Review

Biological Psychiatry

Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges, and Future Directions

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ABSTRACT

Posttraumatic stress disorder (PTSD) is a stress-related psychiatric disorder that is thought to emerge from complex interactions among traumatic events and multiple genetic factors. Epigenetic regulation lies at the heart of these interactions and mediates the lasting effects of the environment on gene regulation. An increasing body of evidence in human subjects with PTSD supports a role for epigenetic regulation of distinct genes and pathways in the pathogenesis of PTSD. The role of epigenetic regulation is further supported by studies examining fear conditioning in rodent models. Although this line of research offers an exciting outlook for future epigenetic research in PTSD, important limitations include the tissue specificity of epigenetic modifications, the phenomenologic definition of the disorder, and the challenge of translating molecular evidence across species. These limitations call for studies that combine data from postmortem human brain tissue and animal models, assess longitudinal epigenetic changes in living subjects, and examine dimensional phenotypes in addition to diagnoses. Moreover, examining the environmental, genetic, and epigenetic factors that promote resilience to trauma may lead to important advances in the field.

Keywords: DNA Methylation, Epigenetics, Fear Conditioning, Hypothalamic-Pituitary-Adrenal Axis, Posttraumatic Growth, Posttraumatic Stress Disorder

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Traumatic events, as defined by DSM-IV, have been estimated to occur in 90% of individuals in the general population at some point in their lives (1). However, only a small percentage of traumatized individuals meet the criteria for posttraumatic stress disorder (PTSD) (2), and some individuals have been reported to undergo positive psychological changes after trauma, denoted as posttraumatic growth (3). The ability to predict individual responses to traumatic events, which are ubiquitous and often inevitable, could offer the opportunity to target preventive strategies and early interventions to vulnerable individuals.

Predictive ability can be enhanced by gaining insights into the mechanisms that contribute to PTSD development after exposure to trauma. PTSD, similar to other diagnostic entities in psychiatry, is clinically heterogeneous and defined on a phenomenologic basis, not adequately reflecting the underlying pathophysiology, a limitation that has been increasingly recognized and attributed to knowledge gaps in the etiological underpinnings of psychiatric disorders (4). Elucidating the molecular mechanisms underlying PTSD could contribute to a more accurate diagnostic definition and ultimately to the development of individualized treatment interventions.

Among the molecular processes implicated in PTSD and related conditions, this review focuses on epigenetics. The role of epigenetic regulation in the pathogenesis of PTSD, its potential utility for the development of biomarkers and novel treatments, and the challenges and future directions of this line of research are discussed.

WHY EXAMINE EPIGENETICS IN PTSD?

The complex phenotype of PTSD is thought to emerge from interactions among multiple genetic and environmental factors. Disentangling the mechanisms through which these factors contribute to PTSD pathogenesis could enable identification of individuals predisposed to show maladaptive responses to trauma. Although heritability studies have repeatedly supported a genetic contribution to the pathogenesis of the disorder (5,6), efforts to consistently identify specific genetic predictors of the disorder have met with little success. This shortcoming can be attributed to several factors, including interindividual variability in the type, timing, and severity of traumatic exposure and the likely polygenic risk for the disorder with only small odds ratios for the individual variants. The awaited results of large meta-analyses performed within the Psychiatric Genomics Consortium for PTSD may lead to the identification of the first robust genetic factors.

An important regulation of gene function and phenotypic expression occurs at the level of epigenetic regulation. Epigenetic changes consist of numerous biochemical processes, including DNA methylation and hydroxymethylation, posttranslational histone modifications, and noncoding RNAs. These processes are shown to be influenced by environmental

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exposure and collectively shape the transcriptional activity of genes without changing the underlying genetic code (7,8). The advent of epigenetics has challenged the previous dichotomy between "nature and nurture" because the epigenome can be viewed as a molecular interface between the environment and the genome that is influenced by genetic sequence but constantly receives regulatory feedback by environmental cues and can shape gene function and phenotypic expression in response to the environment. It is important to consider epigenetic changes in the context of the environmental exposure at the level of the neural circuit and behavior outcome.

