The Role of Genetics and Epigenetics in the Pathogenesis of Posttraumatic Stress Disorder

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ABSTRACT

Posttraumatic stress disorder (PTSD) represents a common psychiatric disorder that can emerge after a traumatic life event. Despite a high incidence of trauma exposure (40%-90%) in the general population, only a minority (7%-12%) will eventually develop the disorder. As indicated by twin and family studies, genetic factors are an important contributor to PTSD, suggesting an individual genetic vulnerability to the disorder. Studies exclusively focusing on genetic main effects have shown limited results, likely because environmental factors play a key role in this disorder. Gene and environment interaction (GxE) studies may represent a more promising approach to better understand the pathophysiology of this disorder because they jointly consider the genetic predisposition as well as the environmental trigger. On a molecular level, such GxE and long-lasting effects of these interactions on transcriptional regulation may be mediated by epigenetic modifications. A number of studies suggest that the etiology of PTSD is the result of a complex interplay of genetics, environmental factors, and epigenetic regulation. This article reviews current genetic and epigenetic findings in the field of PTSD, focusing both on candidate gene and genome-wide approaches. Although there has been some initial progress, the field still lacks large-scale studies on the genetic level, but some are currently underway within the Psychiatric Genomics Consortium PTSD. Finally, the reviewed studies support that a combination of different approaches, integrating genetic and epigenetic data, will be necessary to better understand the underlying molecular mechanisms of PTSD. [Psychiatr Ann. 2016;46(9):510-518.]
With a prevalence of about 5% in the general population and an overall lifetime prevalence of 7% to 12%, posttraumatic stress disorder (PTSD) is a common psychiatric disorder. Per definition, PTSD is a disorder with long-lasting symptoms occurring after exposure to a traumatic life event. These symptoms include intrusive memories, avoidance and numbing, and hyperarousal. Women are twice as likely as men to develop the disease.1

Although environmental triggers are well-defined, a key question that remains unanswered is why only a small percentage of people that experience trauma go on to develop PTSD.2,3 The ratio between a high lifetime trauma incidence and the relatively low prevalence of PTSD suggests that exposure to a trauma does not inevitably lead to development of the disorder.4 Some of the environmental factors that seem to be responsible for an altered response to traumatic life events are the type and intensity of the trauma, exposure to previous trauma, and living in unsafe neighborhoods. Nonetheless, inter-individual differences in susceptibility to the disease exist and these may be mediated by genetic factors. As indicated by twin and family studies, genetics represent an important factor in accounting for the risk of developing this disorder. Several of these studies have consistently shown the estimated genetic contribution to be between 30% and 40%.5 It should be noted, however, that heritability research for PTSD is complex, as it is depends on comparable environmental exposures in relatives.

Although there is a clear indication of genetic contribution to this disorder, so far the investigation of the main effects of genetics in PTSD has provided only limited results.6 As mentioned above, environmental factors, in this case traumatic life events, play a decisive role in the pathogenesis of the disorder. Some of the lasting effects caused by these factors are likely mediated by epigenetic changes. These are changes that do not affect the sequence of the DNA but rather its accessibility to transcription factors or effects mediated by noncoding RNA that shape the transcriptional response of affected tissues.7 Consequently, research in the field of PTSD has now increasingly focused on the interplay of genetics, environment, and epigenetic factors.

One main approach to the study of the genetic component of PTSD was to investigate candidate genes. These genes were selected for their involvement in systems possibly relevant for PTSD. These include genes involved in the serotonergic and dopaminergic system but also more specifically, genes from molecular pathways that are thought to be involved in the pathogenesis of PTSD, such as genes involved in the stress-hormone system or relevant for different aspects of fear conditioning.8 However, as with other complex disorders, candidate gene studies are fraught with inconsistent replication and the risk of false-positive associations.9 To overcome this issue, The Psychiatric Genomics Consortium PTSD Workgroup has recently been formed to enable genome-wide association studies (GWAS).10 It is hoped that large-scale GWAS may provide novel, hypothesis-free, genetic risk variants for PTSD.

