.1016/j.bbi.2010.10.003.

Paten, B., Herrero, J., Beal, K., Fitzgerald, S., and Birney, E. (2008). Enredo and Pecan: genome-wide mammalian consistency-based multiple alignment with paralogs. *Genome Res* 18**,** 1814-1828. doi: 10.1101/gr.076554.108.

Pavasovic, M., Richardson, N.A., Anderson, A.J., Mann, D., and Mather, P.B. (2004). Effect of pH, temperature and diet on digestive enzyme profiles in the mud crab, *Scylla serrata*. *Aquaculture* 242**,** 641-654.

Perroud, N., Paoloni-Giacobino, A., Prada, P., Olie, E., Salzmann, A., Nicastro, R., Guillaume, S., Mouthon, D., Stouder, C., Dieben, K., Huguelet, P., Courtet, P., and Malafosse, A. (2011). Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl Psychiatry* 1**,** e59. doi: 10.1038/tp.2011.60.

Perroud, N., Salzmann, A., Prada, P., Nicastro, R., Hoeppli, M.E., Furrer, S., Ardu, S., Krejci, I., Karege, F., and Malafosse, A. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Transl Psychiatry* 3**,** e207. doi: 10.1038/tp.2012.140.

Phillips, J.E., and Corces, V.G. (2009). CTCF: master weaver of the genome. *Cell* 137**,** 1194-1211. doi: 10.1016/j.cell.2009.06.001.

Pigliucci, M. (2010). "Phenotypic Plasticity," in *Evolution: The Extended Synthesis,* eds. M. Pigliucci & G.B. Muller. (Cambridge, MA: Massachusetts Institute of Technology Press).

Pitman, R.K. (2013). A Brief Nosological History of PTSD. *J Trauma Stress Disor Treat* 2. doi: 10.4172/2324-8947.1000101.

Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., Milad, M.R., and Liberzon, I. (2012). Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci* 13**,** 769-787. doi: 10.1038/nrn3339.

Plutarch *Plutarch's Lives.* New York, NY: Random House.

Poulter, M.O., Du, L., Weaver, I.C., Palkovits, M., Faludi, G., Merali, Z., Szyf, M., and Anisman, H. (2008). GABAA receptor promoter hypermethylation in suicide brain: implications for the involvement of epigenetic processes. *Biol Psychiatry* 64**,** 645-652. doi: 10.1016/j.biopsych.2008.05.028.

Provencal, N., Suderman, M.J., Caramaschi, D., Wang, D., Hallett, M., Vitaro, F., Tremblay, R.E., and Szyf, M. (2013). Differential DNA methylation regions in cytokine and transcription factor genomic loci associate with childhood physical aggression. *PLoS One* 8**,** e71691. doi: 10.1371/journal.pone.0071691.

Pruessner, J.C., Dedovic, K., Pruessner, M., Lord, C., Buss, C., Collins, L., Dagher, A., and Lupien, S.J. (2010). Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations - 2008 Curt Richter Award Winner. *Psychoneuroendocrinology* 35**,** 179-191. doi: 10.1016/j.psyneuen.2009.02.016.

Ptak, C., and Petronis, A. (2010). Epigenetic approaches to psychiatric disorders. *Dialogues Clin Neurosci* 12**,** 25-35.

Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. (1995). MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res* 23**,** 4878-4884.

Radant, A., Tsuang, D., Peskind, E.R., Mcfall, M., and Raskind, W. (2001). Biological markers and diagnostic accuracy in the genetics of posttraumatic stress disorder. *Psychiatry Res* 102**,** 203-215.

Rasmusson, A.M., Vythilingam, M., and Morgan, C.A., 3rd (2003). The neuroendocrinology of posttraumatic stress disorder: new directions. *CNS Spectr* 8**,** 651-656, 665-657.

Reik, W., Dean, W., and Walter, J. (2001). Epigenetic reprogramming in mammalian development. *Science* 293**,** 1089-1093. doi: 10.1126/science.1063443.