The possible relevance of epigenetic regulation in PTSD lies in its role in mediating long-term effects of trauma exposure on gene expression and brain function during development and in the mature central nervous system. Psychological trauma has been shown to induce epigenetic changes that can have short-term and long-lasting effects on neuronal function, brain plasticity, and behavioral adaptations to psychological stressors (9,10). Epigenetic changes can provide a molecular mechanism for the development of distinct phenotypes after exposure to trauma. In most cases, trauma exposure does not lead to the development of PTSD, so that epigenetic changes following trauma exposure may accompany learning of new behaviors to avoid trauma exposure or other adaptive mechanisms. It will be important to disentangle these adaptive changes from the maladaptive ones that lead to PTSD. The difference in epigenetic changes related to disease outcome could be moderated by genetic predisposition (11), previous epigenetic embedding of another (trauma) experience, or specificity to developmental windows. These mechanistic implications and the potentially reversible nature of epigenetic changes have led to increasing interest in the epigenetics of PTSD.

OVERVIEW OF EPIGENETIC STUDIES IN PTSD

To date, most epigenetic studies in PTSD have examined the methylation status of cytosine residues of genomic DNA. The particular interest in DNA methylation in the context of PTSD was spurred by numerous studies in animals and humans showing that DNA methylation changes can be embedded by early adverse experiences and these markers may confer vulnerability to subsequent life adversity (12–22). Although DNA methylation initially was thought to be a nonreversible modification based on its role in cell differentiation, it was later shown to be dynamically regulated by active enzymatic methylation and demethylation processes (23,24). This dynamic regulation does not preclude that some stress-induced changes in DNA methylation can be stabilized and inherited as shown in animal models (25–29) and suggested in humans (15,30,31). Identifying such DNA methylation markers may offer particular insights into the pathogenesis of the disorder.

Human studies that have examined DNA methylation changes in PTSD are summarized in Table 1. These studies assessed peripheral blood and largely examined a priori biologically plausible candidate genes. Overall, epigenetic regulation in PTSD has been supported for genes involved in stress responses (17,31–36), neurotransmitter activity (37–40), immune regulation (41), or repetitive genomic elements (42). Far fewer studies have followed an epigenome-wide approach or used epigenome-wide markers (43–46). The latter approaches offer a less biased way of interrogating the epigenome and the potential to identify novel candidate genes and biological pathways implicated in the pathogenesis of PTSD. The potential mechanisms, limitations, and implications of these epigenetic findings and potential directions for future research are discussed in more detail in the following sections.

EXAMINING EPIGENETIC MECHANISMS OF PTSD IN HUMANS

Human studies are crucial for understanding epigenetic processes in PTSD, but the tissue-specific nature of epigenetic modifications and the inability to access brain tissue of living humans impede the ability of such studies to offer mechanistic insights. This shortcoming may be partially overcome by examining postmortem brain tissue. The need for postmortem studies in PTSD has long been highlighted (47), but, to our knowledge, no studies so far have examined epigenetic markers in postmortem brains of subjects with PTSD. Such studies could offer valuable insights into brain region–specific epigenetic markers that might show differences between patients with PTSD and control subjects. Analysis of postmortem data from different brain regions could be used to understand how epigenetic regulation works at a circuit, brain region, or whole-brain level in PTSD. System-level approaches using postmortem tissue have shown promise in other fields of psychiatric research (48). Despite these strengths, postmortem studies would still face important limitations, including the confounding effect of lifestyle factors known to affect epigenetic changes and the inability to discern the temporal relationship among trauma exposure, epigenetic modifications, and PTSD development.

The importance of examining living subjects and the demand for easily accessible biomarkers that can be used in clinical settings necessitate the use of peripheral tissue. The premise is that epigenetic changes in peripheral tissues, such as in blood or saliva, either could be driven by processes initiated in the central nervous system and may reflect similar changes in the brain or could be peripheral disease-associated changes that may or may not be involved in the pathogenesis of the disorder and can serve as biomarkers. Two potential mechanisms that could mediate effects of trauma exposure on the periphery include persistent neuroendocrine alterations, in particular, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and immune dysregulation.

