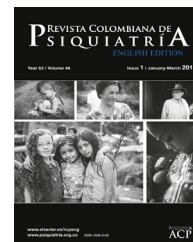




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Review Article

Genetic advances in post-traumatic stress disorder[☆]

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ARTICLE INFO

Article history:

Received 19 July 2016

Accepted 2 December 2016

Available online 11 April 2018

Keywords:

Genetics

Post-traumatic stress disorder

Epigenetics

ABSTRACT

Post-traumatic stress disorder, or PTSD, is a condition that affects a subgroup of individuals that have suffered a previous traumatic event capable of generating changes at a psychological and behavioural level. These changes affect the personal, family, and social environment of those who suffer from this condition. Different genes have been identified as risk markers for development of this disorder. The population heterogeneity and individual differences (genetic and environmental) of each subject have made it difficult to identify valid markers in previous studies. For this reason, studies of Gene $\times$ Environment ($G \times E$) have gathered importance in the last two decades, with the aim of identifying of the phenotypes of a particular disease. These studies have included genes such as SLC64A, FKBP5, and ADCYAP1R1, among others. Little is known about the interaction between the genes, pathways, and the molecular and neural circuitry that underlie PTSD. However their identification and association with stimuli and specific environments that stimulate the development of PTSD makes it focus of interest for identify genomic variations in this disorder. In turn, the epigenetic modifications that regulate the expression of genes involved in the hypothalamic–pituitary–adrenal (HPA) axis and the amygdala–hippocampal–medial prefrontal cortex circuits play a role in the identification of biomarkers and endophenotypes in PTSD. In this review, the advances in genetic and epigenetic that have occurred in the genomic era in PTSD are presented.

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DOI of original article: <https://doi.org/10.1016/j.rcp.2016.12.001>.

[☆] Please cite this article as: Guillén-Burgos HF, Gutiérrez-Ruiz K. Avances genéticos en el trastorno por estrés postraumático. Rev Colomb Psiquiat. 2018;47:108–118.

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<https://doi.org/10.1016/j.rcpeng.2018.03.001>

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Avances genéticos en el trastorno por estrés postraumático

R E S U M E N

Palabras clave:

Genética

Trastorno por estrés

postraumático

Epigenética

El trastorno por estrés postraumático (TEPT) es una condición que afecta a un subgrupo de individuos que han sufrido un evento traumático con la capacidad de generar cambios psicológicos y conductuales. Estos cambios interfieren el entorno personal, familiar y social de los individuos. Diferentes genes se han identificado como marcadores de riesgo de esta enfermedad; sin embargo, la heterogeneidad poblacional y las diferencias individuales (genéticas y ambientales) han hecho difícil la identificación de marcadores genéticos válidos en los estudios realizados. Por tal motivo, han cobrado gran importancia en las últimas dos décadas los estudios de relación entre gen y ambiente con la intención de identificar fenotipos propios de la enfermedad. Se han estudiado ampliamente genes como SLC64A, FKBP5 y ADCYAP1R1, entre otros. Poco se conoce de su interacción con las vías y los circuitos moleculares y neuronales que subyacen al TEPT, pero su identificación y asociación con estímulos y ambientes específicos condicionantes de la aparición del TEPT los hacen centro de interés para el estudio de identificación de variables genómicas en TEPT. A su vez, las modificaciones epigenéticas que regulan la expresión de genes vinculados en el eje hipotálamo-pituitario-adrenal y en el circuito amígdala-corteza prefrontal media-hipocampo son de gran interés para la identificación de biomarcadores y endofenotipos en TEPT. En esta revisión se podrá elucidar los avances en genética y epigenética que han acontecido en la comprensión genómica del TEPT.

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Introduction

A traumatic event is an event which has the capacity to generate fear, helplessness or horror in response to the threat of injury or death.¹ Approximately two thirds of the population may suffer a traumatic event at some point in their lives.² Post-traumatic stress disorder (PTSD) is a condition that affects a subgroup of people who have suffered a traumatic event which results in psychological and behavioural changes, with maladaptive symptoms for the mental and physical health of the sufferer and limitations in the social and family environments.^{3–5} In the United States, the prevalence of PTSD over the course of people's lives is estimated to be 6.8%.⁶ In Colombia, according to the 2015 Mental Health Survey, 40.2% of adults aged 18–44 and 41.4% of those over 45 have suffered at least one traumatic event in their lives. The prevalence for measuring the positive risk indicator for PTSD is 3.29% for men and 3.84% for women.⁷

Little is known about the neurobiological bases involved in the development of PTSD. The systems that regulate the response to stress include endocrine components and neurotransmission pathways in brain areas, such as the amygdala, involved in the response to fear and stress.⁸ The hypothalamic–pituitary–adrenal (HPA) axis acts as the main organiser of the endocrine response to stress^{9–11} and several studies have identified dysfunctions in the HPA axis in the response to stress,¹² anxiety¹³ and PTSD.¹⁴ Neurotransmitter alterations have been found involving catecholamines, serotonin, glutamate and GABA, which have been implicated in the neurocircuits that regulate the response to fear and stress.⁸

These alterations have been theorised about and subsequently investigated in the development of PTSD.

Advances in genome sequencing techniques have led to different genes being identified that confer risk of suffering from PTSD.^{15–18} However, many of the genes involved have lost statistical validity, as their association has been weak in sequencing studies and on some occasions they have not been identified in different study populations due to the heterogeneity of the population in PTSD (maltreated children, female victims of sexual abuse, male and female combatants in war, etc.).¹⁹ This has led over the last 20 years to a more translational approach to genetic studies of PTSD. Studies of genetic variations associated with an environmentally determined condition to identify a phenotype of interest have gained importance in population genetic studies in neuropsychiatric diseases.²⁰ Similarly, Genome-wide Association Studies (GWAS) and exome sequencing have discovered new genetic markers linked to the risk of suffering from PTSD.^{19,21} One of the interesting aspects of this disorder as a study model is that it develops as a result of a previous traumatic experience. This has led research groups to consider questions relating to genetic variations that confer risk of becoming ill or determine the severity of the symptoms, or the inheritable epigenetic changes that modify the expression of genes linked to the development of the disorder without generating changes in the sequence of these genes.

The purpose of this study is to publish the advances and new perspectives in the genetics and epigenetics of PTSD. We carried out a narrative review of the literature in the PubMed database in May 2016. We selected review articles, observational studies and analytical studies related to the purpose

of our project. The following MeSH terms were included in the search: ("genetics" OR "gene" OR "epigenetic" OR "genetic advances") AND ("post-traumatic stress disorder" OR "ptsd"). We selected articles from 2000 to date.

Genetic studies in families and identical twins

Family studies have demonstrated the link of genetic variations in families of patients with PTSD. A study conducted by Yehuda et al. showed that the children of parents who survived the holocaust and had PTSD had a higher risk of PTSD than the children of parents who survived the holocaust and were not diagnosed with PTSD ($p < 0.0005$).²² However, these studies faced the difficulty of not being able to differentiate between the environmental exposure that each member of the family may have been subject to and the genetic risks inherent to each of the families.