Renaud, S., Loukinov, D., Abdullaev, Z., Guilleret, I., Bosman, F.T., Lobanenkov, V., and Benhattar, J. (2007). Dual role of DNA methylation inside and outside of CTCF-binding regions in the transcriptional regulation of the telomerase hTERT gene. *Nucleic Acids Res* 35**,** 1245-1256. doi: 10.1093/nar/gkl1125.

Resnick, H.S., Kilpatrick, D.G., Dansky, B.S., Saunders, B.E., and Best, C.L. (1993). Prevalence of civilian trauma and posttraumatic stress disorder in a representative national sample of women. *J Consult Clin Psychol* 61**,** 984-991.

Ressler, K.J., Mercer, K.B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., Norrholm, S.D., Kilaru, V., Smith, A.K., Myers, A.J., Ramirez, M., Engel, A., Hammack, S.E., Toufexis, D., Braas, K.M., Binder, E.B., and May, V. (2011). Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 470**,** 492-497. doi: 10.1038/nature09856.

Reyes, J.C., Robles, R.R., Colon, H.M., Negron, J., Matos, T.D., Calderon, J., and Perez, O.M. (2008). Neighborhood disorganization, substance use, and violence

among adolescents in Puerto Rico. *J Interpers Violence* 23**,** 1499-1512. doi: 10.1177/0886260508314311.

Rhee, I., Bachman, K.E., Park, B.H., Jair, K.W., Yen, R.W., Schuebel, K.E., Cui, H., Feinberg, A.P., Lengauer, C., Kinzler, K.W., Baylin, S.B., and Vogelstein, B. (2002). DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* 416**,** 552-556. doi: 10.1038/416552a.

Robertson, K.D., Uzvolgyi, E., Liang, G., Talmadge, C., Sumegi, J., Gonzales, F.A., and Jones, P.A. (1999). The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. *Nucleic Acids Res* 27**,** 2291-2298.

Rohleder, N., Wolf, J.M., and Wolf, O.T. (2010). Glucocorticoid sensitivity of cognitive and inflammatory processes in depression and posttraumatic stress disorder. *Neurosci Biobehav Rev* 35**,** 104-114. doi: 10.1016/j.neubiorev.2009.12.003.

Rosenbloom, K.R., Dreszer, T.R., Pheasant, M., Barber, G.P., Meyer, L.R., Pohl, A., Raney, B.J., Wang, T., Hinrichs, A.S., Zweig, A.S., Fujita, P.A., Learned, K., Rhead, B., Smith, K.E., Kuhn, R.M., Karolchik, D., Haussler, D., and Kent, W.J. (2010). ENCODE whole-genome data in the UCSC Genome Browser. *Nucleic Acids Res* 38**,** D620-625. doi: 10.1093/nar/gkp961.

Roth, T.L., Lubin, F.D., Funk, A.J., and Sweatt, J.D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biol Psychiatry* 65**,** 760-769. doi: 10.1016/j.biopsych.2008.11.028.

Rusiecki, J.A., Byrne, C., Galdzicki, Z., Srikantan, V., Chen, L., Poulin, M., Yan, L., and Baccarelli, A. (2013). PTSD and DNA Methylation in Select Immune Function

Gene Promoter Regions: A Repeated Measures Case-Control Study of U.S. Military Service Members. *Front Psychiatry* 4**,** 56. doi: 10.3389/fpsyt.2013.00056.

Rusiecki, J.A., Chen, L., Srikantan, V., Zhang, L., Yan, L., Polin, M.L., and Baccarelli, A. (2012). DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members. *Epigenomics* 4**,** 29-40. doi: 10.2217/epi.11.116.

Rutter, M., Moffitt, T.E., and Caspi, A. (2006). Gene-environment interplay and psychopathology: multiple varieties but real effects. *J Child Psychol Psychiatry* 47**,** 226-261. doi: 10.1111/j.1469-7610.2005.01557.x.

