Original article

A review of epigenetic contributions to post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) is a syndrome which serves as a classic example of psychiatric disorders that result from the intersection of nature and nurture, or gene and environment. By definition, PTSD requires the experience of a traumatic exposure, and yet data suggest that the risk for PTSD in the aftermath of trauma also has a heritable (genetic) component. Thus, PTSD appears to require both a biological (genetic) predisposition that differentially alters how the individual responds to or recovers from trauma exposure. Epigenetics is defined as the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself, and more recently it has come to refer to direct alteration of DNA regulation, but without altering the primary sequence of DNA, or the genetic code. With regards to PTSD, epigenetics provides one way for environmental exposure to be "written" upon the genome, as a direct result of gene and environment (trauma) interactions. This review provides an overview of the main currently understood types of epigenetic regulation, including DNA methylation, histone regulation of chromatin, and noncoding RNA regulation of gene expression. Furthermore, we examine recent literature related to how these methods of epigenetic regulation may be involved in differential risk and resilience for PTSD in the aftermath of trauma.

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Introduction

Post-traumatic stress disorder (PTSD) is a *Diagnostic and Statistical Manual of Mental Disorders* 5th ed. (*DSM-5*) diagnosis marked by the development of stressor-related symptoms following one or more traumatic events.[1] Outlined in the *DSM-5*, a traumatic event is defined as exposure to actual or threatened death, serious injury, or sexual violence that is experienced directly, witnessed, experienced vicariously through family or close friends, or experienced repeatedly or with extreme exposure to aversive details of the traumatic event. Diagnostic criteria and symptomology include the following: intrusive symptoms; avoidance of stimuli associated with the traumatic event; negative alterations in cognition and mood; alterations in arousal and reactivity associated with the traumatic event; and in some cases, dissociative symptoms.[1]

While around 50% to 60% of the population will experience traumatic stress over the course of their lifetime, the lifetime prevalence for PTSD using *DSM-IV* criteria has been estimated at around 8.7%.[2,3] The disparity between trauma exposure and the development of trauma-related disorders has garnered much interest, and our understanding of what contributes to this susceptibility or resilience is still limited.[4,5] The old debate of nature versus nurture sought answers in a single domain; however, as our understanding continues to evolve, it has become clear that—like many

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other mental disorders–PTSD development is heavily influenced by an interplay between environmental factors and genetic predisposition or heritability. The study of epigenetics bridges both sides of this debate and focuses on the changes in gene expression that may be caused by our environment. In our review on the epigenetics of PTSD, we will discuss the heritability of this disorder and give an overview of epigenetic mechanisms, targets, genome-wide association studies (GWAS), and epigenome-wide association studies (EWAS) conducted to date, and future directions for the field.

Epigenetic approaches may afford novel targeted therapeutic approaches to enhance treatment and prevention of PTSD

Heritability

Diagnosis of PTSD is reliant on environmental influence through a traumatic event. Given this fact, it may seem backwards then to study the heritability of a disorder that requires an outside event for its manifestation. Contrary to what may seem intuitive of a disorder with this diagnostic criterion, research suggests that genes do play a role, and perhaps a significant one, in the risk of developing PTSD.

Twin studies serve as an invaluable tool to parse out genetic and environmental factors and contribute in concert with newer molecular and genetic methods to help piece together a complete picture.[6] In one twin study, heritable influences accounted for 46% of the variance in PTSD, and for 71% of variance in females.[7,8] Another study suggested that exposure to assaultive trauma (robbery, sexual assault, and other life-threatening events) may not be entirely random and is influenced by individual and familiar risk factors.[9] Indeed, it is known that parental post-traumatic stress can cause negative psychological outcomes and potential biological alterations in their offspring, with several studies indicating that severity of a parent's PTSD symptoms may contribute to a child's psychological difficulties—namely anxiety, depression, and behavior problems.[10,11] Furthermore, childhood adversity has been strongly implicated in the development of many psychiatric disorders, and individuals who experience these early life adversities are at greater risk for PTSD in adulthood.[12,13] The psychological collateral of trauma-related distress can percolate through the family unit, potentially exacerbating risk factors that may lead to the development of future psychological distress or disorder.

In addition, PTSD has common comorbidity with other mental disorders, namely major depression, substance abuse, and other anxiety disorders.[14] While the *DSM-5* is limited by its definitions and diagnostic criteria, genetic evidence suggests that these disorders may fall on a spectrum rather than being entirely independent entities.

Epigenetic mechanisms

Data from twin studies suggests that PTSD is at least partially heritable and, by definition, requires influence from environmental trauma.[9] Epigenetics is defined as the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself, and more recently it has come to refer to direct alteration of DNA regulation, but without altering the primary sequence of DNA, or the genetic code. With regards to PTSD, epigenetics provides one way for environmental exposure to be "written" upon the genome, as a direct result of gene and environment (trauma) interactions. The epigenome is influenced by both genetic and environmental factors—the environment in effect is written onto the genes themselves. While epigenetics does not change the sequence of the DNA code, it does alter the expression of genes and may contribute to long-lasting–in some cases intergenerational—phenotypic effects.[15] There are several mechanisms that drive this process, three of which have been widely studied.