Epigenetic Regulation of the HPA Axis as a "Window to the Brain"

HPA axis dysregulation has been repeatedly linked with trauma exposure and stress-related psychiatric disorders (49,50), and although discrepancies exist among studies, PTSD has been associated with suppressed cortisol levels that have been attributed to hypersensitivity of the glucocorticoid receptor (GR) and enhanced negative feedback inhibition (51,52). These HPA axis abnormalities may drive

Table 1. Human Studies Supporting the Role of DNA Methylation in PTSD

Reference	Sample Size (*N*)	Approach	Identified Genetic Loci	Method of DNA Methylation Assessment	Primary Finding
Uddin *et al.*, 2010 (44)	100	Epigenome-wide	Genes involved in innate and adaptive immune responses	Bisulfite followed by Infinium HumanMethylation 27K BeadChip (Illumina, San Diego, California)	Immune system functions are overrepresented among the uniquely unmethylated genes in subjects with PTSD
Koenen *et al.*, 2011 (37)	100	Candidate genetic loci	*SLC6A4*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	At high numbers of traumatic events, lower *SLC6A4* methylation levels increase risk for PTSD, whereas higher methylation levels protect from the disorder
Uddin *et al.*, 2011 (88)	100	Candidate genetic loci	*MAN2C1*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	Increased lifetime risk for PTSD in individuals with higher *MAN2C1* methylation levels and exposed to greater numbers of potentially traumatic events
Smith *et al.*, 2011 (45)	110	Epigenome-wide	*TPR, CLEC9A, APC5, ANXA2, TLR8*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	PTSD is associated with increased global methylation and differential methylation of genes associated with inflammation
Ressler *et al.*, 2011 (34)	94	Candidate genetic loci	*ADCYAP1R1*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	*ADCYAP1R1* methylation levels were associated with PTSD symptoms in subjects with heavy exposure to trauma
Rusiecki *et al.*, 2012 (42)	150	Candidate genetic loci	LINE-1, Alu	Bisulfite mapping using pyrosequencing	Postdeployment hypermethylation of Alu in veterans with PTSD and LINE-1 in veterans without PTSD
Chang *et al.*, 2012 (38)	83	Candidate genetic loci	*SLC6A3*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	Nine-repeat allele of *SLC6A3* doubles PTSD risk only in the presence of high *SLC6A3* promoter methylation levels
Norrholm *et al.*, 2013 (39)	270	Candidate genetic loci	*COMT*	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	Higher methylation levels of the *COMT* promoter are associated with impaired fear inhibition. The Met/Met *COMT* genotype was associated with increased *COMT* promoter methylation
Klengel *et al.*, 2013 (11)	76	Candidate genetic loci	*FKBP5*	Bisulfite mapping using pyrosequencing	Allele-specific childhood trauma–dependent *FKBP5* demethylation increases PTSD risk
Rusiecki *et al.*, 2013 (41)	150	Candidate genetic loci	*IGF2, H19, IL8, IL16, IL18*	Bisulfite mapping using pyrosequencing	Deployment resulted in increased *IL18* methylation in veterans who developed PTSD, but decreased *H19* and *IL18* methylation levels in veterans without PTSD
Mehta *et al.*, 2013 (43)	169	Epigenome-wide	Enrichment of overlapping and nonoverlapping pathways between groups	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	Compared with PTSD cases without childhood abuse, PTSD cases with childhood abuse show distinct and almost nonoverlapping gene expression and DNA methylation profiles
Uddin *et al.*, 2013 (89)	100	Epigenome-wide	Several genes involved in neuronal function and synaptic transmission	Bisulfite followed by Infinium HumanMethylation 27K BeadChip	Socioeconomic position moderates the relationship between methylation levels of genes involved in neuronal function and PTSD symptoms
Yehuda *et al.*, 2013 (32)	16	Candidate genetic loci	*NR3C1, FKBP5*	Bisulfite mapping using clonal sequencing	Pretreatment methylation levels of the *NR3C1* exon 1F predicted response to prolonged exposure psychotherapy, and changes in methylation of the *FKBP5* promoter occurred concomitantly with recovery from PTSD
Labonte *et al.*, 2014 (35)	46	Candidate genetic loci	*NR3C1*	Sequenom EpiTYPER (Agena Bioscience, San Diego, California)	PTSD is associated with higher *NR3C1* expression and lower overall methylation levels in 1B and 1C promoters. Methylation levels were inversely correlated with *NR3C1* expression
Yehuda *et al.*, 2014 (31)	95	Candidate genetic loci	*NR3C1*	Bisulfite mapping using clonal sequencing	Offspring with paternal PTSD showed higher *NR3C1* 1F promoter methylation if maternal PTSD was not present. Offspring with maternal and paternal PTSD showed lower methylation
Yehuda *et al.*, 2014 (33)	122	Candidate genetic loci	*NR3C1*	Bisulfite mapping using clonal sequencing	Lower *NR3C1* 1F promoter methylation was observed in combat veterans with PTSD compared with combat veterans without PTSD. *NR3C1* 1F promoter methylation inversely correlated with symptoms