This article provides an overview of the genetic and epigenetic mechanisms possibly involved in the pathophysiology of PTSD, as well as recent findings and developments in this field.

CANDIDATE GENES

Because dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been shown to play a decisive role in the pathogenesis of PTSD,11 genes involved in its regulation are of particular interest. The HPA axis represents the most important system regulating the neuroendocrine stress response of an organism.12 It acts through a complex interplay of direct interactions and negative feedback loops. In response to stress, the release of corticotropin-releasing hormone (CRH) in the hypothalamus sets off a cascade of reactions, promoting the release of a number of hormones from the adrenal glands, including cortisol. Cortisol then acts on two nuclear hormone receptors—the mineralocorticoid and glucocorticoid receptor (GR). A set of downstream effects, including transcriptional ones, allows the organism to adapt to stress exposure, but once the stressor has passed, also decreases HPA activity through negative feedback mediated by the GR. This stress hormone system is dysregulated in PTSD, making genetic variants of key regulators in this system prime candidates in understanding the genetics of PTSD. These candidates include the receptors for CRH, particularly CRHR1, the gene encoding the GR (NR3C1), a co-chaperone of the GR, (FKBP5), as well as the important pituitary peptide PACAP (ADCYAP1 gene) and its receptor.

Although case/control association studies are the main method used for the study of many disorders, they often fail for PTSD due to the strong environmental component. Therefore, a number of genetic studies in PTSD have sought to incorporate interactions between genes and trauma exposure.7 CRH and its receptor CRHR1 play a role in several stress-related disorders, including PTSD. In animal studies, in-
tracerebroventricular infusion of CRH led to anxiety and particularly PTSD-like behavior, and these effects were reversed by the administration of CRHR1 antagonists. In humans, increased levels of CRH in the cerebrospinal fluid of patients with PTSD compared to controls represents another interesting finding linking CRH to the disorder. Three CRHR1 polymorphisms that have previously been associated with the development of depression after child abuse have been tested in the context of PTSD. These polymorphisms not only interact with child abuse to predict depression, but also withendocrine dysregulation, with an exaggerated stress response seen in risk-allele carriers with exposure to early trauma. Imaging studies also indicate that these polymorphisms moderate neural responses to emotional stimuli. Although these specific single nucleotide polymorphisms (SNPs) were not associated with PTSD, two recent studies reported associations of other CRHR1 SNPs and the disorder. First, rs12944712 was significantly related to PTSD severity in a prospective study of children who had experienced medical trauma. Second, the major alleles of two polymorphisms within the CRHR1 gene increased the risk for posthurricane PTSD symptoms. Despite being a main regulator of the HPA axis and, therefore, thought to be crucially involved in the pathogenesis of PTSD, investigations into possible associations of genetic variants of the GR with the disorder have not been successful. For example, Bachmann et al. studied GR polymorphisms in the context of PTSD in a cohort of 118 combat veterans diagnosed with PTSD. The authors did not detect altered frequencies of the tested polymorphisms in cases compared to controls. FKBPs is a heat shock protein 90 associated co-chaperone of the GR complex. Among its other functions, FKBPs regulates GR sensitivity. Cortisol binding to the complex leads to a change in affinity, which results in an exchange of FKBPs with other co-chaperones such as FKBP4. Subsequently, the increased binding of FKBP4 promotes the recruitment of dynein, which leads to translocation of the GR to the nucleus, where it acts as a transcription factor. Activation of the GR by glucocorticoids enhances, among many other genes, FKBPs transcription, resulting in the formation of an intracellular ultrashort feedback mechanism. Increased levels of FKBPs inhibit GR activity.