Histone modification

Histone proteins (H2A, H2B, H3, H4) help organize DNA into structured units called nucleosomes. Nucleosomes are packaged units formed by spooling the DNA sequence of 200 nucleotide base pairs around eight histones (octamer), which help to compact the DNA. The nucleosomes can be thought of as "beads" and are connected by linker DNA forming a collection of "beads on a string" called chromatin. Chemical alteration or modification of histones–through acetylation or deacetylation–influences the structure of chromatin, remodeling it to either coil or uncoil and altering the ability of RNA polymerase to transcribe genes. Thus, histone acetylation or deacetylation regulates the extent to which a gene is expressed by altering chromatin structures. Histone regulation has been implicated in a number of activ-

ities in the brain related to emotion regulation, for example traumatic memory encoding and fear extinction,[16] which is an important process that is dysregulated in PTSD.

Histone acetylation occurs when the enzyme histone acetyltransferase (HAT) interacts with the histone protein, adding an acetyl group to lysine residues in the N-terminal tail of the histone protein. Acetylation of histones by HAT causes the uncoiling or loosening of DNA, creating decondensed and "open" chromatin structure (euchromatin), which allows access to the DNA by proteins involved in the transcriptional machinery, copying the DNA sequence into RNA. Conversely, histone deacetylation occurs when histone deacetylase (HDAC) removes the acetyl group added by the HATs. Deacetylation of histones by HDAC causes the coiling of DNA, creating condensed and "closed" chromatin structure (heterochromatin) making it densely packed and more difficult to transcribe—in effect inhibiting or repressing gene expression. Histone methylation provides yet another mechanism of histone regulation, mediated by histone methyltransferases. Generally, histone methylation is thought to have an opposite effect to histone acetylation, generally consistent with condensing chromatin structure, though this is also dependent on which specific amino acid components of the histones are modified. In summary, without changing the DNA sequence or even directly modifying the DNA chemical structure, this process of histone modification allows genes to be turned on and off by making regions of DNA either accessible or inaccessible to the transcriptional machinery.

DNA methylation

DNA modification through direct methylation is one epigenetic process that has been widely studied in PTSD. Although there are more than 20 identified DNA modifications,[17] 5-methylcytosine (5-mc) and 5-hydroxymethylcytosine (5-hmc)[18] are two types of methylation-related modifications that are highly prevalent in neurons related to known processes involved in PTSD, such as learning and extinction of conditioned fear.[19-21] DNA methylation changes within a gene can occur at any stage during the life cycle of a cell,[22] and they have been characterized to make a long-term impact on transcriptional response due to different stress-related environmental factors, including early adverse life events.[23,24]

There have been many reports of increased and decreased DNA methylation in response to exposure of stressful life events. Thus, it is important to understand the mechanism of both addition and removal of methyl groups. Methylation is mediated by DNA methyltransferase proteins, DNMT3a and DNMT3b, to add a methyl group to an unmethylated cytosine C5 position.[25] The oxidation of 5-methylcytosine to 5-hydroxymethylcytosine is mediated by ten-eleven translocation proteins (TET1, TET2, and TET3); hydroxymethylcytosine is an intermediate step in DNA demethylation. DNA-binding proteins have also shown to be involved in active demethylation of DNA, and other proteins involved in dynamic transcriptional activation or repression can also "recruit" DNMT and TET proteins, leading to a longer-lasting alteration in DNA methylation status.[26,27]

One example of DNA methylation findings are the role of differential methylation at the FKBP5 gene, which is further outlined below as a critical regulator of the HPA cortisol response. DNA demethylation at glucocorticoid receptor binding site (GREs) within the *FKBP5* gene in peripheral blood cells and hippocampal progenitor cells was found to be associated with prior exposure to childhood abuse.[23] Recent studies also suggest that epigenetic marks might be transmitted down to the next generation, influencing the risk of diseases in offspring,[28,29] though these have typically been small or underpowered studies that require expansion and replication.

Noncoding RNA

Noncoding RNAs (ncRNAs) are transcripts from DNA, but unlike other RNAs, ncRNAs are not translated into a polypeptide or protein sequence. ncRNAs are functional and are involved in the processing and regulation of other RNAs such as messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).[30,31] Recent more detailed reviews highlight the role of different types of ncRNAs, such as micro RNA (miRNA), long noncoding RNA (lncRNA), and retrotransposons, that may become useful biomarkers for trauma-related brain disorders such as PTSD.[32]

miRNAs are characterized by 21 to 24 nucleotides in length. They are thought to generally bind to the 3' untranslated region or other untranslated regions of their target mRNAs to regulate gene expression.[33] Studies have reported the miRNA expression level changes in experimental stress models.[34-36] One of those studies linked to hypothalamus-

pituitary-adrenal (HPA) axis pathways identified a specific miRNA, miR-34c, to be upregulated in a stress-dependent manner in mouse amygdala tissue.[37] In rodents, miRNA also seem to be regulating glucocorticoid receptor (GR) function through post-transcriptional effects that are sensitive to stress exposure, thus influencing the regulation of GR-regulated downstream genes to alter the behavioral response to stress.[34]

lncRNAs are characterized by longer than 200 nucleotides in length and are also involved in regulation of gene expression in PTSD.[38] Although there are not many studies on the role of lncRNAs in PTSD, Guffanti et al previously identified a single nucleotide variant lncRNA- lncRNA-ALINC01090 (previously called AC068718.1) that reached genome-wide significance in a GWAS of PTSD.[39]

Candidate gene studies

While twin studies suggest a genetic component to PTSD, candidate gene studies aid in identifying specific genes that may be associated with the disorder. Candidate gene designs examine the main effect of specific genes on expression of a disorder and typically focus on biological candidates that are selected using existing biological evidence.[4,5] That said, there has been much pushback, even controversy, as to the use of candidate gene studies in recent years, as large-scale GWAS studies have been possible and have expanded markedly. Few if any prior candidate genes have been replicated in a well-powered GWAS, though the Million Veteran's Program study appears to have found an HPA-related gene, corticotrophin releasing factor receptor (*CRFR1*), associated with hyperarousal in PTSD.[40] Additionally, without well-defined pathophysiology of a disease—as is the case for psychiatric disorders—it is not always straightforward to define candidate genes. Overall, prior candidate gene studies need to be regarded with skepticism until larger-scale replications and extensions have more definitively demonstrated their involvement or lack thereof.