Genetic factors associated with the risk of exposure to traumatic events have been identified in studies of identical twins.^{15,16} Lyon et al. studied a sample of male twins from the Vietnam registry to examine genetic and environmental factors associated with exposure to traumatic events during combat. Inheritability explained 35–47% of the variance in exposure to the traumatic event during combat, but the family environment was not found to have a significant effect on the variable of interest.^{17,18} Other studies, such as the estimation of the inheritability of the symptoms of PTSD,²³ the comorbidity^{24–29} and the physical condition,^{30–32} and gene–environment interaction studies³³ have been carried out in monozygotic and dizygotic twins, with positive results for the association or linkage of genes. However, despite indicating that there is a risk of inheritability in the development of PTSD, these studies were unable to identify which gene is the direct link that confers the risk of PTSD, because of the

multifactorial and polygenic component of this disorder and neuropsychiatric diseases in general.

Multifactorial correlation in PTSD: studies of genes $\times$ environment

Research into neuropsychiatric disorders and the multifactorial nature of mental illness have led us to understand that they cannot be explained by one single gene and, furthermore, that the gene–environment interaction goes beyond simple epistemological assumptions.³⁴ Consequently, gene $\times$ environment ($G \times E$) interaction studies have become increasingly important in recent years. The first studies in psychiatry to assess the interaction of genetic conditions and exposure to stimuli or environments and identify different disease phenotypes were in maltreated children who had a genotype conferring low concentrations of the enzyme monoamine oxidase A (MAOA), compared to maltreated children with high concentrations of MAOA, in relation to the appearance of behavioural disorders, antisocial behaviour and violence.³⁵

Those studies led researchers to ask which psychiatric disorders would be best to examine for $G \times E$ studies? PTSD can be considered an ideal candidate for $G \times E$ studies as one of the criteria for diagnosis is previous exposure to the traumatic event or exposure to a hostile environment that facilitates occurrence of the traumatic event²⁰ (Fig. 1).

Of the $G \times E$ studies, the one conducted by Ressler et al. identifies a positive association for genetic risk between the single nucleotide polymorphism (SNP) rs2267735 in African-American women with a history of childhood and adult trauma and the risk of suffering PTSD.³⁶ This study confers the association of risk of PTSD on the pituitary adenylate-cyclase 1 (PAC1) receptor, involved in the development of the nervous

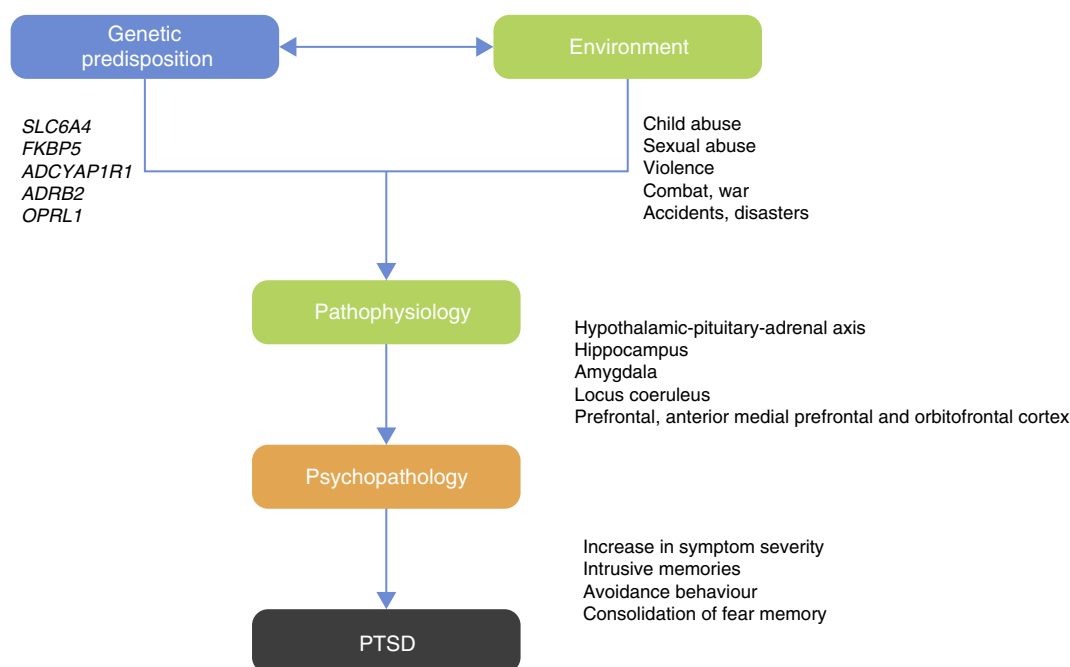


Fig. 1 – Gene–environment interaction with the pathophysiological development of post-traumatic stress disorder.

system, endocrine homeostasis, metabolism and the response to chronic stress.^{36,37} These findings suggest that the pituitary adenylate cyclase-activating polypeptide (PACAP)/PAC1 system is involved in the psychological and physiological response to stress resulting from trauma.

Another study to find a positive association between a gene and the environment contributing to traumatic events is that conducted by Kolassa et al. with patients who were refugees from the genocide in Rwanda. The study analysed 408 subjects (218 males and 190 females) living in a refugee camp in southwestern Uganda. All the subjects had experienced multiple traumatic situations. This study reported that the SLC6A4 gene that encodes the 5-hydroxytryptamine transporter gene, involved in previous studies of depression and response to medications,³⁸ confers a risk from association of suffering PTSD after traumatic events.³⁹ Other studies have associated different genes and polymorphisms linked to environmental variables (Table 1).

Hypothalamic-pituitary-adrenal axis in the neural circuit of PTSD

The HPA axis is involved in the neuroendocrine response to stress. The hypothalamus controls the secretion of corticotropin (adrenocorticotrophic hormone; ACTH) from the anterior pituitary gland and stimulates the consequent secretion of cortisol by the adrenal gland.⁴⁰ Under conditions of acute stress, the pulsatile release of corticotropin-releasing hormone (CRH) and vasopressin (arginine vasopressin, AVP) are increased, thereby increasing the release of ACTH and cortisol and other factors, such as angiotensin II, various cytokines and inflammatory mediators⁴⁰⁻⁴² (Fig. 2). The neurobiological interaction between genes that act on the HPA axis has been studied extensively in different neuropsychiatric disorders such as depression,⁴³ bipolar disorder,⁴⁴ substance abuse,⁴⁵ alcoholism⁴⁶ and PTSD.²⁰

Table 1 – Published studies of gene-environment interaction in PTSD.

