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***Joanne Ryan[1,2,3], Isabelle Chaudieu[2], , ¶Marie-Laure Ancelin[2¶], Richard Saffery[1]**

[1]Cancer & Disease Epigenetics Group, Murdoch Children's Research Institute, and Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia.

[2]Inserm, U1061, University of Montpellier, Montpellier, France,

[3]Department of Epidemiology and Preventive Medicine, School of Public Health and Preventive Medicine, Monash University, Prahran, Australia.

¶These authors contributed equally

*Author for correspondence: Tel.: +61 39936 6621; Fax: +61 393481391; joanne.ryan@mcri.edu.au;

Abstract

Certain individuals are more susceptible to stress and trauma, as well as the physical and mental health consequences following such exposure, including risk for post-traumatic stress disorder (PTSD). This differing vulnerability is likely to be influenced by genetic predisposition and specific characteristics of the stress itself (nature, intensity and duration), as well as epigenetic mechanisms. In this review we provide an overview of research findings in this field. We highlight some of the key genetic risk factors identified for PTSD, and the evidence that epigenetic processes might play a role in the biological response to trauma, as well as being potential biomarkers of PTSD risk. We also discuss important considerations for future research in this area.

Keywords:

Trauma; Stress; Post-traumatic stress disorder (PTSD); early-life; genetics; epigenetics; DNA methylation; biomarkers; humans

The majority of individuals will encounter some form of trauma or severe stress over their lifetime, however most have the capacity to recover from such events without any long term health consequences [1]. Post-traumatic stress disorder (PTSD) is a chronic and highly debilitating psychiatric disorder which develops in a small number of people following exposure to a single traumatic event or multiple/chronic exposures over time [2]. This includes through direct involvement of the individual, as well as witnessing or hearing of the event occurring to a close friend or family member. PTSD is highly heterogeneous and can manifest in different ways, with 20 clinical symptoms as defined in the Diagnostic and Statistical Manual of Mental Disorders version 5 [3]. The core symptoms are re-experiencing (e.g. flashbacks, intrusive memories, and nightmares), avoidance behaviours, negative alteration in cognitions and mood, and hyperarousal [3]. The prevalence rates of PTSD range from about two to eight percent [4, 5], and it is more common in those experiencing certain types of trauma, such as having a child with a serious illness [6] and severe trauma which may pose a threat to life (particularly violence and military combat, and to a lesser extent severe accidents and disasters) [7]. However, even for individuals experiencing the same trauma, there is considerable inter-individual variability in their resilience and risk of PTSD, most likely due to underlying differences in biological processes, possibly genetic or epigenetically driven.

Here, we aim to provide a brief overview of the role of genetic and epigenetics in trauma, by highlighting some of the main findings to date. Recommendations for future research which will help advance our knowledge in this field are also discussed. While we acknowledge the essential contribution of animal studies, this review will focus predominantly on findings in humans. The majority of studies have investigated PTSD due to its direct etiological link with trauma, providing a model for gene and environment interactions, including those mediated by epigenetics.

Heritability of experiencing trauma and PTSD

Family-linkage studies which investigate patterns of diseases within families provide good evidence for the heritability of PTSD. Among Holocaust survivors for example, those with PTSD were much more likely to have children who also developed PTSD following exposure to trauma [8], although this estimate is likely inflated due to the shared family environment. Twin studies have been invaluable in this context, by comparing the degree of similarity in traits between monozygotic (MZ) and dizygotic (DZ) twins, enabling the genetic contribution of a disorder to be estimated. Such studies of PTSD indicate a relative high heritability explaining up to 45% of the variability in risk [9], with ranges from 13-69% when individual PTSD symptoms are considered.

The persisting and unique complication in estimating the underlying genetic contribution of PTSD however, is that the risk is directly tied to the occurrence of a traumatic event and cannot be estimated in individuals who have not been exposed. Further, an individual's likelihood of experiencing trauma, in particular certain types of trauma, may also have a genetic component. Studies of male MZ twin pairs have found a two-fold higher concordance in volunteering for and serving in the military [10], as well as a significantly higher intra-pair correlation in self-reported combat experiences [10, 11]. In a study of 2591 adult twins and their siblings, additive genetic factors were estimated to account for 60% of the variance of high-risk trauma (e.g. childhood sexual and physical abuse, neglect), while for other traumas this dropped to 47% [9]. By comparing the intra-pair correlations of exposure between 222 MZ (0.53) and 184 DZ twins (0.23), Stein and colleagues also found a moderate genetic component to the risk of exposure for some assaultive traumas (i.e. robbery and sexual assault), but not for motor vehicle

accidents or natural disasters (intra-pair correlation 0.35 and 0.26 for MZ and DZ twins respectively) [12].