Table 1. Continued

Reference	Sample Size (*N*)	Approach	Identified Genetic Loci	Method of DNA Methylation Assessment	Primary Finding
Vukojevic *et al.*, 2014 (36)	152	Candidate genetic loci	*NR3C1*	Bisulfite mapping using pyrosequencing	In male but not female survivors of the genocide in Rwanda, increased *NR3C1* 1F promoter methylation was associated with less PTSD risk and re-experiencing symptoms, lower *NR3C1* expression, reduced picture recognition, and differences in recognition memory-related brain activity
Boks *et al.*, 2015 (46)	96	Use of epigenome-wide based marker ("epigenetic clock")	—	Bisulfite followed by Infinium HumanMethylation 450K BeadChip	Exposure to military combat trauma was significantly associated with accelerated epigenetic aging, whereas development of PTSD symptoms was inversely correlated with epigenetic aging

Studies are reported in chronologic order. Sample sizes reflect the number of subjects with DNA methylation data that were included in the respective analyses. All studies examined peripheral blood from human subjects except for the study by Vukojevic *et al.*, 2014 (36), which examined saliva. Presented studies were retrieved by searching the National Library of Medicine PubMed database using the terms "DNA methylation," "epigenetics," "histone modifications," "histone acetylation," "histone deacetylation," "histone methylation," "histone demethylation," "epigenetic modifications," and "epigenetic changes" for the epigenetic component combined with the terms "PTSD," "posttraumatic stress disorder," and "posttraumatic growth." This search strategy and further reading of the literature yielded 18 original articles in humans published between 2009 and 2015 that are summarized in the table.

PTSD, posttraumatic stress disorder.

persistent changes of the neuroendocrine milieu, which could effect epigenetic changes in cells throughout the body. A detailed description of the HPA axis is beyond the scope of this article and has been provided elsewhere (9,53). The HPA axis culminates in peripheral secretion of glucocorticoids. After activation by glucocorticoids, the GR dissociates from this complex, translocates into the nucleus, and transactivates or transrepresses a large number of glucocorticoid-responsive genes. In addition to dynamic changes in gene expression, repetitious transactivation or transrepression of GR-responsive genes in the periphery may result in lasting changes in DNA methylation. Such changes have been observed in glucocorticoid-responsive genes after exposure to glucocorticoids (11,54) and have been suggested to correlate with similar changes in specific brain regions (55).

Among genes involved in regulation of the HPA axis, the most extensively studied is the gene encoding the GR, *NR3C1*, which is regulated through multiple promoter regions located in its noncoding exon 1 variants that contain numerous glucocorticoid response elements. Particular attention was drawn to this locus by studies in rodents and humans linking early life adversity with increased methylation levels of the *NR3C1* exon 1_F promoter (*Nr3c1* 1_7 in rodents) in peripheral blood and in brain tissue (12,13,15,56). In a study examining combat veterans, subjects with PTSD were shown to have lower peripheral blood methylation levels in the *NR3C1* exon 1_F promoter compared with combat veterans without PTSD (32). In addition, *NR3C1* exon 1_F methylation inversely correlated with dexamethasone suppression of cortisol and PTSD symptoms. In a separate study, increase in *NR3C1* exon 1_F methylation levels predicted favorable PTSD response to prolonged exposure psychotherapy, supporting the usefulness of *NR3C1* methylation as a biomarker for the disorder (32). In line with these findings, civilians with lifetime diagnosis of PTSD were shown to have lower T-cell *NR3C1* exon 1_B and 1_C methylation levels and higher GR expression, but no differences were observed between individuals with current and remitted PTSD (35).