Gene	Variation	Trauma	Environment variable	Population	Author	Year
SLC6A4	5HTTLPR	Hurricane in Florida	Hurricane and social support	Caucasian	Kilpatrick	2007
FKBP5	rs4713916, rs1360780, rs3800373, rs992105, rs9296158, rs737054, rs1334894, rs9470080	Miscellaneous trauma	Childhood and adulthood trauma	African	Binder	2008
SLC6A4	5HTTLPR	Hurricane in Florida	Unemployment and crime rates	Caucasian	Koenen	2009
	5HTTLPR	Miscellaneous trauma	Childhood and adulthood trauma	African-Americans and European-Americans	Xie	2009
SLC6A4	5HTTLPR, rs25531	Miscellaneous trauma	Miscellaneous trauma	Caucasian	Grabe	2009
FKBP5	rs3800373, rs9296158, rs1360780, rs9470080	Child abuse	Childhood maltreatment	African-Americans and European-Americans	Xie	2009
RGS2	rs4606	Hurricane in Florida	Hurricane and social support	Caucasian	Armstadter	2009
GABRA2	rs279836, rs279826, rs279858, rs279871	Miscellaneous trauma	Childhood trauma	Australian	Nelson	2009
SLC6A4	5HTTLPR	Genocide in Rwanda	Events related to war and non-violence	African	Kolassa	2010
ADCYAP1R1	rs2267735	Childhood and adulthood trauma	Miscellaneous trauma	African-Americans	Ressler	2011
	PAC1 (rs2267735-C allele)	Childhood maltreatment	Miscellaneous trauma	African-Americans	Almli	2013
COBL	rs406001	Childhood and adulthood trauma	Miscellaneous trauma	African-Americans and European-Americans	Xie	2013
ADRB2	rs2400707-A allele	Childhood trauma	Miscellaneous trauma	African-Americans	Liberzon	2014
COBL	rs406001	Childhood maltreatment	Miscellaneous trauma	African-Americans	Almli	2014

5HTTLPR: serotonin-transporter-linked polymorphic region; ADCYAP1R1: adenylate cyclase-activating polypeptide 1 (pituitary) receptor type I; ADRB2: beta-2-adrenergic receptor, surface; COBL: Cordon-bleu WH2 repeat protein; FKBP5: FK506 Binding Protein 5; GABRA 2: gamma-aminobutyric acid (GABA) A receptor, alpha 2; PAC1: pituitary adenylate cyclase-activating polypeptide type I receptor; RGS2: regulator of G-protein signalling 2; SLC6A4: solute carrier family 6 (neurotransmitter transporter) member 4.

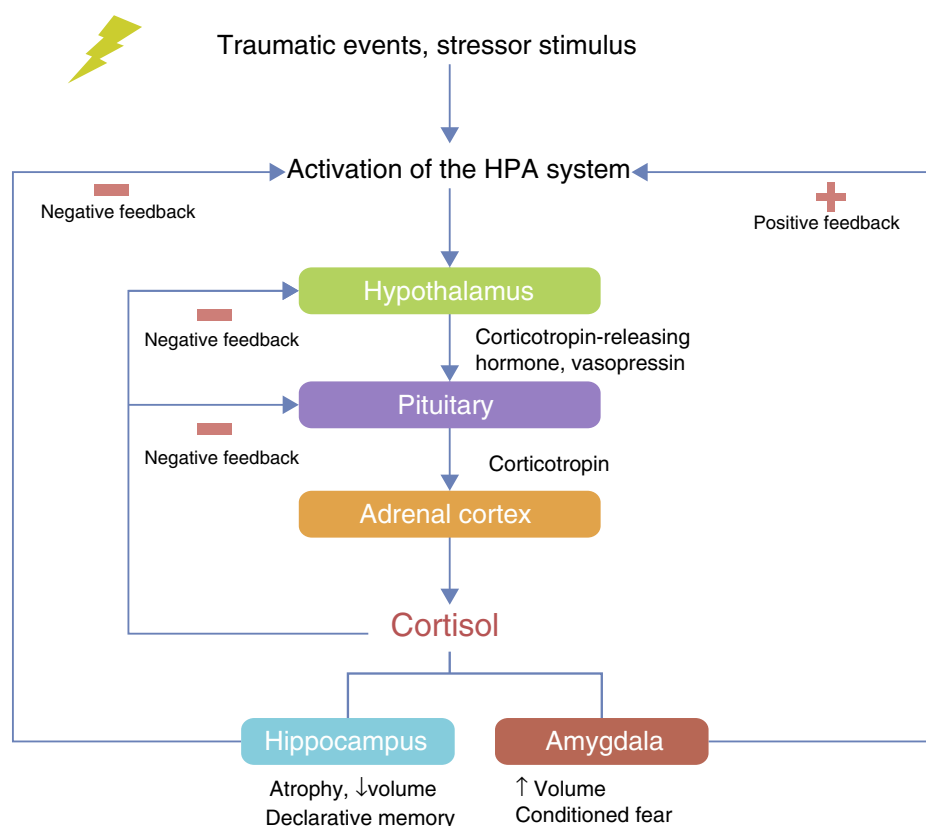


Fig. 2 – Hypothalamic-pituitary-adrenal (HPA) axis.

Immunophilins (cytosolic proteins) were discovered because of their ability to bind and mediate the immunosuppressive effect of drugs such as ciclosporin and rapamycin.⁴⁷ The FKBP5 gene (FK506 Binding Protein 5) encodes one of the immunophilins that binds to the immunosuppressant FK506 and rapamycin⁴⁸ and plays a role as a regulatory gene for the HPA axis.⁴⁹ Several studies have identified the FKBP5 gene in the development of PTSD⁵⁰⁻⁵²; one of the first groups to associate this gene was Koenen et al. in 2005; their study included 46 children who had already suffered car accidents, gunshot or stab wounds and other types of trauma. The polymorphisms rs3800373 and rs1360780 were strongly associated with the development of peri-traumatic dissociation in this group of patients.⁵² In a study of 900 predominantly Caucasian subjects, 4 SNPs of FKBP5 interacted with the severity of the sexual abuse of children as predictors of PTSD symptoms in adulthood.⁵³

A recent study evaluated the FKBP5 polymorphisms (rs9296158, rs3800373, rs1360780, rs947008) in two populations of European-American US military veterans. The first population was of 1585 veterans and the second, of 577. The results of this study showed that the four polymorphisms were associated with the PTSD symptom severity in both samples.⁵⁰ This genetic variation is a risk factor for later PTSD in adulthood, a link which had already been made in children who were victims of sexual abuse.^{53,54} The results indicate that child abuse and the presence of these polymorphisms in a population exposed to a traumatic event in life (such as the populations

in this study) confer risk of suffering severe PTSD symptoms in adulthood.