With those caveats in mind, we feel it is still worthwhile, even if for historical purposes, to mention the work to date. The current literature on the genetic markers for PTSD spans more than 100 studies published since 1991: for a detailed overview of this literature, we direct readers to several comprehensive reviews.[4,5,41-44] The present review focuses on the HPA axis and FK506 binding protein 51 (*FKBP5*), discussing several of the more robust studies examining this target.

Repeated exposure to trauma alters endocrine mechanisms involved in the stress response. The hypothalamic-pituitary-adrenal (HPA) stress axis—in addition to filling many other roles such as immune and metabolic function—guides the endocrine response to stress.[45] HPA axis dysregulation has been observed in both depression and PTSD, though each disorder appears to have unique manifestations.[45] The effects of this dysregulation change the HPA axis function, altering its response to cortisol feedback. This is thought to be associated with physiological and psychological emotion dysregulation and stress hyper-responsiveness, both of which are implicated in PTSD.[41] Evidence also suggests that early life adversity may contribute to epigenetic changes in the HPA axis which may impact the development of PTSD.[45]

FK506 binding protein 51 gene

The FK506 binding protein 51 (*FKBP5*) gene is perhaps the most comprehensively studied candidate among genes related to the HPA axis. It is believed to be an important regulator of stress response through altering GR sensitivity.[5,46] Through its role as both an inhibitor of GR translocation to the nucleus, but also an exquisitely stress- and GR-responsive gene, it is thought to act as a rapid, intracellular feedback regulator of GR sensitivity within the cell. *FKBP5* has shown strong association with PTSD in conjunction with a history of childhood trauma/abuse when examined in gene-environment interaction (GxE) studies.[23,47,48] One such study identified allele-specific, early trauma exposure-dependent demethylation of CpGs in *FKBP5*, which suggests a *FKBP5* x child abuse interaction resulting in differential (?upregulated) transcriptional activation of FKBP5 in response to childhood abuse.[23] Wang et al systematically reviewed interactions between FKBP5, early life stress, and risk for PTSD. Results from this meta-analysis revealed a significant interaction between the T allele of *rs1360780* and early life stress in those with PTSD. The C-allele of *rs3800373* and the T-allele of *rs9470080* also interacted with early life stress and predicted higher risk for PTSD.[49] A second more recent meta-analysis reaffirmed these findings and adds to the mounting evidence of an overall effect of *FKBP5* interacting with trauma exposure on PTSD.[50] Although the precise mechanisms are still to be understood, a working

hypothesis is that exposure to prior trauma, particularly early life stress, interacts with stress-sensitive genotypes through long-lasting DNA methylation changes in *FKBP5*, leading to greater stress responsiveness later in life through altered GR sensitivity.

Epigenetics of intergenerational transmission of stress

Recent mechanistic studies using animal models have investigated the effects of stress on epigenetic machinery and how future generations may be affected. Rodent studies examining parental care influences revealed that differences in maternal phenotypes, namely grooming behavior, had effects on their pup's development of behavioral and HPA responses to stress as adults.[51] Further studies examining DNA methylation in high vs low maternal care parental behaviors revealed elevated DNA methylation levels in the offspring after low maternal care, and lower methylation in those with high maternal care—potentially contributing to reduced transcriptional activation of the GR in the low-maternal care offspring.[23,51] In humans, Yehuda et al examined intergenerational effects of trauma in Holocaust survivors and their offspring through measuring cytosine methylation within the *FKBP5* gene. Results revealed differential findings for survivors and their offspring, with higher levels of methylation in survivors compared with controls, and lower in offspring, further demonstrating the potential of trauma influences on epigenetic mechanisms to have intergenerational effects.[52] While these studies have small sample sizes and need replication in much larger cohorts, as discussed below with genome-wide studies, they provide intriguing initial insight into gene regulation as a function of epigenetic alterations in trauma-related symptoms and syndromes.

Genome-wide association studies

The current most powerful and robust method to study the interplay between genetics and PTSD uses genome-wide association studies (GWAS) to provide an unbiased approach to identify loci in the genome that have association with PTSD. Large-scale GWAS compare hundreds of thousands of single-nucleotide polymorphisms (SNP) across the entire genome to identify variants that may have a causal effect on the disorder. Only in recent years has the fiscal feasibility of using this method become possible with a drastic 2000-fold reduction in cost per genotype in a 10-year period.[44] A major challenge with this approach, however, is amassing a large enough sample to achieve the required statistical power to detect these loci—with a statistical *P*-value threshold of $5x10^{-8}$ required for the multiple test correction after examining roughly a million SNPs per individual.[44] The Psychiatric Genomic Consortium (PGC) was organized in 2007 to centralize the GWAS from around the world and to adequately power analyses.[42] Subsequently they are now the largest collaboration in the history of psychiatry, with more than 250 000 subjects, and the inclusion of more than 500 scientists from 100 countries.[42]