A likely explanation for the high heritability of experiencing specific traumas may relate to personality traits that can influence behaviour and lifestyle choices, including risk taking behaviours. Indeed, antisocial personality traits, self-harming behaviour and substance misuse have been shown to predict the risk of violent assaultive trauma, and these are partly mediated by genetic factors [13]. Few studies however, have yet attempted to identify the individual genes involved in influencing risk. Further, it remains unclear to what extent genetic risk factors for trauma and PTSD overlap. A Norwegian study of 2794 adults determined that only one fifth of the familial liability (genetic and common environmental factors) of PTSD symptoms overlapped with the liability for trauma exposure [14]. However another study reported a very high correlation between genetic risk factors for trauma and PTSD [9], highlighting the importance of controlling for the risk of trauma in genetic association studies of PTSD.

Genetic risk variants for PTSD

The majority of studies investigating genetic risk factors for PTSD have been candidate gene association studies, where genes were selected based on their known or perceived involvement in the etiology, pathology or neurobiology of the disease. Functional variants within these genes are often targeted. Currently more than 25 genes have been examined and some of the main findings are summarized in **Table 1**.

The early genetic association studies targeted dopaminergic signalling, given the long established role of dopamine in the stress response [15]. Dopamine is released following stress [16] and dopamine levels have been directly correlated with the magnitude of the cortisol response to stress [17]. A number of studies have investigated single nucleotide polymorphisms (SNPs) in the dopamine receptor *DRD2* or a specific variant (*rs1800497*) in the closely positioned ankyrin repeat and kinase domain containing 1 (*ANKK1*) gene which can regulate *DRD2* [18]. Almost all studies involved Caucasian military men exposed to combat, however the findings have been variable. In regards to *rs1800497*, a few studies report that the T allele is associated with increased PTSD risk [19, 20], however other larger studies have found no association [21]. The other SNPs which have been investigated have varied across studies [22, 23]. A more recent and larger study of men and women exposed to a range of traumas (651 PTSD cases, 1098 controls), reported a significant association with a specific SNP (*rs12364283*) in *DRD2* [24]. However this was a case-control study of heroin dependence, and the associations identified were predominantly limited to amphetamine-dependent individuals. More consistent findings have been reported for the dopamine transporter *DAT1*, where the shorter variable number tandem repeat (VNTR) increased the risk of PTSD, even in those exposed to very different traumas (i.e. natural disasters, war and violence) [25, 26].

Dysfunction of serotonergic signalling is thought to play a role in the pathophysiology of PTSD [27], and some treatments for PTSD target this pathway, with mixed success [28]. The serotonin transporter gene (*5-HTT*), essential for neurotransmitter signalling, has been extensively studied, with most studies focusing on a functional variant that affects gene transcription [29]. The serotonin transporter linked polymorphism (*5-HTTLPR*) consists of a 44-bp insertion/deletion and the short ("S") compared to the long ("L") allele leads to reduced gene transcription. Several, but not all studies [30, 31], have reported that *5-HTTLPR* (with or without consideration of the proximal *rs25531* variant), influences the risk of PTSD [32]. The S allele has been associated with an increased risk of PTSD following exposure to natural

disasters, civilian war, physical and childhood traumas, and in different ethnic populations. On the other hand, a couple of studies have reported reversed associations, in that individuals homozygous for the S allele actually had a reduced PTSD risk [33, 34]. These conflicting findings may be explained by the differential susceptibility hypothesis [35], which posits that certain genetic variants are more responsive to the environment, being risk factors under certain conditions but conferring resilience in others. In support of this, a study of 590 individuals found that the S allele increased the risk of PTSD following a natural disaster, however only for individuals in high risk environments [36]. For individuals living in areas with low unemployment and crime rates, the S allele was associated with a decreased risk [36]. Other studies have also shown that the S allele is a risk factor for PTSD only in the absence of social support [37], in individuals having experienced both a childhood trauma and a later event in adulthood [38], or in response to severe traumas but not milder events [39].