Furthermore, FKBP5 as a co-chaperone in the regulation of the glucocorticoid receptor (GCCR/NR3C1) plays a fundamental role in the consolidation of intrusive memories. Memories of this type are repetitive and unwanted and generate an involuntary sensory/perceptual experience which is usually associated with negative memories in patients with PTSD.⁵⁵ Various studies have reported that cortisol enhances the consolidation of emotional memory.^{56,57} The SNPs rs3800373, rs9296158, rs1360780 and rs9470080 present in FKBP5 were analysed in a study of 46 subjects included for genetic analysis, and it was concluded that the participants homozygous for the risk alleles of the studied polymorphisms were more likely to consolidate memories of negative images because of the modulation by FKBP5 of the GCCR/NR3C1 receptor in the response to stress.⁵⁸

Amygdala, hippocampus and prefrontal cortex in patients with PTSD

The relationship between the amygdala, the hippocampus and the prefrontal cortex has been the subject of neurobiological research in PTSD. These brain regions are rich in glucocorticoid receptors and very sensitive to stress, which makes them a very important circuit for the conditioning of fear.⁵⁹⁻⁶²

Neuroimaging studies have investigated the activation of these areas during exposure to stimuli related to traumatic experience. It was found that the medial prefrontal cortex (mPFC), which includes the anterior cingulate cortex, the subcallosal cortex and the middle frontal gyrus, is involved in the processes of extinction of conditioned fear.^{63,64} Increases in cerebral blood flow in the amygdala and reduced flow in the middle frontal gyrus have been detected by positron-emission tomography in combat veterans.⁶⁵ This would indicate hypersensitivity in the amygdala's response to stimuli related to a traumatic event.⁶⁶ Kirby et al.⁶⁷ demonstrated that emotions affect the formation of memories. In situations of fear, in the hippocampus, which is involved in the formation of declarative memories, the basolateral amygdala induces the generation of new neurons from a single population of neural stem cells in the dentate gyrus of the adult hippocampus. The formation of these new neurons has been associated with the fact that emotional events are remembered more easily and for longer than everyday experiences, with this having important implications in the pathophysiology of PTSD.⁶⁸ In a recent study involving maltreated young people with PTSD, a decrease in the volume of the ventromedial prefrontal cortex (vmPFC) was found on functional magnetic resonance imaging.⁶²

Studies of the expression of genes identified in areas involved in the neural circuitry of PTSD have produced different genetic markers which are of interest in the genetic and molecular study of this condition, both in animal models and in humans. One of these is the gene *OPRL1* (opioid receptor-like 1), which encodes the nociceptin/orphanin FQ receptor. This receptor-ligand system regulates a variety of biological functions, such as the response to stress,⁶⁹ anxiety,⁷⁰ learning and memory,⁷¹ locomotor activity⁷² and immune and inflammatory responses.⁷³ In the murine animal model, expression of *OPRL1* was found in the central amygdala. Using the microarray technique, regulation of *OPRL1* mRNA was greater in the group of animals with fear expression than in the control group.⁷⁴ In that same study, Andero et al. used the peptide SR-8993, a new selective nociceptin receptor agonist, to potentiate impairment in fear learning (conditioning). The findings from that study indicate that intraperitoneal injection in the animal model of SR-8993 prevents fear memory consolidation.⁷⁴ Andero et al. also sought to find association between polymorphisms present in the *OPRL1* gene and the development of PTSD. Using 1847 subjects who had already suffered traumatic events, they found a positive association between the SNP rs6010719 and the increased symptoms in individuals with PTSD with a history of child abuse.

The *OPRL1* gene is worthy of more attention and further research in biomedical and in $G \times E$ studies, as it has previously been linked to alcoholism,⁷⁵ a condition frequently shared by individuals who have PTSD.⁷⁶⁻⁷⁸ *OPRL1* also plays an important role in the HPA axis circuit in the stress response. There is evidence implicating the nociceptin system in the stress response through a control of regulation on the HPA axis.⁷⁹⁻⁸¹ Devine et al. demonstrated that the administration of

nociceptin/orphanin FQ increases plasma cortisol in experimental rats.⁷⁹

As already mentioned, the *FKBP5* gene modulates glucocorticoid receptor activity and has high expression levels in the hippocampus. A positive association has been found between the "risk" T allele of the SNP rs1360780 and an increase in the transcription of *FKBP5*, with a consequent increase in the inhibition of glucocorticoid signalling.⁸² Low concentrations of glucocorticoids have been associated with risk of PTSD.⁶⁵ This allele has also been related to structural differences in the left posterior cingulate cortex characterised by a decrease in fibre density, axonal diameter and myelination in this region.⁸³ The polymorphism rs1360780 (TT/TC) has been associated with alterations in the structure of the left hippocampus in the CA1 region and increased attention to threat in attention tasks.⁸⁴

Other genes of interest related to differences in brain function in PTSD are *SLC6A4* (serotonin transporter gene) and *ADCYAP1R1*. The SNP alleles rs16965628 G and 5-HTTLPR S are associated with decreased expression of *SLC6A4*; the first affects the activity of the ventrolateral prefrontal cortex (vlPFC), while the second affects the activity of the amygdala.⁶⁵ The PAC1 receptor is encoded by the *ADCYAP1R1* gene. This receptor is widely expressed in the amygdala, the hypothalamus and the hippocampus, and is associated with an increased risk of PTSD, especially in women.⁸⁵ The *ADCYAP1R1* SNP rs2267735 C risk allele has been associated with a decrease in the expression of *ADCYAP1R1* and less of its mRNA; this allele modulates the activity of the amygdala and the hippocampus, which leads to greater activation of these structures in response to threatening stimuli.⁸⁶

There are other genetic variations associated with differences in brain structure in PTSD which involve the *COMT* and *BDNF* genes. The genetic variations in the *COMT* SNP rs4680 are associated with decreased grey matter volume in the anterior cingulate cortex (ACC)⁸⁷ and those in the *BDNF* SNP rs6265 are associated with an alteration in fear extinction in humans and murine animal models.⁸⁸ *BDNF* has been found to interact with glucocorticoid signalling (important for brain plasticity), and higher levels of *BDNF* are associated with an improvement in glucocorticoid signalling and a lower level of vulnerability to stress.⁸⁹

These data suggest that the linkage of genes involved in the amygdala-mPFC-hippocampus circuit could be worthy of study for the identification of biological markers of PTSD in individuals with previous exposure to traumatic events.

Noradrenergic system

Noradrenaline is an important element in the pathophysiology of PTSD. This catecholamine acts as a neurotransmitter synthesised in the locus coeruleus in the brainstem,^{90,91} and as a hormone when released by the adrenal medulla.⁹² Noradrenaline carries out its biological function in the groups of alpha and beta adrenergic receptors,⁹³ and as the main neurotransmitter in the sympathetic nervous system.⁹⁴ Some studies have linked the noradrenergic system with the response to stress and fear generated in patients with

PTSD.⁹⁵ One group of researchers managed to identify tonic concentrations of noradrenaline in the cerebrospinal fluid (CSF) of American war veterans with PTSD compared to the controls.^{96,97} This confirms the hypothesis that the noradrenergic system becomes hyperactive in subjects with PTSD, in addition to over-consolidation of fear memory and associated intrusive memories.¹⁶

Catechol-O-methyltransferase is an enzyme involved in the catalysis and inactivation of catecholamines such as dopamine, noradrenaline and adrenaline. The COMT Val158Met polymorphism has been associated with the development of PTSD and the abolition of the conditioned fear response.⁹⁸⁻¹⁰⁰ This polymorphism has been the object of experimentation in previous studies which linked it to anxiety disorders, especially in homozygous Met/Met carriers.¹⁰¹ A recent study associated Val158Met with a reduction in the capacity of human beings to recover from traumatic events or painful situations, known as resilience.¹⁰² It was also found that methylation of CpG islands in COMT regions, including the promoter region, was influenced by the presence of the Val158Met polymorphism; Val158Met regulated gene expression and the function of the enzyme^{103,104} and was associated with impaired inhibition of fear previously generated in a conditioning paradigm model.¹⁰⁴