Already other mental disorders such as schizophrenia, depression, and bipolar disorder have utilized GWAS successfully to identify genes and molecular pathways of interest, and only recently has focus turned to PTSD—with the first GWAS recently published in 2013.[53] We have identified 12 successful GWAS in PTSD to-date (*Table 1*) from individual studies, which discovered several genes of interest due to their prior associations with stress or epigenetic regulation of neuronal function, including the following: *LINC01090, BC036345, ZNRD1-ASI*, and *RORA*.[40,42,46,54-62] Most of these cohorts and many more have been combined for the meta-analytic approaches of the PGC, and are currently examining GWAS for PTSD in >150 000 subjects. Thus, it is still relatively early in the field and further research is required. GWAS are the first step in identifying these genes, and further studies using an array of molecular and clinical methods must still be employed to validate these findings.[5] Given the multiple genes of small effect size that are found in large-scale GWAS, it is not yet entirely clear how these findings will lead to actionable interventions. There are several thoughts about this, which remain to be determined: (i) while any given SNP or gene may have small effects, "hub"genes or combined pathways may be triangulated and together have much larger effect size and serve as important targets representing additive risk from multiple genes and SNPs; (ii) while the common variant findings indeed have very small effects when the entire syndrome of PTSD is considered, there may be yet-to-be-determined biological subtypes of PTSD, each of which is determined by a smaller number of larger effect size variants with less biological heterogeneity; or iii) while the common variants and genes themselves in a causal fashion are of limited effect, their identification will lead to novel understanding of the biology of PTSD,

STUDY	SAMPLE SIZE	COHORT	SNP(S)	GENE	*P* VALUE	HIGHLIGHTS
Logue et al, 2013	Discovery: N=491	Discovery: white European American military veterans	rs8042149	Retinoid-related orphan receptor alpha (RORA)	2.50E-08	
	Replication: N=600	Replication: African American military veterans				
Xie et al, 2013	Discovery: N=4344	European American and African American	rs6812849	Tolloid-Like 1 (TLL 1)	3.10E-09	
	Replication: N=2643	European American and African American				
Guffanti et al, 2013	Discovery: N=413	Discovery: DNHS women	rs10170218	LINC01090 (long noncoding RNA)	5.09E-08	
	Replication: N=2541	Replication: NHSII women				
Wolf et al, 2014	Discovery: N=484	European American military veterans	rs263232	Adenylyl cyclase 8 (ADCY8)	6.12E-07	
Nievergelt et al, 2015	Discovery: N=3494	MRS military veterans	rs6482463	Phosphoribosyl transferase domain containing 1 (PRTFDC1)	2.04E-09	
	Replication: N=491					
Almli et al, 2015	Discovery: N=147	Discovery: Military veterans	rs717947	BC036345 (long noncoding RNA)	1.28E-08	
	Replication: N=2006	Replication: GTP Large urban community cohort				

STUDY	SAMPLE SIZE	COHORT	SNP(S)	GENE	*P* VALUE	HIGHLIGHTS
Ashley-Koch et al, 2015	Discovery: N=1708	Non-Hispanic black (NHB) Non-Hispanic white (NHW)	rs7866350 (NHW Cohort)	TBC1domain family member 2 (TBC1D2)	1.10E-06	
Stein et al, 2016	Discovery: N=7774	American military veterans	rs159572	Ankyrin repeat domain 55 (ANKRD55)	2.34E-08	
	Replication: N=5916		rs11085374	Zinc finger prot. 626 (ZNF626)	4.59E-08	
Kilaru et al, 2016	Discovery: N=3678	GTP Large urban community cohort	N/A	Neuroligin 1 (NLGN1)	minSNP: 1.00E-06	
	Replication: N=205	DCHS pregnant south African women	N/A	ZNRD1-AS1 (long noncoding RNA)	VEGAS: 1.00E-06	
Melroy-Greif et al, 2017	Discovery: N=512	Mexican Americans and American Indians	rs6681483 rs6667389 rs10888255 rs10888257	Olfactory receptor family 11 subfamily L Member 1 (OR11L1)	1.83E-06	
Duncan et al, 2017	Discovery: N=20730	Trauma exposed adults from 11 contributing studies	rs139558732 African American	Kelch-like protein 1 (KLHL1)	3.33E-08	PGC-PTSD
Morey et al, 2018	Discovery: N=157	European American and African American military veterans	rs6906714 rs17012755 rs76832471 rs9499406	LINC02571	5.99E-08 6.05E-08 6.51E-08 8.19E-08	
	Replication: N=133	GTP African American women				

Table I. Genome-wide association studies in PTSD. GTP- Grady Trauma Project; DCHS-Drakenstein Child Health Study; NHSII- Nurses' Health Study II.