Because of its critical role in regulating GR sensitivity, genetic polymorphisms in FKBPs are a target of PTSD research. In several different cohorts, a haplotype tagging a functional polymorphism that alters the induction of FKBPs mRNA by GR has been associated with PTSD, but only when in combination with childhood trauma exposure. One study has also shown that depending on the environment and trauma, the FKBPs “risk” allele may also confer protective features, as it has been associated with posttraumatic growth in people who experienced Hurricane Katrina. As detailed later in this article, this interaction may be mediated by allele-specific DNA methylation changes in the FKBPs locus that further disinhibit FKBPs transcription. A number of animal studies have shown that increased FKBPs, especially in the amygdala, is associated with key endophenotypes often related to PTSD, such as stress coping and increased anxiety, but also altered fear extinction. In addition, in humans, the genetic polymorphisms associated with increased FKBPs expression have not only been associated with PTSD but also with related endophenotypes. For example, the risk allele has been associated with an enhanced GR suppression as measured by the low-dose dexamethasone suppression test. Imaging studies point to an important role of FKBPs not only in amygdala reactivity to threat but also structure, function, and connectivity of the hippocampus, a brain region consistently implicated in PTSD. For example, people carrying two risk alleles exhibited lower structural as well as functional connectivity between the anterior cingulate cortex and the hippocampus, a connection critical for adaptive cognitive and emotional processing. FKBP5 risk alleles have also been associated with behavioral endophenotypes for PTSD, such as bias toward threat and an increase in intrusions, even in healthy people. They are also associated with peri-traumatic dissociation in children who experienced acute medical injury, a strong predictor for PTSD later in life. Finally, these genetic polymorphisms were also associated with response to PTSD treatment. In a cohort of 43 people exposed to trauma during civil war in Uganda, the authors evaluated the treatment effect of narrative exposure therapy dependent on an FKBPs genotype (rs1360780). After 10 months of therapy, people carrying the risk (T) allele had a significant increase in relapse of PTSD symptoms compared to noncarriers.

The pituitary adenylate cyclase activating polypeptide (PACAP) represents another key mediator of the stress response and, therefore, has been studied in the context of PTSD. A study by Ressler et al. examined a civilian cohort of highly traumatized people and patients with PTSD. The authors identified a genetic polymorphism (rs2267735) in the PACAP receptor type 1 gene (PAC1R; ADCYAP1R1) that was significantly associated with PTSD in women only. In another study using a cohort of similarly traumatized people (n = 1,160), the finding was not replicated, but interestingly an association between PTSD and the interaction of rs2267735 and trauma load was observed, and again the association was restricted to women.
Further, an interaction of the same SNP and childhood maltreatment was found to be associated with PTSD symptoms in 495 adult women. Finally, there seems to be an ADCYAP1RI genotype effect on individual brain function. The activity of PTSD-relevant brain regions, the amygdala and hippocampus, was analyzed in 49 traumatized women after exposure to threatening and neutral stimuli. The authors observed increased amygdala activity after the threat stimulus as well as lower functional connectivity with the hippocampus in carriers of the risk genotype (CC).

Despite some promising findings in PTSD research using the candidate gene approach, a single gene is unlikely to explain the complex phenotype of the disorder. By combining different candidate genetic risk variants to form a cumulative risk score, Boscarino et al. aimed to develop a possible PTSD prediction tool. The authors used a cumulative risk allele count including polymorphisms of different genes (CRHR1, FKBP5, COMT, CHRNA5, CRHR1) to show an interaction effect of the risk score and trauma exposure level on PTSD symptom severity. In a subsequent study, they incorporated this genetic risk-allele information to their previously developed PTSD screening instrument, which already included data concerning mental health status, substance abuse, and other psychosocial measures. Adding the genetic information to the existing screening tool significantly increased the ability to predict PTSD.

In a pilot study, Rothbaum et al. also found evidence that a composite additive risk score derived from polymorphisms in 10 previously identified genes associated with stress response (ADCYAP1RI, COMT, CRHR1, DBH, DRD2, FAAH, FKBP5, NPY, NTRK2, and PCLO) predicted the development of PTSD symptoms after trauma exposure in patients recruited in the emergency department and observed prospectively. This risk could be attenuated by early intervention.