Epigenetics in PTSD

Epigenetic studies have been attracting increasing amounts of interest in recent years. Epigenetics is the "study of changes in gene function which are mitotically inheritable and do not involve a change in the DNA sequence".¹⁰⁵ Identifying epigenetic modifications in experimental studies, they have been standardised as: (a) DNA methylation; (b) histone post-translational modification; (c) chromatin remodelling; (d) histone variants, and (e) non-coding RNA.¹⁰⁶ Epigenetic studies in PTSD could explain individual differences in subjects exposed to trauma who are vulnerable to PTSD. Such studies allow a molecular approach considering family vulnerability, predisposition to certain environments and prolonged exposure to certain trauma effectors.¹⁰⁷

There is evidence that DNA methylation is involved in all stages of consolidation, reconsolidation and maintenance of memory formation in both the hippocampus and the cerebral cortex.¹⁰⁸ The studies in murine animal models clarified that the acetylation of histone H3 in the CA1 area of the hippocampus occurred in the conditioned fear model, and, with the use of inhibitors of the histone deacetylase enzyme, it was possible to enhance the formation of the memory.^{108,109}

A group of researchers led by Weaver¹¹⁰ discovered that changes in the DNA methylation of the glucocorticoid receptor gene in the hippocampus caused by variations in the maternal behaviour of rats towards their offspring generated a regulation in the HPA axis. The breeding model, in which the mother rats were removed early after having given birth to their offspring to prevent natural interaction (licking and grooming their offspring), generated inheritable changes in the expression of the glucocorticoid receptor gene in the hippocampus. These changes were generated by hypermethylation of the

gene promoter. DNA methylation has also been demonstrated in immune response cells in peripheral blood of subjects with PTSD.¹¹¹

Discussion

Genetic studies identifying polymorphisms and genes associated with illness in PTSD have linked the genes SLC6A4,^{38,39,65,86} FKBP5,^{49-53,58,82-84,100} ADCYAP1R1,^{85,86} ADRB2¹¹² and OPRL1,⁷⁴ among others, as candidates in the risk of PTSD. The difficulty posed by genetic association studies is the lack of reproducibility in different population groups.¹⁹ This may be due to differences in the environment to which the study subjects are exposed and sometimes also to the size of the samples used by researchers. However, studies of the G × E relationship are of great interest, particularly in psychiatric conditions such as PTSD, where it is essential to meet the diagnostic criterion of having experienced a traumatic event before the onset of symptoms.²⁰ It is important to note that most of the studies of G × E have been conducted with the diagnostic considerations pre-established in the DSM-IV for the diagnosis of PTSD. However, since the publication of the DSM-V in 2013 with the diagnostic modifications and the inclusion of new criteria for the diagnosis of PTSD, further studies could be conducted not only to examine those who have suffered directly from the traumatic event, but also people who have indirectly shown symptoms associated with PTSD. Another aspect to look at would be whether the genetic variations most associated with risk of disease in those who directly witnessed the traumatic event might be the same for people who have the symptoms of PTSD but through indirect contact with the trauma.

The first molecular studies on PTSD focused on the HPA axis.^{20,49} However, whole-genome sequencing studies (genome-wide association studies [GWAS]) in large population samples have identified new candidate genes which are of interest when analysing the molecular mechanisms in which these genes are involved.^{19,21} The genetic variations present in neuropsychiatric diseases involve magnitudes of risk of suffering from a certain disorder. However, they are polygenic and multifactorial mental illnesses^{15,20} and more precise study of the interaction is therefore required: genetic variation due to intracellular and extracellular signalling pathways; genetic variation through gene expression; genetic variation due to structural changes; genetic variation resulting from epigenetic modifications. All of this can be transferred to the experimental field by way of cellular and molecular studies and with experimentation in animal models.

The amygdala-mPFC-hippocampal system requires closer examination in genetics and molecular studies, particularly in relation to the genes involved in the synaptic and neurotransmission mechanisms in the processes of fear, anxiety, learning and memory. Different neurotransmitters and their respective receptors have been linked to cognitive processes such as memory, learning and executive functions.⁸ These cognitive components are related to the symptoms of patients with PTSD, especially the intrusive memories and memories of the event.⁶²⁻⁶⁶

Conclusions

PTSD is a serious psychiatric condition which becomes disabling. Although susceptibility to suffering a traumatic event in life is >50%, depending on the population studied, the prevalence of PTSD is 7–12%, suggesting that genetic and environmental predisposition leads to differences between subjects who suffer from the disorder.^{6,113} Studies of G × E interaction seek to associate genetic variations and environmental characteristics as factors linked to the development of the disorder. For these polygenic and complex mental disorders, epidemiological research through G × E studies is an interesting and useful tool for addressing multifactorial relationships in PTSD.

The HPA axis, the noradrenergic system and the amygdala-mPFC-hippocampal circuit are considered pivotal in the neurobiological circuit involved in the development of PTSD. However, different genes related to synaptic transmission and neurotransmitters such as serotonin, glutamate, GABA and dopamine have also been involved in animal experimentation and population studies.

Epigenetic mechanisms, such as methylation of CpG islands in promoter regions of genes involved in the development of PTSD, indicate inheritable mechanisms that are worth examining, as they could help identify risk biomarkers and/or explain disease progression. Continuity in the studies of genetic association with large population samples and their reproducibility in cell and animal models will help us to better understand the cellular mechanisms that underlie this condition.

Conflicts of interest

The authors have no conflicts of interest to declare.