Another glucocorticoid-responsive gene that has drawn increasing attention is the gene encoding FK506 binding protein 51 (FKBP51), *FKBP5*. Among other functions, FK506 binding protein 51—induced by GR activation—also acts as a cochaperone of the GR, creating an intracellular ultrashort negative feedback loop that decreases GR signaling and maintains homeostasis of the HPA axis (57–59). FKBP51 is an important modulator of GR activity and stress responsivity. The role of the *FKBP5* gene in PTSD has been supported by many studies conducted by our group and others showing that *FKBP5* alleles confer increased risk for PTSD, especially in the context of early adversity, and are associated with worse PTSD symptoms (60–62). We further showed that not only genetic but also epigenetic regulation of the *FKBP5* gene in response to early trauma is implicated in PTSD pathogenesis. In particular, cytosine-phosphate-guanine (CpG) sites located near glucocorticoid response elements of the intron 7 regulatory region of the *FKBP5* gene were shown to undergo demethylation in response to childhood, but not adulthood, trauma in risk allele carriers (11). Similar DNA methylation changes were observed after glucocorticoid exposure in human progenitor hippocampal neuronal cells, suggesting that *FKBP5* demethylation in response to excessive glucocorticoids release following early life trauma may represent an epigenetic signature that is stable across tissues in humans. Methylation levels of the *FKBP5* promoter also were shown to accompany treatment response in PTSD, suggesting potential usefulness of this marker in tracking disease course (31). These preliminary findings await replication in a larger sample.

Finally, epigenetic regulation in the context of PTSD has been examined at the stress-responsive genes that encode the pituitary adenylate cyclase–activating polypeptide (*ADCYAP1*) and its receptor (*ADCYAP1R1*), which, among its pleiotropic functions, modulates stress responses. In peripheral blood, pituitary adenylate cyclase–activating polypeptide levels were associated with PTSD diagnosis and symptoms in highly traumatized female subjects, and *ADCYAP1R1* CpG island methylation levels predicted PTSD symptoms in both sexes (34). These results were corroborated by rodent

experiments showing induction of *ADCYAP1R1* transcription by fear conditioning, an established animal model of PTSD. Future studies are needed to assess to what extent epigenetic changes of HPA genes in the periphery reflect alterations in brain tissue and whether such changes contribute to the pathogenesis of PTSD or merely represent an epiphenomenon of endocrine dysregulation.

Epigenetics and Immune Dysregulation in PTSD

A growing body of evidence has linked PTSD with alterations in immune function (63–68). This relationship has been suggested to be twofold: 1) Trauma exposure and PTSD may dysregulate peripheral immune function via persistent disturbances of the HPA axis, and 2) immune dysregulation in the periphery can contribute to vulnerability for the development of PTSD via alterations in brain function. It is plausible that traumatic exposure induces lasting epigenetic signatures at immune-related genetic loci that may lead to immune dysregulation and increased risk for PTSD.

Epigenetic changes in immune-related genes have been observed in numerous studies examining DNA methylation changes of immune-related genes as well as genome-wide studies that identified epigenetic alterations in biological pathways involved in immune function. Methylation levels of the gene encoding interleukin (IL)-18 were found to increase after deployment in military service members who developed PTSD, but to decrease in deployed subjects who did not develop PTSD (41). Because IL-18 is a cytokine that has been linked with risk for cardiovascular disease (69), these findings may have implications for the elevated risk of cardiovascular risk observed in patients with PTSD (70). In a separate study that used an unbiased, epigenome-wide approach, immune-related functions were found to be overrepresented among uniquely unmethylated genetic loci and among genes showing decreased methylation with increasing traumatic exposure (44). Another epigenome-wide study showed that subjects with PTSD have differential methylation levels at five genes involved in inflammatory processes (*TPR, CLEC9A, APC5, ANXA2,* and *TLR8*) accompanied by increased levels of IL-4, IL-2, and tumor necrosis factor α (45).

Nonetheless, an important limitation of these studies is the measurement of DNA methylation in either serum (41) or whole blood (44,45), without controlling for cell composition. The observed findings could reflect differences in immune cell counts in PTSD, rather than trauma-specific or disease-specific epigenetic changes within a cell type. Future studies should account for cell type composition either by using bioinformatics approaches (71) or, optimally, by sorting cells with flow cytometry and subsequently measuring methylation levels in homogeneous cell populations.