Genes of the HPA-axis are obvious candidates for studies of PTSD given that this axis is activated in response to stress and influences a broad range of biological processes (**Figure 1**). A study of corticotropin-releasing hormone receptor 1 gene (*CRHR1)* identified variants that were associated with PTSD symptoms in adults following a hurricane exposure. One, *rs12938031*, also predicted PTSD diagnosis [40], but this has not been replicated [41]. Two complementary studies found that severe childhood trauma interacts with genetic variation in the FK506 binding protein 5 (*FKBP5)* gene to increase the risk of PTSD in adults. In the Grady Trauma Project, four highly linked SNPs (*rs9296158, rs3800373, rs1360780, rs9470080*) interacted with the severity of childhood abuse to predict risk of PTSD symptoms in adulthood [42]. A subsequent study reported that three of these four variants were independently associated with the risk of PTSD in a similar ethnic group (African-Americans), but there were no significant association in a non-Hispanic white population [43]. These ethnic-specific findings may be explained by differences in genotype frequencies, including a higher frequency of risk variants in African-Americans and varying degrees of linkage disequilibrium with other potential risk alleles, as well as environmental specific factors which also contribute to risk. Interestingly in that study, childhood adversity was a stronger risk factor for PTSD in European rather than the Africa Americans [43]. One of these genetic variants (*rs9470080*) also interacted with childhood adversity to increase PTSD risk, thus replicating the earlier findings [42]. The *rs1360780* genetic variant may also influence the effectiveness of psychotherapy in PTSD patients [44]. A single PTSD study found no association with variants in the glucocorticoid receptor gene (*NR3C1),* despite it being the most extensively studied gene in epigenetic analysis (discussed later) [45].

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) and its PAC1 receptor have been shown to be unregulated following chronic stress and they in turn can activate corticotropin-releasing hormone (*CRH*) transcription [46, 47]. The genes coding for PACAP and PCA1 (*ADCYAP1* and *ADCYAP1R1* respectively) have been investigated in a highly traumatised African-American population, comparing PTSD cases and controls matched on type of trauma [48]. The *rs2267735* SNP of *ADCYAP1R1* was associated with diagnosis of PTSD and PTSD symptoms in females only [48] and subsequently replicated [49]. *ADCYAP1R1* gene expression has been shown to be modulated by the female sex-hormone, estrogen [48], and *rs2267735* is positioned in a putative estrogen response element, within the gene, which may help explain the gender-specific associations. A Chinese study investigating this variant, reported no association with total PTSD symptoms, but it predicted the severity of specific symptoms in women who had lost a child in a natural disaster [50].

The brain-derived neurotrophic factor gene (*BDNF*) is a neurotrophin that plays a key role in the formation, plasticity and integrity of neurons in brain circuits regulating emotion. Stress appears to regulate BDNF signalling [51], resulting in increased BDNF serum levels [46]

which have also been observed in PTSD [52]. A variant of this gene, *rs6265*, has been associated with the risk of PTSD in male war veterans [53] and therapy response in patients [54], as well as specific features of PTSD, such as fear conditioning. In contrast, earlier studies of civilian populations experiencing various types of other traumas reported no associations, even with compatible sample sizes [55].

Other candidate genes investigated include catechol-o-methyltransferase (*COMT*), implicated in the metabolism of catecholamines [56]; protein kinase C alpha *(PRKCA)* implicated in diverse signalling processes [57, 58], the cannabinoid receptor gene (*CNR1)*, which is involved in dopamine regulation and stress [59] and Apolipoprotein E *(APOE)* thought to influence stress reactivity [60]. C-reactive protein (*CRP*) is a pro-inflammatory marker and increased circulating levels of this protein have been observed in individuals with PTSD. The variant *rs1130864* of this gene was associated with increased CRP serum levels, PTSD diagnosis and severity of PTSD symptoms [61]. A single SNP in the opioid receptor-like 1 gene (*OPRL1*) was associated with both PTSD symptoms and a self-reported history of childhood trauma [62], which supports the potential beneficial effect of opioid analgesia on PTSD opioid analgesia [63]. However, there is currently insufficient evidence to clearly implicate any of these genes in PTSD.