Schwartz, A.C., Bradley, R.L., Sexton, M., Sherry, A., and Ressler, K.J. (2005). Posttraumatic stress disorder among African Americans in an inner city mental health clinic. *Psychiatr Serv* 56**,** 212-215. doi: 10.1176/appi.ps.56.2.212.

Scrimin, S., Moscardino, U., and Natour, M. (2014). Socio-ecological correlates of mental health among ethnic minorities in areas of political conflict: a study of Druze adolescents in Israel. *Transcult Psychiatry* 51**,** 209-227. doi: 10.1177/1363461513520342.

Segman, R.H., Shefi, N., Goltser-Dubner, T., Friedman, N., Kaminski, N., and Shalev, A.Y. (2005). Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol Psychiatry* 10**,** 500-513, 425. doi: 10.1038/sj.mp.4001636.

Seisenberger, S., Peat, J.R., Hore, T.A., Santos, F., Dean, W., and Reik, W. (2013). Reprogramming DNA methylation in the mammalian life cycle: building and

breaking epigenetic barriers. *Philos Trans R Soc Lond B Biol Sci* 368**,** 20110330. doi: 10.1098/rstb.2011.0330.

Shalev, A.Y. (2001). What is posttraumatic stress disorder? *J Clin Psychiatry* 62 Suppl 17**,** 4-10.

Shay, J. (1994). *Achilles in Vietnam: combat trauma and the undoing of character.* New York, NY: Scribner.

Sherin, J.E., and Nemeroff, C.B. (2011). Post-traumatic stress disorder: the neurobiological impact of psychological trauma. *Dialogues Clin Neurosci* 13**,** 263-278.

Shrout, P.E.a.F., J. (1979). Intraclass Correlations: Uses in Assessing Rater Reliability. *Psychological Bulletin* 86**,** 420-428.

Siegmund, A., and Wotjak, C.T. (2006). Toward an animal model of posttraumatic stress disorder. *Ann N Y Acad Sci* 1071**,** 324-334. doi: 10.1196/annals.1364.025.

Siegmund, K.D., Connor, C.M., Campan, M., Long, T.I., Weisenberger, D.J., Biniszkiewicz, D., Jaenisch, R., Laird, P.W., and Akbarian, S. (2007). DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. *PLoS One* 2**,** e895. doi: 10.1371/journal.pone.0000895.

Silove, D. (1998). Is posttraumatic stress disorder an overlearned survival response? An evolutionary-learning hypothesis. *Psychiatry* 61**,** 181-190.

Simpson, T.L. (2002). Women's treatment utilization and its relationship to childhood sexual abuse history and lifetime PTSD. *Subst Abus* 23**,** 17-30. doi: 10.1080/08897070209511472.

Sipahi, L., Wildman, D., Aiello, A., Koenen, K., Galea, S., Abbas, A., and Uddin, M. (2014). Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder. *Psychol Med*. doi: http://dx.doi.org/10.1017/S0033291714000968.

Slater, E., and Slater, P. (1944). A HEURISTIC THEORY OF NEUROSIS. *J Neurol Psychiatry* 7**,** 49-55.

Smith, A.K., Conneely, K.N., Kilaru, V., Mercer, K.B., Weiss, T.E., Bradley, B., Tang, Y., Gillespie, C.F., Cubells, J.F., and Ressler, K.J. (2011a). Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 156b**,** 700-708. doi: 10.1002/ajmg.b.31212.

Smith, A.K., Conneely, K.N., Kilaru, V., Mercer, K.B., Weiss, T.E., Bradley, B., Tang, Y., Gillespie, C.F., Cubells, J.F., and Ressler, K.J. (2011b). Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* 156**,** 700-708. doi: 10.1002/ajmg.b.31212.

Solovieff, N., Roberts, A.L., Ratanatharathorn, A., Haloosim, M., De Vivo, I., King, A.P., Liberzon, I., Aiello, A., Uddin, M., Wildman, D.E., Galea, S., Smoller, J.W., Purcell, S.M., and Koenen, K.C. (2014). Genetic Association Analysis of 300 Genes Identifies a Risk Haplotype in SLC18A2 for Post-traumatic Stress Disorder in Two Independent Samples. *Neuropsychopharmacology*. doi: 10.1038/npp.2014.34.