REFERENCES

1. Yehuda R. Post-traumatic stress disorder. *N Engl J Med*. 2002;346:108–14.
2. Galea S, Nandi A, Viahov D. The epidemiology of post traumatic stress disorder after disasters. *Epidemiol Rev*. 2005;27:78–91.
3. Krystal JH, Kosten TR, Southwick S, Mason JW, Perry BD, Giller EL. Neurobiological aspects of PTSD: review of clinical and preclinical studies. *Behav Ther*. 1989;20:177–98.
4. Bisson J, Andrew M. Psychological treatment of post-traumatic stress disorder (PTSD). *Cochrane Database Syst Rev*. 2007;3. CD003388.
5. McDonald SD, Calhoun PS. The diagnostic accuracy of the PTSD checklist: a critical review. *Clin Psychol Rev*. 2010;30:976–87.
6. Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). *Arch Gen Psychiatr*. 2005;62:617–27.
7. Gómez-Restrepo C, De Santacruz C, Rodríguez MN, Rodríguez V, Tamayo MN, Matallana D, et al. Encuesta Nacional de Salud Mental Colombia 2015. Protocolo del estudio. *Rev Colomb Psiquiatr*. 2016;45, <http://dx.doi.org/10.1016/j.rcp.2016.04.007>.
8. Sherin JE, Nemeroff CB. Post-traumatic stress disorder: the neurobiological impact of psychological trauma. *Dialogues Clin Neurosci*. 2011;13:263–78.
9. Kudielka BM, Buske-Kirschbaum A, Hellhammer DH, Kirschbaum C. HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender. *Psychoneuroendocrinology*. 2004;29:83–98.
10. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol*. 2004;44:525–57.
11. Herman J. Stress response: neural and feedback regulation of the HPA axis. *Neuroendocrinology*. 2010;75.
12. Adinoff B, Junghanns K, Kiefer F, Krishnan-Sarin S. Suppression of the HPA axis stress-response: implications for relapse. *Clin Exp Res*. 2005;29:1351–5.
13. Van den Bergh BR, Van Calster B, Smits T, Van Huffel S, Lagae L. Antenatal maternal anxiety is related to HPA-axis dysregulation and self-reported depressive symptoms in adolescence: a prospective study on the fetal origins of depressed mood. *Neuropsychopharmacology*. 2008;33:536–45.
14. Klaassens ER, Giltay EJ, Cuijpers P, van Veen T, Zitman FG. Adulthood trauma and HPA-axis functioning in healthy subjects and PTSD patients: a meta-analysis. *Psychoneuroendocrinology*. 2012;37:317–31.
15. Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. *Am J Psychiatry*. 2002;159:1675–81.
16. Skelton K, Ressler KJ, Norrholm SD, Jovanovic T, Bradley-Davino B. PTSD and gene variants: new pathways and new thinking. *Neuropharmacology*. 2012;62:628–37.
17. Lyons MJ, Goldberg J, Eisen SA, True W, Tsuang MT, Meyer JM, et al. Do genes influence exposure to trauma? A twin study of combat. *Am J Med Genet*. 1993;48:22–7.
18. Afifi T, 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;30:101–12.
19. 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;12:313–26.
20. Mehta D, Binder EB. Gene × environment vulnerability factors for PTSD: the HPA-axis. *Neuropharmacology*. 2012;62:654–62.
21. 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;74:656–63.
22. Yehuda R, Halligan SL, Bierer LM. Relationship of parental trauma exposure and PTSD to PTSD, depressive and anxiety disorders in offspring. *J Psychiatr Res*. 2001;35:261–70.
23. Roy-Byrne P, Arguelles L, Vitek ME, Goldberg J, Keane TM, True WR, et al. Persistence and change of PTSD symptomatology: a longitudinal co-twin control analysis of the Vietnam Era Twin Registry. *Soc Psychiatry Psychiatr Epidemiol*. 2004;39:681–5.
24. Chantarujkapong SI, Scherrer JF, Xian H, Eisen SA, Lyons MJ, Goldberg J, et al. A twin study of generalized anxiety disorder symptoms, panic disorder symptoms and post-traumatic stress disorder in men. *Psychiatr Res*. 2001;103:133–45.
25. Collimore KC, Asmundson GJG, Taylor S, Jang KL. Socially related fears following exposure to trauma: environmental and genetic influences. *J Anxiety Disord*. 2009;23:240–6.
26. Xian H, Chantarujkapong SI, Scherrer JF, Eisen SA, Lyons MJ, Goldberg J, et al. Genetic and environmental influences on

- posttraumatic stress disorder, alcohol and drug dependence in twin pairs. *Drug Alcohol Depend.* 2000;61:95-102.
27. Koenen KC, Lyons MJ, Goldberg J, Simpson J, Williams WM, Toomey R, et al. Co-twin control study of the relationship among combat exposure, combat-related PTSD, and other mental disorders. *J Trauma Stress.* 2003;16:433-8.
 28. Koenen KC, Nugent NR, Amstadter AB. Gene-environment interaction in posttraumatic stress disorder: review, strategy and new directions for future research. *Eur Arch Psychiatr Clin Neurosci.* 2008;258:82-96.
 29. Koenen KC, Fu Q, Ertel K, Lyons MJ, Eisen SA, True WR, et al. Common genetic liability to major depression and posttraumatic stress disorder in men. *J Affect Disord.* 2008;105:109-15.
 30. Eisen SA, Neuman R, Goldberg J, True WR, Rice J, Scherrer JF, et al. Contributions of emotionally traumatic events and inheritance to the report of current physical health problems in 4042 Vietnam era veteran twin pairs. *Psychosom Med.* 1998;60:533-9.
 31. Goodwin RD, Fischer ME, Goldberg J. A twin study of post-traumatic stress disorder symptoms and asthma. *Am J Respir Crit Care Med.* 2007;176:983-7.
 32. Afari N, Wen Y, Buchwald D, Goldberg J, Plesh O. Are post-traumatic stress disorder symptoms and temporomandibular pain associated? A twin study. *J Orofac Pain.* 2008;22:41.
 33. Jang KL, Taylor S, Stein MB, Yamagata S. Trauma exposure and stress response: exploration of mechanisms of cause and effect. *Twin Res Hum Genet.* 2007;10:564-72.
 34. Newton-Cheh C, Hirschhorn JN. Genetic association studies of complex traits: design and analysis issues. *Mutat Res.* 2005;573:54-69.
 35. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, et al. Role of genotype in the cycle of violence in maltreated children. *Science.* 2002;297:851-4.
 36. Ressler KJ, Mercer KB, Bradley B, Jovanovic T, Mahan A, Kerley K, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature.* 2011;470:492-7.
 37. Botia B, Basille M, Allais A, Raoult E, Falluel-Morel A, Galas L, et al. Neurotrophic effects of PACAP in the cerebellar cortex. *Peptides.* 2007;28:1746-52.
 38. Wilkie MJ, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al. Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. *Pharmacogenomics J.* 2009;9:61-70.
 39. Kolassa IT, Ertl V, Eckart C, Glöckner F, Kolassa S, Papassotiropoulos A, et al. Association study of trauma load and SLC6A4 promoter polymorphism in posttraumatic stress disorder: evidence from survivors of the Rwandan genocide. *J Clin Psychiatry.* 2010;71:543-7.
 40. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res.* 2002;53:865-71.
 41. Holmes MC, Antoni FA, Aguilera G, Catt KJ. Magnocellular axons in passage through the median eminence release vasopressin. *Nature.* 1986;319:126-9.
 42. Phillips MI. Functions of angiotensin II the central nervous system. *Annu Rev Physiol.* 1987;49:413-35.
 43. Pariante CM, The Lightman SL. HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* 2008;31:464-8.
 44. Watson S, Gallagher P, Ritchie JC, Ferrier IN, Young AH. Hypothalamic-pituitary-adrenal axis function in patients with bipolar disorder. *Br J Psychiatry.* 2004;184:496-502.
 45. Kreek MJ, Nielsen DA, Butelman ER, LaForge KS. Genetic influences on impulsivity, risk taking, stress reactivity and vulnerability to drug abuse and addiction. *Nat Neurosci.* 2005;8:1450-7.
 46. Clarke TK, Treutlein J, Zimmermann US, Kiefer F, Skowronek MH, Rietschel M, et al. Review: HPA-axis activity in alcoholism: examples for a gene-environment interaction. *Addict Biol.* 2008;13:1-4.
 47. Marks AR. Cellular functions of immunophilins. *Physiol Rev.* 1996;76:631-49.
 48. Genecards.org [sede Web]. FKBP5 Gene Rehovot. Weizmann Institute of Science; 1996. Available from: <http://www.genecards.org/cgi-bin/carddisp.pl?gene=FKBP5> [updated 2016; cited 10.10.16].
 49. Supriyanto I, Sasada T, Fukutake M, Asano M, Ueno Y, Nagasaki Y, et al. Association of FKBP5 gene haplotypes with completed suicide in the Japanese population. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011;35:252-6.
 50. Watkins LE, Han S, Harpaz-Rotem I, Mota NP, Southwick SM, Krystal JH, et al. FKBP5 polymorphisms, childhood abuse, and PTSD symptoms: results from the National Health and Resilience in Veterans Study. *Psychoneuroendocrinology.* 2016;69:98-105.
 51. Kohrt BA, Worthman CM, Ressler KJ, Mercer KB, Upadhyaya N, Koirala S, et al. Cross-cultural gene-environment interactions in depression, post-traumatic stress disorder, and the cortisol awakening response: FKBP5 polymorphisms and childhood trauma in South Asia: G x E interactions in South Asia. *Int Rev Psychiatry.* 2015;27:180-96.
 52. Koenen KC, Saxe G, Purcell S, Smoller JW, Bartholomew D, Miller A, et al. Polymorphisms in FKBP5 are associated with peritraumatic dissociation in medically injured children. *Mol Psychiatry.* 2005;10:1058-9.
 53. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, et al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA.* 2008;299:1291-305.
 54. Kaplow JB, Dodge KA, Amaya-Jackson L, Saxe GN. Pathways to PTSD, Part II: Sexually abused children. *Am J Psychiatry.* 2005;162:1305-10.
 55. Clark DA, Rhyno S. Unwanted intrusive thoughts in nonclinical individuals: implications for clinical disorders. In: Clark DA, editor. *Intrusive thoughts in clinical disorders: theory, research, and treatment.* New York: Guilford Press; 2005. p. 1-29.
 56. Kuhlmann S, Wolf OT. Arousal and cortisol interact in modulating memory consolidation in healthy young men. *Behav Neurosci.* 2006;120:217.
 57. Payne JD, Jackson ED, Hoscheidt S, Ryan L, Jacobs WJ, Nadel L. Stress administered prior to encoding impairs neutral but enhances emotional long-term episodic memories. *Learn Mem.* 2007;14:861-8.
 58. Cheung J, Bryant RA. FKBP5 risk alleles and the development of intrusive memories. *Neurobiol Learn Mem.* 2015;125:258-64.
 59. Davis M, Whalen PJ. The amygdala: vigilance and emotion. *Mol Psychiatry.* 2001;6:13-34.
 60. Ledoux JE. Emotion circuits in the brain. *Annu Rev Neurosci.* 2003;23:155-84.
 61. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci.* 2009;10:434-45.
 62. Morey RA, Haswell CC, Hooper SR, De Bellis MD. Amygdala, hippocampus, and ventral medial prefrontal cortex volumes differ in maltreated youth with and without chronic posttraumatic stress disorder. *Neuropsychopharmacology.* 2016;41:791-801.
 63. Milad MR, Quirk GJ. Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature.* 2002;420:70-4.
 64. Quirk GJ, Russo GK, Barron JL, Lebron K. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci.* 2000;20:6225-31.