STUDY	SAMPLE SIZE	COHORT	SIGNIFICANT CPG SITE(S)	GENE	*P* VALUE	HIGHLIGHTS
Uddin et al, 2010	N=100	DNHS	cg17709873 cg25831111	Retinoid-related orphan receptor alpha (RORA) Coenzyme A synthase (COASY)	3.00E-3 (unadjusted) 1.00E-3 (unadjusted)	
Smith et al, 2011	N=110	African Americans	cg24577137 cg08081036 cg20098659 cg07967308 cg07759587	Translocated promoter region, nuclear basket protein (TPR) Annexin A2 (ANXA2) C-type lectin domain family 9 member a (CLEC9A) Acid phosphatase 5, Tartrate resistant (ACP5) TLR8 toll like receptor 8 (TLR8)	1.90E-06 9.30E-06 4.30E-06 8.00E-06 1.10E-05	
Uddin et al, 2013	N=100	DNHS	118 CpG sites/ 116 genes		1.00E-2 (unadjusted)	
Mehta et al, 2013	N=168	GTP	458 CpG sites/ 164 genes		<5.00E-2	
Mehta et al, 2017	Discovery: N=211 Replication: N=115	Australian male Vi-etnam war veterans GTP males	cg26499155 cg02357741 cg09325682 cg17750109 cg16277944	Intergenic (43 kb from leucine-rich repeat contain-ing 3B [LRRC3B]) BR Serine/threonine kinase 1 (BRSK1) Lipocalin 8 (LCN8) Nerve growth factor (NGF) Dedicator of cytokinesis 2 (DOCK2)		7.94E-07 2.24E-06 3.28E-06 3.06E-06 4.95E-06
Rutten et al, 2017	Discovery: N=93 Replication: N=98	Male Dutch military veterans Male American mili-tary veterans	17 DMPs and 12 DMRs	Dual specificity phosphatase 22 (DUSP22) Histone cluster 1 H2A pseudogene 2 (HIST1H2APS2) Hook microtubule tethering Protein 2 (HOOK2) Ninjurin 2 (NINJ2) Paired box 8 (PAX8) Ring finger protein 39 (RNF39) Zinc finger protein 57 (ZFP57)	<5.00E-2	

STUDY	SAMPLE SIZE	COHORT	SIGNIFICANT CPG SITE(S)	GENE	*P* VALUE	HIGHLIGHTS
Hammamieh et al, 2017	Training set: N=99 Test set: N=60 Merged: N=159	OEF/OIF Male Amer-ican military veterans	5578 diffe-rential-ly methylated CpG islands	3662 DMGs 3339 DMGs	<5.00E-2 (unad-justed)	
Kuan et al, 2017	N=473	WTC	cg05693864 cg06182923 cg08696494 cg25664402 cg05569176 cg09370982 cg07654569	Zinc finger DHHC-type containing 11 (ZDHHC11) CUB and sushi multiple domains 2 (CSMD2) Collagen type IX alpha 3 chain (COL9A3) Intergenic Programed cell death 6 Interacting protein (PDCD6IP) TBC1 domain family member 24 (TBC1D24) Family with sequence similarity 164, member A (FAM164A)	1.73E-06 4.73E-05 5.39E-05 5.80E-05 7.82E-05 8.97E-05 9.91E-05	
Ratanathara-thorn et al, 2017	N=147	Four milita-ry cohorts (MRS, PRISMO, VA-M, and VA-NCPTSD) Three civilian cohorts (DNHS, GTP, and WTC)		Proposed – Consortium Study Description		Planned meta-analysis
Uddin et al, 2018	N=545	Civilian trauma ex-posed cohorts	cg23637605 cg19577098	Neuregulin 1 (NRG1) Hepatocyte growth factor-regulated tyrosine ki-nase substrate (HGS)	4.66E-08 1.47E-07	Meta-analysis

Table II. Epigenome-wide association studies in post-traumatic stress disorder. OEF/OIF-Operation Enduring/Iraqi Freedom; MRS- Marine Resiliency Study; PRISMO- Prospective Research in Stress-Related Military Operations; VA-M- Veterans Affairs' Mental Illness Research, Education and Clinical Centers; VA-NCPTSD- National Center for PTSD; WTC- World Trade Center 9/11 First Responders study.

leading to novel more powerful interventions. In summary, the field is hopeful that many robust GWAS gene candidates will be identified with these tools, potentially transforming our approach to the biology of PTSD.

Epigenome-wide association studies

Following a similar unbiased approach, epigenome-wide association studies (EWAS) offer a novel approach to finding candidate gene pathways through examining epigenetic mechanisms at a genome-wide level. EWAS studies to-date have focused primarily on DNA methylation, which has been the most cost-effective to examine in large data sets.[30] We have identified 10 EWAS studies to date that examined DNA methylation (*Table II*).[24,63-71] Recent EWAS focusing on combat veterans identified evidence for a relationship between combat trauma and PTSD symptoms, which may be mediated by longitudinal changes in DNA methylation.[66-68] Analysis revealed a number of gene associations to PTSD symptom severity including the following: *BRSK1, LCN8, NFG, DOCK2, ZFP57*, and *RNF39*.[66-68] The PGC-PTSD has also commissioned a work group to focus on building a data set to adequately power large-scale PTSD research examining DNA methylation and other epigenetic mechanisms.[70] A recent publication by the PGC-PTSD outlines a framework for using meta-analysis with modest sample sizes to create well-powered epigenetic association.[70] Further, Uddin et al conducted a meta-analysis using three civilian cohorts and identified *NRG1* (*cg23637605*) and *HGS* (*cg19577098*) as biomarkers for PTSD.[71]

Conclusions and future directions

Epigenetics provides potentially the best approach for understanding the interaction of genetics with environmental exposure to trauma in PTSD. Here we have reviewed some recent progress in understanding DNA methylation, histone regulation, and noncoding RNA approaches to epigenetic regulation in PTSD. The most profound recent progress in the biology of PTSD has been the onset of large-scale collaborations to support enormous studies in the genetic architecture of PTSD through the Psychiatric Genomics Consortium and collaborative GWAS studies, allowing compilation of hundreds of thousands of samples. These consortia are also beginning to allow combined datasets examining DNA methylation arrays and RNA sequencing studies, which soon may also be very well-powered. Together such approaches offer great promise for determining the true genetic and epigenetic architecture of risk vs resilience in PTSD. Such progress may afford novel targeted therapeutic approaches to enhance treatment and prevention of PTSD in the aftermath of trauma. ■