Springer, S.A., Crespi, B.J., and Swanson, W.J. (2011). Beyond the phenotypic gambit: molecular behavioural ecology and the evolution of genetic architecture. *Mol Ecol* 20**,** 2240-2257. doi: 10.1111/j.1365-294X.2011.05116.x.

Stein, M.B., Jang, K.L., Taylor, S., Vernon, P.A., and Livesley, W.J. (2002). Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. *Am J Psychiatry* 159**,** 1675-1681.

Sterner, K.N., Weckle, A., Chugani, H.T., Tarca, A.L., Sherwood, C.C., Hof, P.R., Kuzawa, C.W., Boddy, A.M., Abbas, A., Raaum, R.L., Gregoire, L., Lipovich, L., Grossman, L.I., Uddin, M., Goodman, M., and Wildman, D.E. (2012). Dynamic gene expression in the human cerebral cortex distinguishes children from adults. *PLoS ONE* 7**,** e37714. doi: 10.1371/journal.pone.0037714.

Su, Y.A., Wu, J., Zhang, L., Zhang, Q., Su, D.M., He, P., Wang, B.D., Li, H., Webster, M.J., Rennert, O.M., and Ursano, R.J. (2008). Dysregulated mitochondrial genes and networks with drug targets in postmortem brain of patients with posttraumatic stress disorder (PTSD) revealed by human mitochondria-focused cDNA microarrays. *Int J Biol Sci* 4**,** 223-235.

Surget, A., and Belzung, C. (2008). Involvement of vasopressin in affective disorders. *Eur J Pharmacol* 583**,** 340-349. doi: 10.1016/j.ejphar.2007.11.065.

Szyf, M. (2011). DNA methylation, the early-life social environment and behavioral disorders. *J Neurodev Disord* 3**,** 238-249. doi: 10.1007/s11689-011-9079-2.

Tolin, D.F., and Foa, E.B. (2006). Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychol Bull* 132**,** 959-992. doi: 10.1037/0033-2909.132.6.959.

Toyokawa, S., Uddin, M., Koenen, K.C., and Galea, S. (2012). How does the social environment 'get into the mind'? Epigenetics at the intersection of social and psychiatric epidemiology. *Soc Sci Med* 74**,** 67-74. doi: 10.1016/j.socscimed.2011.09.036.

True, W.R., Rice, J., Eisen, S.A., Heath, A.C., Goldberg, J., Lyons, M.J., and Nowak, J. (1993). A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch Gen Psychiatry* 50**,** 257-264.

Tsigos, C., and Chrousos, G.P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* 53**,** 865-871.

Tyler, K.A., and Johnson, K.A. (2006). A longitudinal study of the effects of early abuse on later victimization among high-risk adolescents. *Violence Vict* 21**,** 287-306.

Tyrka, A.R., Price, L.H., Marsit, C., Walters, O.C., and Carpenter, L.L. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS One* 7**,** e30148. doi: 10.1371/journal.pone.0030148.

Uddin, M., Aiello, A.E., Wildman, D.E., Koenen, K.C., Pawelec, G., De Los Santos, R., Goldmann, E., and Galea, S. (2010). Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A* 107**,** 9470-9475. doi: 0910794107 [pii]

10.1073/pnas.0910794107.

Uddin, M., Chang, S.C., Zhang, C., Ressler, K., Mercer, K.B., Galea, S., Keyes, K.M., Mclaughlin, K.A., Wildman, D.E., Aiello, A.E., and Koenen, K.C. (2013). Adcyap1r1 genotype, posttraumatic stress disorder, and depression among

women exposed to childhood maltreatment. *Depress Anxiety* 30**,** 251-258. doi: 10.1002/da.22037.