TRANSLATING EPIGENETIC FINDINGS IN PTSD

The limitations inherent to examining mechanisms of PTSD in humans create the need for animal models with good construct validity for PTSD, such as the Pavlovian fear conditioning, avoidance learning, and predator-exposure models. A detailed description of these models and related epigenetic studies is beyond the scope of this article and has been provided elsewhere (72,73). The rodent studies have provided valuable insights into the neural circuitry and epigenetic mechanisms that are involved in the consolidation, maintenance, and extinction of fear memories. Alterations in fear processes and related neural circuitry have been observed in animal models and subjects with PTSD, suggesting that these models might be valuable paradigms for translating research in PTSD (74,75).

Despite the role of fear processes in the pathogenesis of PTSD, studies linking peripheral epigenetic markers with fear conditioning or similar endophenotypes in humans are scarce. A study addressing this question found that in DNA from peripheral blood, higher methylation levels of CpG sites within the promoter of the gene encoding catechol-*O*-methyltransferase (*COMT*), the enzyme responsible for the inactivation of catecholamine neurotransmitters, are associated with impaired fear inhibition (39). Future studies need to explore further the role of epigenetic regulation in fear conditioning and other endophenotypes with good construct validity for PTSD.

GENE-TRAUMA-EPIGENETIC INTERACTIONS IN PTSD

An important concept that emerges from epigenetic studies in PTSD is that DNA methylation changes induced by trauma may be allele-specific and may interact in a complex manner with genetic background and trauma exposure. These interactions may affect the expression of genes involved in stress responses, neurotransmitter function, and immune regulation, eventually contributing to vulnerability/resilience endophenotypes and, ultimately, to a continuum of phenotypes ranging from PTSD to posttraumatic growth. This model of gene-trauma-epigenetic regulation in PTSD and related phenotypes and endophenotypes is described in more detail and presented schematically in Figure 1. Examining the moderating role of multiple genetic factors on trauma-mediated epigenetic changes at a systems level would require very large sample sizes and longitudinal approaches, but such an approach may pave the way for a mechanistic understanding of epigenetic regulation in PTSD.

TIMING OF TRAUMA AND THE NEED FOR LONGITUDINAL STUDIES

An important point that has not been adequately addressed by previous studies is the timing of traumatic exposure and its temporal relationship to epigenetic changes and development of PTSD. Sensitive periods of trauma exposure previously were highlighted in gene-environment interaction; in particular, trauma early in life was associated with lasting epigenetic changes and more robust effects on PTSD phenotypes (10,11,57,76–82). The importance of early trauma on epigenetic changes in PTSD was highlighted by a genome-wide study using a design that compared trauma-exposed control subjects without PTSD with patients with PTSD with or without child trauma. Patients with different traumatic histories showed distinct peripheral blood gene expression and concomitantly distinct DNA methylation profiles (43). Compared with control subjects, patients with PTSD without childhood abuse showed 244 differentially regulated transcripts, whereas patients with PTSD with childhood abuse showed 303 differentially regulated transcripts. The overlap of differentially