**GENOME-WIDE ASSOCIATION STUDIES**

In contrast to the above-presented candidate gene studies, GWAS represent a hypothesis-free tool to identify the most common genetic variations associated with the disease on a genome-wide level.

In the past 10 years, GWAS studies have decisively broadened our knowledge about new loci associated with susceptibility to common complex disorders not only in psychiatry, but across many medical disorders. To achieve this, international GWAS consortia were established to analyze sample sizes large enough to reach adequate power, which is one of the critical factors for these analyses. The Psychiatric Genomics Consortium represents such a consortium that has been successful in identifying robust genetic risk variants for a number of disorders, in particular schizophrenia. Although a few GWAS results for PTSD have been published, they are mostly limited by the relatively small sample sizes. To overcome this, the first PTSD GWAS consortium (Psychiatric Genomics Consortium-PTSD) was recently formed.

The first PTSD GWAS was conducted in 2012 by Logue et al. The authors found one associated SNP after correction for multiple testing. This SNP was located in the retinoid-related orphan receptor alpha gene (RORA). The significant SNP did not reach genome-wide significance in two African-American replication samples, but several other RORA SNPs were found to be nominally significant in these samples. The RORA gene was previously found to be associated with other psychiatric disorders such as bipolar disorder and major depressive disorder. Three other GWAS that were published recently identified genome-wide significant polymorphisms in genes previously connected to neurobiological pathways and processes implicated in PTSD.

The most recent GWAS found a SNP (rs717947) that was significantly associated with PTSD on a genome-wide level. Having used a small discovery sample (n = 147) the authors were able to replicate their finding in a much larger cohort where the SNP remained significant in women diagnosed with PTSD but not in men (n = 2,006). Interestingly, the discovered SNP correlated with an intermediate neural phenotype (more precisely with altered medial and dorsolateral prefrontal activation to fearful faces) identified using functional magnetic resonance imaging (fMRI) data in a subset of the replication sample. Although these studies brought to light some promising results, they only represent a first step in identifying “true” and replicable genetic risk loci for PTSD. In addition to the challenge faced by all other psychiatric disorders (ie, small genetic effect sizes with the need of samples exceeding several thousand samples and diagnostic heterogeneity), differences in environmental exposure and gene and environment (GxE) interactions also need to be incorporated in the models.

**EPIGENETICS**

The genetic background plays a decisive role and provides an important contribution to disease pathogenesis. However, the complex phenotype of PTSD cannot be explained by genotype alone. Like most psychiatric disorders, the etiology of PTSD is multifactorial in nature, where environment represents another important contributor to the disease. Although environmental factors do not affect the genetic code itself, they can alter gene function by epigenetic changes such as DNA methylation, histone modification, or noncoding RNAs. These modifications are candidate mechanisms for mediating effects by the environ-
ment on the DNA, which most often result in an alteration of gene transcription and protein translation. Such effects can be long lasting but also lead to a rather short-term change (eg, RNA expression). Epigenetic changes can occur at multiple stages throughout life, including in adulthood, and are not limited to early developmental phases as previously assumed. They represent adaptations or maladaptations to a changed environment and these epigenetic consequences can be moderated by genetic variation, thus providing a molecular mechanism for GxE interaction.

In the context of PTSD, the environmental factors affecting a person are, by definition, different kinds of trauma exposure. Several studies have shown that stressful life events can lead to alterations in epigenetic marks. Here, DNA methylation represents the most examined and best known mechanism. Briefly, cytosine bases (mainly in CpG sites) are converted to 5-methylcytosine by covalent modification. This results in decreased transcription factor binding to these loci which, if located in the promoter region of a gene, subsequently suppresses its transcription.64

Pioneering work in this field was performed by Weaver et al.65,66 in animals showing epigenetic modification induced by early life experience. More specifically, the authors observed that low levels of maternal licking and grooming led to hypermethylation of the hippocampal GR gene in pups followed by decreased GR expression. This study is of great relevance for PTSD because early adverse life experience is an important risk factor for the disorder and the investigated animal model reflects PTSD-related phenotypes such as decreased cortisol levels at baseline as well as increased cortisol suppression after the dexamethasone suppression test65,66 (reviewed by Anacker et al.,67 Szyf,68 and Zhang et al.69).