Uddin, M., Galea, S., Chang, S.C., Aiello, A.E., Wildman, D.E., De Los Santos, R., and Koenen, K.C. (2011a). Gene expression and methylation signatures of MAN2C1 are associated with PTSD. *Dis Markers* 30**,** 111-121. doi: 10.3233/dma-2011-0750.

Uddin, M., Koenen, K.C., Aiello, A.E., Wildman, D.E., De Los Santos, R., and Galea, S. (2011b). Epigenetic and inflammatory marker profiles associated with depression in a community-based epidemiologic sample. *Psychol Med* 41**,** 997-1007. doi: 10.1017/s0033291710001674.

Uddin, M., and Sipahi, L. (2013). "Epigenetic influences on mental illness over the life course," in *A Life Course Approach to Mental Disorders,* ed. K.R. Koenen, S.; Susser, E.; Galea, S. (Oxford: Oxford University Press).

Unternaehrer, E., Luers, P., Mill, J., Dempster, E., Meyer, A.H., Staehli, S., Lieb, R., Hellhammer, D.H., and Meinlschmidt, G. (2012). Dynamic changes in DNA methylation of stress-associated genes (OXTR, BDNF) after acute psychosocial stress. *Transl Psychiatry* 2**,** e150. doi: 10.1038/tp.2012.77.

Valena, S., and Moczek, A.P. (2012). Epigenetic Mechanisms Underlying Developmental Plasticity in Horned Beetles. *Genetics Research International* 2012. doi: http://dx.doi.org/10.1155/2012/576303.

Veldic, M., Caruncho, H.J., Liu, W.S., Davis, J., Satta, R., Grayson, D.R., Guidotti, A., and Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively

overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proc Natl Acad Sci U S A* 101**,** 348-353. doi: 10.1073/pnas.2637013100.

Veldic, M., Guidotti, A., Maloku, E., Davis, J.M., and Costa, E. (2005). In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. *Proc Natl Acad Sci U S A* 102**,** 2152-2157. doi: 10.1073/pnas.0409665102.

Villagomez, R.E., Meyer, T.J., Lin, M.M., and Brown, L.S., Jr. (1995). Post-traumatic stress disorder among inner city methadone maintenance patients. *J Subst Abuse Treat* 12**,** 253-257.

Wadhwa, P.D., Buss, C., Entringer, S., and Swanson, J.M. (2009). Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. *Semin Reprod Med* 27**,** 358-368. doi: 10.1055/s-0029-1237424.

Wang, H., Maurano, M.T., Qu, H., Varley, K.E., Gertz, J., Pauli, F., Lee, K., Canfield, T., Weaver, M., Sandstrom, R., Thurman, R.E., Kaul, R., Myers, R.M., and Stamatoyannopoulos, J.A. (2012). Widespread plasticity in CTCF occupancy linked to DNA methylation. *Genome Res* 22**,** 1680-1688. doi: 10.1101/gr.136101.111.

Waterland, R.A., and Michels, K.B. (2007). Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr* 27**,** 363-388. doi: 10.1146/annurev.nutr.27.061406.093705.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J. (2004). Epigenetic programming by maternal behavior. *Nat Neurosci* 7**,** 847-854. doi: 10.1038/nn1276

nn1276 [pii].

Weaver, I.C., Champagne, F.A., Brown, S.E., Dymov, S., Sharma, S., Meaney, M.J., and Szyf, M. (2005). Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci* 25**,** 11045-11054. doi: 10.1523/jneurosci.3652-05.2005.

Wells, C.L., and Pigliucci, M. (2000). Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3**,** 1-18.

West-Eberhard, M. (2003). *Developmental Plasticity and Evolution.* New York: Oxford University Press.

Whirl-Carrillo, M., Mcdonagh, E.M., Hebert, J.M., Gong, L., Sangkuhl, K., Thorn, C.F., Altman, R.B., and Klein, T.E. (2012). Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther* 92**,** 414-417. doi: 10.1038/clpt.2012.96.

Wong, C.M. (2002). Post-traumatic stress disorder: advances in psychoneuroimmunology. *Psychiatr Clin North Am* 25**,** 369-383, vii.