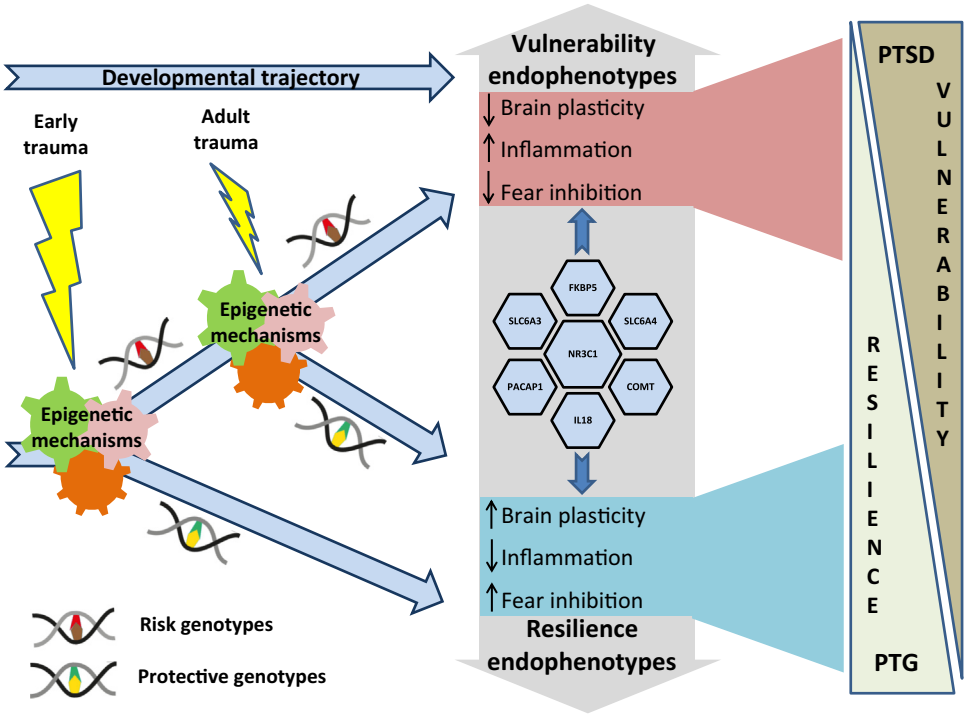

Figure 1. Simplified schematic representation of gene-trauma-epigenetic interactions in posttraumatic stress disorder and related phenotypes. This model is supported, for example, for the methylation status of the *SLC6A4* promoter, shown to interact with the 5HTTLPR polymorphism of the gene promoter to predict psychological responses to trauma. In particular, individuals carrying the short (risk) allele were more prone to develop unresolved responses to trauma at lower methylation levels, but less prone to develop maladaptive responses at higher methylation levels (40). In line with these findings, the nine-repeat allele of the *SLC6A3* gene was shown to double the risk for posttraumatic stress disorder only in the presence of high methylation levels of the *SLC6A3* promoter (38). Genetic variation of the *COMT* gene was also shown to predict methylation levels of cytosine-phosphate-guanine (CpG) sites within the gene promoter, with higher methylation levels being associated with the Met/Met *COMT* genotype and predicting impaired fear inhibition (39). In addition to moderation of the associations of epigenetic factors with trauma responses, genetic variants may directly moderate trauma-induced epigenetic changes. A study conducted by our group showed that exposure to childhood abuse leads to demethylation of CpG sites in the functional glucocorticoid response element in intron 7 of the *FKBP5* gene only in rs1360780 T-allele (risk) carriers and not carriers of the opposite (protective) genotype (11). In this case, a genetically driven change in systemic glucocorticoid release likely alters DNA methylation changes after early trauma exposure. The risk *FKBP5* allele, which is located close to a functional glucocorticoid response element, was shown to increase binding of an enhancer region located in intron 2 to the transcription start site of the gene. This leads to enhanced induction of *FKBP5* expression after glucocorticoid receptor activation and subsequent glucocorticoid receptor resistance, impairing the negative feedback of the axis. In addition to such indirect effects, the effects of trauma on transcription and DNA methylation changes of sensitive loci may be directly moderated by genetic variants that affect transcription factor binding, remove or create CpG dinucleotides, or lead to altered expression of epigenetic readers and writers that command subsequent epigenetic changes. The term "epigenetic mechanisms" is used for simplification purposes and denotes the entirety of epigenetic processes, including DNA methylation, posttranslational histone modifications, noncoding RNAs, and three-dimensional changes in chromatin conformation, which act in concert to regulate gene transcription. PTG, posttraumatic growth; PTSD, posttraumatic stress disorder.

expressed transcripts between the two PTSD subgroups was only 2%, and the DNA methylation changes that accompanied these gene-expression differences were up to 12-fold higher in the PTSD subgroup with childhood abuse. These data suggest that PTSD-related gene expression changes in peripheral blood seem to be accompanied to a different extent by DNA methylation changes depending on the prior trauma history, especially in childhood. Different epigenetic mechanisms could be implicated in the pathogenesis of the disorder depending on trauma history, and distinct biomarker profiles may be relevant to distinguish subtypes of PTSD. The importance of timing likely extends beyond childhood, and accounting for traumatic exposures at other vulnerable time points, such as during gestation or in old age, could provide invaluable insights into epigenetic mechanisms of PTSD. In addition, studies should explore the potential kindling effect of repetitive trauma exposure or the synergistic effect of early traumatic exposure and adult stressors throughout the life span. A kindling hypothesis has been supported in other psychiatric disorders (83), and this effect could be mediated by cumulative epigenetic changes induced by repetitive stressor exposure.