65. Shin LM, Orr SP, Carson MA, Rauch SL, Macklin ML, Lasko NB, et al. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female vietnam veterans with ptsd. *Arch Gen Psychiatry*. 2004;61:168-76.
66. Lebois LA, Wolff JD, Ressler KJ. Neuroimaging genetic approaches to posttraumatic stress disorder. *Exp Neurol*. 2016;284 Pt B:141-52.
67. Kirby ED, Friedman AR, Covarrubias D, Ying C, Sun WG, Goosens KA, et al. Basolateral amygdala regulation of adult hippocampal neurogenesis and fear-related activation of newborn neurons. *Mol Psychiatry*. 2012;17:527-36.
68. DeCarolis NA, Eisch AJ. Hippocampal neurogenesis as a target for the treatment of mental illness: a critical evaluation. *Neuropharmacology*. 2010;58:884-93.
69. Green MK, Devine DP. Nociceptin/orphanin FQ and NOP receptor gene regulation after acute or repeated social defeat stress. *Neuropeptides*. 2009;43:507-14.
70. Goeldner C, Reiss D, Wichmann J, Kieffer BL, Ouagazzal AM. Activation of nociceptin opioid peptide (NOP) receptor impairs contextual fear learning in mice through glutamatergic mechanisms. *Neurobiol Learn Mem*. 2009;91:393-401.
71. Rooszendaal B, Lengvilas R, McGaugh JL, Civelli O, Reinscheid RK. Orphanin FQ/nociceptin interacts with the basolateral amygdala noradrenergic system in memory consolidation. *Learn Mem*. 2007;14:29-35.
72. Rutten K, De Vry J, Bruckmann W, Tzschentke TM. Pharmacological blockade or genetic knockout of the NOP receptor potentiates the rewarding effect of morphine in rats. *Drug Alcohol Depend*. 2011;114:253-6.
73. Lambert DG. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. *Nat Rev Drug Discov*. 2008;7:694-710.
74. Andero R, Brothers SP, Jovanovic T, Chen YT, Salah-Uddin H, Cameron M, et al. Amygdala-dependent fear is regulated by *Oprl1* in mice and humans with PTSD. *Sci Transl Med*. 2013;5:188ra73.
75. Ehlers CL, Lind PA, Wilhelmsen KC. Association between single nucleotide polymorphisms in the mu opioid receptor gene (OPRM1) and self-reported responses to alcohol in American Indians. *BMC Med Genet*. 2008;9:1.
76. Stewart SH. Alcohol abuse in individuals exposed to trauma: a critical review. *Psychol Bull*. 1996;120:83.
77. Epstein JN, Saunders BE, Kilpatrick DG, Resnick HS. PTSD as a mediator between childhood rape and alcohol use in adult women. *Child Abuse Neglect*. 1998;22:223-34.
78. Woodward SH, Kaloupek DG, Streeter CC, Kimble MO, Reiss AL, Eliez S, et al. Hippocampal volume, PTSD, and alcoholism in combat veterans. *Am J Psychiatry*. 2006;163:674-81.
79. Devine DP, Watson SJ, Akil H. Nociceptin/orphanin FQ regulates neuroendocrine function of the limbic-hypothalamic-pituitary-adrenal axis. *Neuroscience*. 2001;102:541-53.
80. Fernandez F, Misilmeri MA, Felger JC, Devine DP. Nociceptin/orphanin FQ increases anxiety-related behavior and circulating levels of corticosterone during neophobic tests of anxiety. *Neuropsychopharmacology*. 2004;29:59-71.
81. Leggett JD, Harbuz MS, Jessop DS, Fulford AJ. The nociceptin receptor antagonist [Nphe 1, Arg 14, Lys 15] nociceptin/orphanin FQ-NH 2 blocks the stimulatory effects of nociceptin/orphanin FQ on the HPA axis in rats. *Neuroscience*. 2006;141:2051-7.
82. Zannas AS, Wiechmann T, Gassen NC, Binder EB. Gene-stress-epigenetic regulation of FKBP5: clinical and translational implications. *Neuropsychopharmacology*. 2015;41:261-74.
83. Fani N, King TZ, Reiser E, Binder EB, Jovanovic T, Bradley B, et al. FKBP5 genotype and structural integrity of the posterior cingulum. *Neuropsychopharmacology*. 2014;39:1206-13.
84. Fani N, Gutman D, Tone EB, Almlil L, Mercer KB, Davis J, et al. FKBP5 and attention bias for threat: associations with hippocampal function and shape. *JAMA Psychiatry*. 2013;70:392-400.
85. Almlil LM, Mercer KB, Kerley K, Feng H, Bradley B, Conneely KN, et al. ADCYAP1R1 genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. *Am J Med Genet B: Neuropsychiatr Genet*. 2013;162B:262-72.
86. Stevens JS, Almlil LM, Fani N, Gutman DA, Bradley B, Norrholm SD, et al. PACAP receptor gene polymorphism impacts fear responses in the amygdala and hippocampus. *Proc Natl Acad Sci U S A*. 2014;111:3158-63.
87. Schulz-Heik RJ, Schaefer M, Eliez S, Hallmayer JF, Lin X, Kaloupek DG, et al. Catechol-O-methyltransferase Val158Met polymorphism moderates anterior cingulate volume in posttraumatic stress disorder. *Biol Psychiatry*. 2011;70:1091-6.
88. Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science*. 2010;327:863-6.
89. Arango-Lievano M, Lambert WM, Bath KG, Garabedian MJ, Chao MV, Jeanneteau F. Neurotrophic-priming of glucocorticoid receptor signaling is essential for neuronal plasticity to stress and antidepressant treatment. *Proc Natl Acad Sci U S A*. 2015;112:15737-42.
90. Aston-Jones G, Bloom FE. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci*. 1981;1:876-86.
91. Wakamatsu K, Tabuchi K, Ojika M, Zucca FA, Zecca L, Ito S. Norepinephrine and its metabolites are involved in the synthesis of neuromelanin derived from the locus coeruleus. *J Neurochem*. 2015;135:768-76.
92. Oster H, Damerow S, Hut RA, Eichele G. Transcriptional profiling in the adrenal gland reveals circadian regulation of hormone biosynthesis genes and nucleosome assembly genes. *J Biol Rhythm*. 2006;21:350-61.
93. Rang HP, Ritter JM, Flower RJ, Henderson G. Rang & Dale's pharmacology. 7.th ed. Amsterdam: Elsevier; 2014. p. 177-96.
94. Hamil RW, Shapiro RE, Vizzard MA. Peripheral autonomic nervous system. In: Robertson D, Biaggioni I, Burnston G, Low PA, Paton JFR, editors. *Primer on the autonomic nervous system*. New York: Academic Press; 2012. p. 17-20.
95. Debiec J, LeDoux JE. Noradrenergic signaling in the amygdala contributes to the reconsolidation of fear memory: treatment implications for PTSD. *Ann N Y Acad Sci*. 2006;1071:521-4.
96. Geraciotti TD Jr, Baker DG, Ekhtor NN, West SA, Hill KK, Bruce AB, et al. CSF norepinephrine concentrations in posttraumatic stress disorder. *Am J Psychiatry*. 2001;158:1227-30.
97. Strawn JR, Geraciotti TD. Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. *Depress Anxiety*. 2008;25:260-71.
98. Kolassa IT, Kolassa S, Ertl V, Papassotiropoulos A, Dominique JF. The risk of posttraumatic stress disorder after trauma depends on traumatic load and the catechol-O-methyltransferase Val(158)Met polymorphism. *Biol Psychiatry*. 2010;67:304-8.
99. Valente NL, Vallada H, Cordeiro Q, Bressan RA, Andreoli SB, Mari JJ, et al. Catechol-O-methyltransferase (COMT) val158met polymorphism as a risk factor for PTSD after urban violence. *J Mol Neurosci*. 2011;43:516-23.