Wong, N., Morley, R., Saffery, R., and Craig, J. (2008). Archived Guthrie blood spots as a novel source for quantitative DNA methylation analysis. *Biotechniques* 45**,** 423-424, 426, 428 passim. doi: 10.2144/000112945.

Xie, P., Kranzler, H.R., Poling, J., Stein, M.B., Anton, R.F., Farrer, L.A., and Gelernter, J. (2010). Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. *Neuropsychopharmacology* 35**,** 1684-1692. doi: 10.1038/npp.2010.37.

Yehuda, R. (2001). Biology of posttraumatic stress disorder. *J Clin Psychiatry* 62 Suppl 17**,** 41-46.

Yehuda, R. (2006). Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications. *Ann N Y Acad Sci* 1071**,** 137-166. doi: 10.1196/annals.1364.012.

Yehuda, R., Cai, G., Golier, J.A., Sarapas, C., Galea, S., Ising, M., Rein, T., Schmeidler, J., Muller-Myhsok, B., Holsboer, F., and Buxbaum, J.D. (2009). Gene expression patterns associated with posttraumatic stress disorder following exposure to the World Trade Center attacks. *Biol Psychiatry* 66**,** 708-711. doi: 10.1016/j.biopsych.2009.02.034.

Yehuda, R., Flory, J.D., Pratchett, L.C., Buxbaum, J., Ising, M., and Holsboer, F. (2010). Putative biological mechanisms for the association between early life adversity and the subsequent development of PTSD. *Psychopharmacology (Berl)* 212**,** 405-417. doi: 10.1007/s00213-010-1969-6.

Yehuda, R., Koenen, K.C., Galea, S., and Flory, J.D. (2011). The role of genes in defining a molecular biology of PTSD. *Dis Markers* 30**,** 67-76. doi: 10.3233/dma-2011-0794.

Yehuda, R., and Ledoux, J. (2007). Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron* 56**,** 19-32. doi: 10.1016/j.neuron.2007.09.006.

Young, A. (1995). *The Harmony of Illusions. Inventing Post-Traumatic Stress Disorder.* Princeton: Princeton University Press.

Zayfert, C., Dums, A.R., Ferguson, R.J., and Hegel, M.T. (2002). Health functioning impairments associated with posttraumatic stress disorder, anxiety disorders, and depression. *J Nerv Ment Dis* 190**,** 233-240.

Zeng, J., Konopka, G., Hunt, B.G., Preuss, T.M., Geschwind, D., and Yi, S.V. (2012). Divergent whole-genome methylation maps of human and chimpanzee brains reveal epigenetic basis of human regulatory evolution. *Am J Hum Genet* 91**,** 455-465. doi: 10.1016/j.ajhg.2012.07.024.

Zhao, J., Goldberg, J., Bremner, J.D., and Vaccarino, V. (2013). Association between promoter methylation of serotonin transporter gene and depressive symptoms: a monozygotic twin study. *Psychosom Med* 75**,** 523-529. doi: 10.1097/PSY.0b013e3182924cf4.

Zieker, J., Zieker, D., Jatzko, A., Dietzsch, J., Nieselt, K., Schmitt, A., Bertsch, T., Fassbender, K., Spanagel, R., Northoff, H., and Gebicke-Haerter, P.J. (2007). Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. *Mol Psychiatry* 12**,** 116-118. doi: 10.1038/sj.mp.4001905.