These findings were also translated to humans. McGowan et al.70 analyzed the human GR promoter (NR3C1) in suicide victims with a history of childhood abuse compared to suicide victims without childhood abuse and control subjects. Comparable to the results of the rodent studies, the NR3C1 promoter showed increased methylation as well as significantly lower GR expression in postmortem hippocampus tissue of suicide victims who were abused as children.70

The fact that epigenetic regulation represents a key mechanism in mediating the long-lasting effects of stressful life events might be of particular interest in PTSD. As a result, numerous studies described in more detail below specifically investigating the epigenetic changes affecting genes regulating the HPA axis have been conducted.

In fact, people with PTSD display significantly lower NR3C1 promoter methylation compared to healthy controls in DNA from peripheral blood.71 and differential methylation at these sites serves as a predictor of treatment outcome. In a cohort of combat veterans diagnosed with PTSD, patients with higher pretreatment promoter methylation responded significantly better to psychotherapy.72 Another study demonstrated that increased NR3C1 promoter methylation in peripheral blood was associated with less intrusive memory of the traumatic event and reduced PTSD risk in men, and also showed that it may be related to differences in recognition memory-related brain activity.73

ADCYAP1R1, previously described as being associated with PTSD symptoms, also shows epigenetic modification. In a study examining ADCYAP1R1 methylation levels in highly traumatized people with or without PTSD, the authors observed a significant positive correlation between methylation of the PAC1R locus and PTSD symptom severity.47

Another recently identified player involved in the pathophysiology of PTSD at the epigenetic level is the spindle and kinetochore associated protein 2 (SKA2). SKA2 plays a role in the activation of the GR and, therefore, may moderate negative feedback of the HPA axis. Methylation levels of this locus were recently described as a predictor of suicidal behavior. A study by Guintivano et al.74 examining human postmortem brain tissue showed significantly increased methylation levels of a CpG (cg13989295) located in the SKA2 gene as well as significantly decreased SKA2 expression levels in suicide completers compared to controls. Because of its molecular function and the association of increased suicide rates among PTSD patients, SKA2 methylation was also investigated in patients with PTSD. A combined predictor using SKA2 methylation at the above-described CpG (cg13989295) and early trauma scores resulted in a significant prediction of PTSD status.75

Two further studies investigated associations between SKA2 methylation at cg13989295 and PTSD.76,77 In a cohort of 200 soldiers exposed to trauma, Sadeh et al.76 examined CpG 13989295 methylation levels and PTSD symptoms. The authors observed a positive correlation between PTSD symptoms and SKA2 methylation levels.76 A study by Boks et al.77 investigating a Dutch military cohort showed that SKA2 methylation together with childhood trauma scores were able to significantly predict postdeployment PTSD symptoms, confirming the findings by Kaminsky et al.75 and again strengthening the role of SKA2 as a possible PTSD biomarker.

Besides genes involved in the regulation of the HPA axis, several other
candidate genes (eg, implicated in the serotonergic or dopaminergic system) have been extensively studied in the context of PTSD. Detailed description of these studies is beyond the scope of this review but is available elsewhere. Instead of focusing on a single candidate gene, several studies examined DNA methylation on a genome-wide level or focused on subsets of genes involved in stress response, immune regulation, or located in repetitive genomic elements. These studies aim to elucidate specific methylation patterns of PTSD patients across multiple genomic loci. Although no single consistent sites have been identified so far, the combined findings suggest that trauma and PTSD are associated with genome-wide changes in DNA methylation and may have a system-wide impact on the organism.