100. Boscarino JA, Erlich PM, Hoffman SN, Zhang X. Higher FKBP5, COMT, CHRNA5, and CRHR1 allele burdens are associated with PTSD and interact with trauma exposure: implications for neuropsychiatric research and treatment. *Neuropsychiatr Dis Treat*. 2012;8:131-9.
101. Olsson CA, Anney RJ, Lotfi-Miri M, Byrnes GB, Williamson R, Patton GC. Association between the COMT Val158Met polymorphism and propensity to anxiety in an Australian population-based longitudinal study of adolescent health. *Psychiatr Gen*. 2005;15:109-15.
102. Armbruster D, Mueller A, Strobel A, Lesch KP, Brocke B, Kirschbaum C. Children under stress-COMT genotype and stressful life events predict cortisol increase in an acute social stress paradigm. *Int J Neuropsychopharmacol*. 2012;15:1229-39.
103. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. *Science*. 2001;293:1068-70.
104. Norrholm SD, Jovanovic T, Smith A, Binder EB, Klengel T, Conneely K, et al. Differential genetic and epigenetic regulation of catechol-O-methyltransferase is associated with impaired fear inhibition in posttraumatic stress disorder. *Front Behav Neurosci*. 2013;7:30.
105. Wu CT, Morris JR. Genes, genetics, and epigenetics: a correspondence. *Science*. 2001;293:1103-5.
106. Dupont C, Armant DR, Brenner CA. Epigenetics: definition, mechanisms and clinical perspective. *Semin Reprod Med*. 2009;27:351-7.
107. Yehuda R, Bierer LM. The relevance of epigenetics to PTSD: implications for the DSM-V. *J Trauma Stress*. 2009;22:427-34.
108. Zovkic IB, Sweatt JD. Epigenetic mechanisms in learned fear: implications for PTSD. *Neuropsychopharmacology*. 2013;38:77-93.
109. Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem*. 2004;279:40545-59.
110. Weaver IC, Szyf M, Meaney MJ. From maternal care to gene expression: DNA methylation and the maternal programming of stress responses. *Endocr Res*. 2002;28:699.
111. Smith AK, Conneely KN, Kilaru V, Mercer KB, Weiss TE, Bradley B, et al. Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B: Neuropsychiatr Genet*. 2011;156:700-8.
112. Liberzon I, King AP, Ressler KJ, Almlil LM, Zhang P, Ma ST, et al. Interaction of the ADRB2 gene polymorphism with childhood trauma in predicting adult symptoms of posttraumatic stress disorder. *JAMA Psychiatry*. 2014;71:1174-82.
113. Newman MG, Llera SJ, Erickson TM, Przeworski A, Castonguay LG. Worry and generalized anxiety disorder: a review and theoretical synthesis of evidence on nature, etiology, mechanisms, and treatment. *Annu Rev Clin Psychol*. 2013;9:275-97.