ABSTRACT

PROXIMATE AND EVOLUTIONARY INSIGHTS INTO THE EPIGENETICS OF POSTTRAUMATIC STRESS DISORDER

by

LEVENT H SIPAHI

May 2017

Advisor: Dr. Derek E. Wildman

Major: Molecular Biology and Genetics

Degree: Doctor of Philosophy

Posttraumatic stress disorder (PTSD) is an important medical and social condition. Although the vast majority of individuals are exposed to traumatic events within their lifetime, a minority subsequently develop diagnosable PTSD. What underlies differential risk and resiliency in the face of trauma is an ongoing research and clinical question with implications for prevention and treatment. Recent work has revealed a putative role of epigenetic variation and modification – most notably DNA methylation – in the etiology of PTSD. That DNA methylation is stable, yet modifiable in response to lived experiences, makes it a strong candidate to mechanistically explain the ontogeny of PTSD by putatively linking genetic and environment effects. Here, I provide proximate and evolutionary insights into this biological capacity through three distinct, but conceptually related research projects. In the first, I compare DNA methylation longitudinally in trauma-exposed individuals with and without PTSD to reveal a complex role of epigenetic variation and modification in PTSD development. In the second, I utilize phylogenetic methods to infer and characterize the evolutionary history of genetic loci necessary for the epigenetic regulation of PTSD, revealing that

this capacity may have ancient origins. In the third, I test, and fail to provide evidence for, genetic associations between PTSD risk and single nucleotide polymorphisms (SNPs) annotated to genes involved in the regulation and activity of the hypothalamic-pituitary-adrenal (HPA) axis. Taken together, these data are suggestive of an evolutionarily-conserved capacity to regulate behavior, physiology, and psychology in response to extreme traumatic experiences. I argue that this capacity is epigenetically regulated and represents an example of adaptive developmental phenotypic plasticity. This model of PTSD etiology has implications for policy, clinical practice, public health, and research.

AUTOBIOGRAPHICAL STATEMENT

LEVENT H SIPAHI

EDUCATION

PhD	2010-2014	Wayne State University School of Medicine, Center for Molecular Medicine and Genetics and the MD/PhD Program
MD	2008-2017	Wayne State University School of Medicine, MD/PhD Program
BA	2004-2008	Kalamazoo College, Biology

ACADEMIC HONORS & AWARDS

2013	Honorable mention poster, 29th Annual Meeting of the International Society for Traumatic Stress Studies
2013	Second place poster, Wayne State University School of Medicine Graduate Student Research Day
2013	First place presentation, Wayne State University MD/PhD Research Day
2013	Ingenuity Systems Grant Program Awardee
2012-2013	Graduate Research Assistantship, Wayne State University Graduate School and Office of the Vice President for Research
2012	Sigma Xi Grant in Aid of Research
2012	Competitive Travel Grant, Society for Molecular Biology and Evolution
2008-2011	Board of Governors Medical Scholarship, Wayne State University
2008	Honors in Biology, Magna Cum Laude, Kalamazoo College
2007	Diebold Research Fellowship, Kalamazoo College Biology Department

PUBLICATIONS

Sipahi L, Wildman DE, Aiello AE, Koenen KC, Galea S, Abbas A, and Uddin M. Longitudinal epigenetic variation of DNA methyltransferase genes is associated with vulnerability to post-traumatic stress disorder. *Psychological Medicine* (2014) DOI: http://dx.doi.org/10.1017/S0033291714000968.

Sipahi L, Uddin M, Hou Z, Aiello AE, Koenen KC, Galea S, and Wildman DE (2014) Ancient evolutionary origins of epigenetic regulation associated with posttraumatic stress disorder. *Front. Hum. Neurosci.* **8:284.** doi: 10.3389/fnhum.2014.00284.

Uddin M, **Sipahi L**, Li J, Koenen KC (2013) Sex differences in DNA methylation may contribute to risk of ptsd and depression: a review of existing evidence. *Depression and Anxiety* 30(12): 1151-60.

Uddin M, **Sipahi L**. Epigenetic influences on mental illness over the life course (2013). In “A Life Course Approach to Mental Disorders” Eds. Karestan C. Koenen, Sasha Rudenstine, Ezra Susser, and Sandro Galea. Oxford University Press, Oxford.

Zhong W, Xie Y, Abdallah M, Awonuga AD, Slater JA, **Sipahi L**, Puscheck EE, Rappolee DA (2010) Cellular stress causes reversible, PRKAA1/2-, and proteasome-dependent ID2 protein loss in trophoblast stem cells. *Reproduction* 140(6):921-30.