Finally, increasing evidence suggests that parental vulnerability to PTSD can be transmitted to the next generation. Here, epigenetics represents a candidate mechanism involved in the transgenerational transmission. Studies by Yehuda et al. have specifically investigated the molecular biologic background of this mechanism, demonstrating alterations in methylation levels of GR and FKBP5 in offspring in relation to parental trauma.

It is important to note that epigenetic alterations caused by the environment can be dependent on DNA sequence. Therefore, the epigenetic response of an organism to trauma exposure and the associated individual susceptibility to PTSD can be influenced by genetic variation. One such example of genotype-specific epigenetic changes associated with trauma has been described for the FKBP5 locus. Here, a combination of a genetic variation that leads to an altered transcriptional response of FKBP5 to glucocorticoids and exposure to trauma during childhood leads to further DNA demethylation in additional glucocorticoids responsive elements. This combination of genetic and epigenetic factors then leads to a disinhibition of FKBP5 regulation that has been associated with PTSD and related endophenotypes.

Noncoding RNAs, in particular microRNAs (miRNAs), represent another important epigenetic mechanism in posttranscriptional regulation of gene expression. miRNAs are non–protein-coding single stranded RNAs about 22 base pairs long that are evolutionarily highly conserved. The biogenesis of miRNAs is a complex multistep biochemical process, which begins in the nucleus and results in the cytoplasm where the mature miRNA becomes functionally active. Here they are incorporated into the RNA-induced silencing complex and perform gene silencing. More precisely, they bind and interact with complementary sites in the 3’UTR region of their target mRNA, which leads to translation repression or degradation of their target (for a detailed review see Ha and Kim).

In the past few years, miRNAs have been progressively emerging as pivotal factors involved in the pathogenesis of psychiatric disorders. Most of the studies examining the role of miRNAs in PTSD were performed in animals and used fear conditioning or predator exposure models. These models are thought to explain the underlying mechanisms of fear and the occurrence of trauma-related symptoms in PTSD.

For example, a study by Haramati et al. showed that acute stress leads to a significant upregulation of miR-34c levels in the amygdala of rodents. The authors observed that anxiety-like behavior induced by an acute stressor was significantly decreased after virus-mediated overexpression of this miRNA. Interestingly, the CRHR1 transcript is one of the main targets of miR-34c.

A recent study in humans identified DICER1 as being involved in the pathogenesis of PTSD. DICER1 is an important enzyme in the biogenesis of miRNAs, converting precursor miRNAs to mature miRNAs. In a cohort of 184 mainly African-American patients with or without diagnosis of PTSD with comorbid depression, the authors observed a significantly lower blood expression of DICER1 in cases versus controls and were able to replicate these findings in two independent cohorts. This was associated with overall lower miRNA levels in patients versus controls. In a follow-up fMRI study, they showed that the decreased DICER1 expression levels were associated with elevated amygdala activation to fearful stimuli, which represents a neural correlate for PTSD.

Although still in their primary stages, these and other studies examining miRNAs role in the underlying molecular mechanisms of PTSD show some promising initial results, which nevertheless need to be intensively further investigated.

**CONCLUSIONS**

Over the last few years, it has become clear that a complex interplay of
genetics, environment, and epigenetic changes underlies the pathophysiology of PTSD. Studying the main genetic effects will not be sufficient to explain the complex and multifactorial etiology of the disease, and environmental factors have to be taken into account. Studies assessing GxE interactions will be important. Here, however, many challenges need to be overcome, ranging from sufficiently large sample size for power to consistent and exhaustive measurement of environmental factors, to statistical issues. Epigenetic modifications may represent a candidate mechanism by which environmental factors interact with genetic predisposition to shape risk and resilience to PTSD.

In the end, a combination of GWAS or better genome-wide GxE interactions in large, well-phenotyped cohorts will be necessary and will need to be combined with mechanistic examinations, including epigenetic measures.

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