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References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Washington DC: American Psychiatric Publishing; 2013.
2. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psychiatry*. 2005;62:134-147. doi:10.7312/atwo91826-016.
3. Ozer EJ, Best SR, Lipsey TL, Weiss DS. Predictors of posttraumatic stress disorder and symptoms in adults: a meta-analysis. *Psychol Bull*. 2003;129(1):52-73. doi:10.1037/1942-9681.S.1.3.
4. Sheerin CM, Lind MJ, Bountress K, Nugent NR, Amstadter AB. The genetics and epigenetics of PTSD: overview, recent advances, and future directions. *Curr Opin Psychol*. 2016;118(24):6072-6078. doi:10.1002/cncr.27633.
5. Smoller JW. The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology*. 2016;41(1):297-319. doi:10.1038/npp.2015.266.
6. Van Dongen J, Slagboom PE, Draisma HHM, Martin NG, Boomsma DI. The continuing value of twin studies in the omics era. *Nat Rev Genet*. 2012;13(9):640-653. doi:10.1038/nrg3243.
7. Sartor CE, McCutcheon VV, Pommer NE, et al. Common genetic and environmental contributions to PTSD and alcohol dependence in young women. *Psychol Med*. 2012;41(7):1497-1505. doi:10.1017/S0033291710002072.
8. Sartor CE, Grant JD, Lynskey MT, et al. Common heritable contributions to low-risk trauma, high-risk trauma, posttraumatic stress disorder, and major depression. *Arch Gen Psychiatry*. 2013;69(3):293-299. doi:10.1001/archgenpsychiatry.2011.1385.
9. Afifi TO, Asmundson GJG, Taylor S, Jang KL. The role of genes and environment on trauma exposure and posttraumatic stress disorder symptoms: A review of twin studies. *Clin Psychol Rev*. 2010;30(1):101-112. doi:10.1016/j.cpr.2009.10.002.
10. Leen-Feldner EW, Feldner MT, Knapp A, Bunaciu L, Blumenthal H, Amstadter AB. Offspring psychological and biological correlates of parental posttraumatic stress: review of the literature and research agenda. *Clin Psychol Rev*. 2013;33(8):1106-1133. doi:10.1016/j.cpr.2013.09.001.
11. Lambert JE, Holzer J, Hasbun A. Association between parents' PTSD severity and children's psychological distress: a meta-analysis. *J Trauma Stress*. 2014;27(1):9-17. doi:10.1002/jts.21891.
12. Widom CS. Posttraumatic stress disorder in abused and neglected children grown up. *Am J*