These hypotheses can be evaluated only by longitudinal studies. To date, there are few such studies in PTSD. In a study assessing epigenetic markers in veterans before and after deployment, differential changes in DNA methylation of the *IL18* and *H19* loci and repetitive genomic elements were observed in veterans exposed to trauma who developed PTSD versus veterans exposed to trauma who did not develop the disorder (41). Another study assessing epigenetic markers as predictors of response to prolonged exposure psychotherapy noted that pretreatment methylation levels of the *NR3C1* exon 1F predicted PTSD responses, and changes in methylation of the FKBP5 promoter occurred concomitantly with recovery from the disorder (31). These data suggest that methylation changes may be dynamic, occur concomitantly with development or recovery from PTSD, and may be useful as biomarkers to track the course of the disorder.

LIMITATIONS OF EPIGENETIC STUDIES IN PTSD AND FUTURE DIRECTIONS

An increasing body of evidence supports a role for epigenetic regulation in PTSD. Although these findings offer an exciting

prospect for future research endeavors, several limitations of epigenetic studies in PTSD should be highlighted. First, most studies to date use either animal models or blood tissue in humans. These studies are inherently limited by the tissue specificity of epigenetic changes and the inability to interrogate brain tissue in living humans. This shortcoming may be overcome to some extent by translational approaches that combine data from animal models, postmortem human brain tissue, and peripheral blood assessments in living humans. Such studies may provide converging evidence for epigenetic signatures that are common in peripheral blood and analogous brain regions in animals and humans. Some studies that have translated evidence across animals and humans suggest that epigenetic changes for certain genetic loci may be observed in brain and periphery (19,34). Second, although epigenetic regulation of some genomic sites in PTSD, such as the 1F promoter of the *NR3C1* gene, have been corroborated by multiple studies in PTSD, replication of existing epigenetic findings is largely lacking and should be sought by future studies. Even for the most replicated finding to date concerning the 1F promoter of the *NR3C1* gene, controversial results have been reported; for example, although 89% of the studies exploring the GR exon variant 1F in humans found increased methylation with early life adversity, only 17% of the studies relating the methylation of this site to stress responsivity and psychopathology found the same direction of effects (84). Another limitation is imposed by the current diagnostic definition of PTSD, which is phenomenologic and dichotomous. Future studies could benefit by examining outcomes based on carefully selected endophenotypes or dimensional phenotypes. In addition, although studies on the epigenetics of PTSD to date have focused on DNA methylation, it is important to consider the full spectrum of epigenetic modifications that have been implicated in the pathogenesis of psychiatric disorders, including posttranslational histone modifications, noncoding RNAs, and three-dimensional changes in chromatin conformation (85,86). Because these epigenetic processes may act in concert to regulate gene function (87), studies examining the interplay among various epigenetic mechanisms could offer a more comprehensive understanding of epigenetic regulation in PTSD. Lastly, efforts should be made to identify epigenetic changes that mediate positive outcomes after trauma exposure, such as posttraumatic growth. Mimicking these changes to promote resilience to trauma would be a desirable effect of preventive strategies for PTSD.

CONCLUSIONS

Beyond their value in promoting mechanistic understanding and use as disease biomarkers, epigenetic modifications are potentially reversible and represent attractive candidates for the development of new treatments for psychiatric disorders. Studies in rodents have used epigenetic drugs successfully to target DNA methyltransferases or histone-modifying enzymes (72,73). Such manipulations showed that DNA methylation and histone acetylation are involved in every step of fear memory, from the initial consolidation to extinction and long-term potentiation, processes that have been shown to be altered in patients with PTSD. Pharmaceutical inhibition of histone deacetylation also was shown to reverse the deleterious effect of early life adversity in rats (11), further supporting the potential utility of epigenetic drugs in the treatment and prevention of trauma-related pathologies. Although the road to safe and effective clinical use of epigenetic drugs is still long, it is hoped that elucidating epigenetic mechanisms of PTSD may improve available treatments for this disorder.

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