Psychiatry. 1999;156(8):1223-1229. doi:10.1176/ajp.156.8.1223.
13. Molnar BE, Buka SL, Kessler RC. Child Sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. *Am J Public Health*. 2001;91(5):753-760.
14. Brady KT, Killeen TK, Brewerton T, Lucerini S. Comorbidity of personality disorders and posttraumatic stress disorder. *J Clin Psychiatry*. 2000;61:22-32. doi:10.1016/j.eurpsy.2007.01.069.
15. Klengel T, Dias BG, Ressler KJ. Models of intergenerational and transgenerational transmission of risk for psychopathology in mice. *Neuropsychopharmacology*. 2016;41(1):219-231. doi:10.1038/npp.2015.249.
16. Andero R, Ressler KJ. Fear extinction and BDNF: Translating animal models of PTSD to genes. *Genes Brain Behav*. 2013;31(9):1713-1723. doi:10.1109/TMI.2012.2196707.
17. Korlach J, Turner SW. Going beyond five bases in DNA sequencing. *Curr Opin Struct Biol*. 2012;22(3):251-261. doi:10.1016/j.sbi.2012.04.002.
18. Khare T, Pai S, Koncevicius K, et al. 5-hmC in the brain is abundant in synaptic genes and shows differences at the exon-intron boundary. *Nat Struct Mol Biol*. 2012;19(10):1037-1043. doi:10.1038/nsmb.2372.
19. Guo JU, Su Y, Zhong C, Ming G, Song H. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell*. 2011;145(3):423-434. doi:10.1016/j.cell.2011.03.022.
20. Lister R, Mukamel EA, Nery JR, et al. Global epigenomic reconfiguration during mammalian brain development. *Science*. 2013;341(6146):1237905. doi:10.1126/science.1237905.
21. Li X, Wei W, Zhao Q-Y, et al. Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. *Proc Natl Acad Sci U S A*. 2014;111(19):7120-7125. doi:10.1073/pnas.1318906111.
22. Sutherland JE, Costa M. Epigenetics and the environment. *Ann N Y Acad Sci U S A*. 2003;983:151-160.
23. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene– childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41. doi:10.1038/nn.3275.
24. Mehta D, Klengel T, Conneely KN, et al. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2013;110(20):8302-8307. doi:10.1073/pnas.1217750110.
25. Novik KL, Nimmrich I, Genc B, et al. Epigenomics: genome-wide study of methylation phenomena. *Curr Issues Mol Biol*. 2002;4(4):111-128.
26. Sardina JL, Collombet S, Tian TV, et al. Transcription factors drive Tet2-mediated enhancer demethylation to reprogram cell fate. *Cell Stem Cell*. 2018;23(5):727-741.e9. doi:10.1016/j.stem.2018.08.016.
27. Gökbuget D, Blelloch R. Epigenetic control of transcriptional regulation in pluripotency and early differentiation. *Development*. 2019;146(19). pii: dev164772. doi:10.1242/dev.164772.
28. Yehuda R, Daskalakis NP, Lehrner A, et al. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. *Am J Psychiatry*. 2014;171(8):872-880. doi:10.1176/appi.ajp.2014.13121571.
29. Bierer LM, Bader HN, Daskalakis NP, et al. Elevation of 11beta-hydroxysteroid dehydrogenase type 2 activity in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. *Psychoneuroendocrinology*. 2014;48:1-10. doi:10.1016/j.psyneuen.2014.06.001.
30. Collins LJ, Schönfeld B, Chen XS. Chapter 4 - The epigenetics of non-coding RNA. In: Tollefsbol T, ed. *Handbook of Epigenetics*. San Diego, CA: Academic Press; 2011:49-61. doi:https://doi.org/10.1016/B978-0-12-375709-8.00004-6.
31. Qureshi IA, Mehler MF. Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease. *Nat Rev Neurosci*. 2012;13(8):528-541. doi:10.1038/nrn3234.
32. Daskalakis NP, Rijal CM, King C, Huckins LM, Ressler KJ. Recent genetics and epigenetics approaches to PTSD. *Curr Psychiatry Rep*. 2018;20(5). doi:10.1007/s11920-018-0898-7.
33. Pasquinelli AE. MicroRNAs and their targets: recognition, regulation and an emerging reciprocal relationship. *Nat Rev Genet*. 2012;13(4):271-282. doi:10.1038/nrg3162.
34. Jung SH, Wang Y, Kim T, et al. Molecular mechanisms of repeated social defeat-induced glucocorticoid resistance: Role of microRNA. *Brain Behav Immun*. 2015;44:195-206. doi:10.1016/j.bbi.2014.09.015.
35. Meerson A, Cacheaux L, Goosens KA, Sapolsky RM, Soreq H, Kaufer D. Changes in brain MicroRNAs contribute to cholinergic stress reactions. *J Mol Neurosci*. 2010;40(1-2):47-55. doi:10.1007/s12031-009-9252-1.
36. Schouten M, Aschrafi A, Bielefeld P, Doxakis E, Fitzsimons CP. microRNAs and the regulation of neuronal plasticity under stress conditions. *Neuroscience*. 2013;241:188-205. doi:10.1016/j.neuroscience.2013.02.065.
37. Haramati S, Navon I, Issler O, et al. MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. *J Neurosci*. 2011;31(40):14191-14203. doi:10.1523/JNEUROSCI.1673-11.2011.
38. Qingzhen L, Jiehua M, Zhiyang Y, Hongjun L, Chunlong C, Weiyan L. Distinct hippocampal expression profiles of lncRNAs in rats exhibiting a PTSD-like syndrome. *Mol Neurobiol*. 2016;53(4):2161-2168. doi:10.1007/s12035-015-9180-8.
39. Guffanti G, Galea S, Yan L, et al. 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*. 2013;38(12):3029-3038. doi:10.1016/j.psyneuen.2013.08.014.
40. Gelernter J, Sun N, Polimanti R, et al. Department of Veterans Affairs Cooperative Studies Program (#575B) and Million Veteran Program.Genome-wide association study of post-traumatic stress disorder reexperiencing symptoms in >165,000 US veterans. Nat Neurosci. 2019;22(9):1394-1401. doi:10.1038/s41593-019-0447-7. Epub 2019 Jul 29. PubMed PMID: 31358989.
41. Almli, Lynn M, Fani, Negar, Smith, Alicia K, Ressler KJ. Genetic approaches to understanding post-traumatic stress disorder. *Intern J Neuropschopharmacol*. 2015;17(2):355-370. doi:10.1017/S1461145713001090.
42. Logue MW, Amstadter AB, Baker DG, et al. The Psychiatric Genomics Consortium Posttraumatic Stress Disorder Workgroup: posttraumatic stress disorder enters the age of large-scale genomic collaboration. *Neuropsychopharmacology*. 2015;40(10):2287-2297. doi:10.1038/npp.2015.118.
43. Cornelis MC, Nugent NR, Amstadter AB, Koenen KC. Genetics of post-traumatic stress disorder: Review and recommendations for genome-wide association studies. *Curr Psychiatry Rep*. 2010;12(4):313-326. doi:10.1007/s11920-010-0126-6.
44. Psychiatric GWAS Consortium Coordinating Committee TPG, Cichon S, Craddock N, et al. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. *Am J Psychiatry*. 2009;166(5):540-556. doi:10.1176/appi.ajp.2008.08091354.
45. McGowan PO. Epigenomic mechanisms of early adversity and HPA dysfunction: Considerations for PTSD research. *Front Psychiatry*. 2013;4:1-6. doi:10.3389/fpsyt.2013.00110.
46. Almli LM, Stevens JS, Smith AK, et al. A genome-wide identified risk variant for PTSD is a methylation quantitative trait locus and confers decreased cortical activation to fearful faces. *Front Mol Neurosci*. 2015;8:68. doi:10.1002/ajmg.b.32315.
47. Binder EB, Bradley RG, Liu W, et al. Association of FKBP5 Polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA*. 2008;299(11):1291-1305. doi:10.1016/j.bmcl.2009.08.098.
48. Xie P, Kranzler HR, Poling J, et al. Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder. *Neuropsychopharmacology*. 2010;35(8):1684-1692. doi:10.1038/npp.2010.37.
49. Wang Q, Shelton RC, Dwivedi Y. Interaction between early-life stress and FKBP5 gene variants in major depressive disorder and post-traumatic stress disorder: A systematic review and meta-analysis. *J Affect Disord*. 2018;118(24):6072-6078. doi:10.1002/cncr.27633.
50. Hawn SE, Sheerin CM, Lind MJ, et al. GxE effects of FKBP5 and traumatic life events on PTSD: A meta-analysis. *J Affect Disord*. 2019:455-462. doi:10.1016/j.jad.2018.09.058.
51. Zhang TY, Labonté B, Wen XL, Turecki

G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology*. 2013;38(1):111-123. doi:10.1038/npp.2012.149.
52. Yehuda R, Daskalakis NP, Bierer LM, et al. Holocaust exposure induced intergenerational effects on FKBP5 methylation. *Biol Psychiatry*. 2016;80(5):372-380. doi:10.1016/j.biopsych.2015.08.005.
53. Logue MW, Ph D, Baldwin C, et al. A genome-wide association study of posttraumatic stress disorder identifies the retinoid-related orphan receptor alpha (RORA) gene as a significant risk locus. *Mol Psychiatry*. 2013;18(8):937-942. doi:10.1038/mp.2012.113.A.
54. Xie P, Kranzler HR, Yang C, Zhao H, Farrer LA, Gelernter J. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. *Biol Psychiatry*. 2013;74(9):656-663. doi:10.1016/j.biopsych.2013.04.013.
55. Duncan LE, Ratanatharathorn A, Aiello AE, et al. Largest GWAS of PTSD (N=20 070) yields genetic overlap with schizophrenia and sex differences in heritability. *Mol Psychiatry*. 2018;23(3):666-673. doi:10.1038/mp.2017.77.
56. Ashley-Koch AE, Garrett ME, Gibson J, et al. Genome-wide association study of posttraumatic stress disorder in a cohort of Iraq- Afghanistan era veterans. *J Affect Disord*. 2016;(184):225-234. doi:10.1016/j.jad.2015.03.049.
57. Kilaru V, Iyer S V., Almli LM, et al. Genome-wide gene-based analysis suggests an association between Neuroligin 1 (NLGN1) and post-traumatic stress disorder. *Transl Psychiatry*. 2016;6(5):e820. doi:10.1038/tp.2016.69.
58. Melroy-Greif WE, Wilhelmsen KC, Yehuda R, Ehlers CL. Genome-wide association study of post-traumatic stress disorder in two high-risk populations. *Twin Res Hum Genet*. 2016;118(24):6072-6078. doi:10.1002/cncr.27633.
59. Morey RA, Garrett ME, Stevens JS, et al. Genome wide association study of hippocampal subfield volume in PTSD cases and trauma-exposed controls. bioRxiv 456988; doi:https://doi.org/10.1101/456988.
60. Nievergelt CM, Maihofer AX, Mustapic M, et al. Genomic predictors of combat stress vulnerability and resilience in U.S. Marines: A genome-wide association study across multiple ancestries implicates PRTFDC1 as a potential PTSD gene. *Psychoneuroendocrinology*. 2015;51(2015):459-471. doi:10.1016/j.psyneuen.2014.10.017.
61. Stein MB, Chen C, Ursano RJ, et al. Genome-wide association studies of posttraumatic stress disorder in two cohorts of US army soldiers. *JAMA Psychiatry*. 2017;73(7):695-704. doi:10.1001/jamapsychiatry.2016.0350.
62. Wolf EJ, Rasmusson AM, Mitchell KS, Logue MW, Baldwin CT, Miller MW. A genome-wide association study of clinical symptoms of dissociation in a trauma-exposed sample. *Depress Anxiety*. 2015;31(4):352-360. doi:10.1002/da.22260.A.
63. Uddin M, Aiello AE, Wildman DE, et al. Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A*. 2010;107(20):9470-9475. doi:10.1073/pnas.0910794107.
64. Smith AK, Conneely KN, Kilaru V, et al. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet Part B Neuropsychiatr Genet*. 2011;156(6):700-708. doi:10.1002/ajmg.b.31212.
65. Uddin M, Galea S, Chang SC, Koenen KC, Wildman DE, Aiello AE. Epigenetic signatures may explain the relationship between socioeconomic position and risk of mental illness: preliminary findings from an urban community based sample. *Biodemography Soc Biol*. 2013;59(1):68-84. doi:10.1080/19485565.2013.774627.
66. Mehta D, Bruenig D, Carillo-Roa T, et al. Genomewide DNA methylation analysis in combat veterans reveals a novel locus for PTSD. *Acta Psychiatr Scand*. 2017;136(5). doi:10.1111/acps.12778.
67. Rutten BPF, Vermetten E, Vinkers CH, et al. Longitudinal analyses of the DNA methylome in deployed military servicemen identify susceptibility loci for post-traumatic stress disorder. *Mol Psychiatry*. 2017;23(5):1145-1156. doi:10.1038/mp.2017.120.
68. Hammamieh R, Chakraborty N, Gautam A, et al. Whole-genome DNA methylation status associated with clinical PTSD measures of OIF/OEF veterans. *Transl Psychiatry*. 2017;7(7):e1169. doi:10.1038/tp.2017.129.
69. Kuan PF, Waszczuk MA, Kotov R, et al. An epigenome-wide DNA methylation study of PTSD and depression in World Trade Center responders. *Transl Psychiatry*. 2017;7(6):e1158. doi:10.1038/tp.2017.130.
70. Ratanatharathorn A, Boks MP, Maihofer AX, et al. Epigenome-wide association of PTSD from heterogeneous cohorts with a common multi-site analysis pipeline. *Am J Med Genet Part B Neuropsychiatr Genet*. 2017;174(6):619-630. doi:10.1002/ajmg.b.32568.
71. Uddin M, Ratanatharathorn A, Armstrong D, et al. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. *Epigenomics*. 2018;10(12). doi:10.2217/epi-2018-0049.