In summary, despite some significant findings, very few of these have so far been successfully replicated [23]. This is likely caused in part by the differences in studies, with varying populations and exposures, as well as the likely small effect sizes which are easily drowned out by heterogeneous study designs. Publication bias would also suggest that there may be many more negative findings, than those discussed here.

Genome-wide association studies of PTSD

Hypothesis-free genome-wide association studies (GWAS) aim to identify novel genes or gene pathways implicated in disease and hold particular promise for PTSD where the exact disease etiology remains unclear. However only a handful of PTSD GWAS have so far been undertaken, the majority in USA veterans or military personnel and their partners [64-69]. A number of novel loci and genes have been identified, although not always at genome-wide significant levels. This includes SNPs in the phosphoribosyl transferase domain containing 1 *(PRTFDC1)* [68], Down syndrome cell adhesion molecule *(DSCAM)* [64], Unc-13 homolog C *(UNC13C*) [64], Tolloid-like 1 *(TLL1)* [69], Neuroligin 1 *(NLGN1)* [66] and the retinoid-related orphan receptor alpha (*RORA)* genes, as well as a long intergenic non-coding RNA [65] [67]. None of these genes however, have been identified across more than one GWAS study, and for most, the role of these genes in PTSD has not been clearly elucidated. *NLGN1* encodes a protein involved in synaptogenesis and has previously been associated with autism. *RORA* encodes a protein that can protect neurons and glial cells for the neurotoxic effects of traumatic stress and it is expressed in brain regions such as the hypothalamus and cerebral cortex [67]. A more recent study using two independent cohorts of individuals with lifetime PTSD diagnosis failed to replicate the overall findings with *RORA* , although a number of nominally significant associations were identified, including an association between *rs11071587* and lifetime PTSD risk in Caucasian females from both cohorts [70]. It may be that this gene is a risk factor for the severity of PTSD symptoms, rather than the disorder itself [71] or with other closely linked forms of psychopathology, such as the fear component of internalizing [72]. A recent study also found evidence that this gene is more closely associated with posttraumatic stress trajectories in individuals exposed to childhood physical abuse [73]. In a discovery sample of 147 military personal with and without PTSD following combat exposure, an intergenic SNP on chromosome 4, *rs717947*, was found to be associated with PTSD at genome-

wide significant levels [74]. Interestingly this association was replicated in community women from the Grady Trauma Project, but not men. The risk variant was also associated with decreased medial and dorsolateral cortical activation to fearful faces, which may be considered an endophenotype of PTSD.

Gene-environment (trauma) interactions

Genetic association studies of PTSD are a special case of a gene-environment interaction, whereby genetic predisposition is considered together with a given environmental condition (trauma) and the risk of disease is determined. Genetic risk factors may interact with trauma to influence other health outcomes as well. One of the most widely cited studies in this area investigated the association between a high number of stressful events and clinical depression in young adults [75]. They reported a significant positive correlation, but only for individuals with the S allele of *5-HTTLPR.* Individuals with the L allele who experienced a high number of stressful events, had no increased depression risk. This research triggered a wave of subsequent studies but the results have not always been in concordance [76, 77] and debate about these findings is ongoing. Numerous other gene-environment interactions have also been reported for early-life trauma and genes previously implicated in behaviour and psychiatric disorders. For example, boys who were maltreated and also carried a gene variant which results in lower monoamine oxidase A (*MAOA*) expression, had an increased risk of antisocial type behaviours in adulthood, but no such risk was observed for untreated boys with the same variant, or maltreated boys without this variant [78]. Once again this finding has not been clearly replicated [79], with most studies inadequately powered to detect such interactions. Despite this controversy, it remains clear that vulnerability to the effects of trauma and the risk of PTSD results from multiple independent, competing and interacting environmental and genetic factors. The question therefore remains of how trauma can have an enduring influence on disease risk, often lasting years, decades and even a lifetime after the actual events have occurred. More recent research has focused on the role of epigenetics in this regard.

The epigenome is responsive to trauma

The epigenome is the collection of potentially reversible modifications that regulate the activity of genes without influencing the DNA sequence. In addition to being regulated by genetic factors, much of the epigenome is responsive to external influences throughout the lifecourse. This plasticity potentially enables optimal adaptation to changing environmental conditions. Exposure to trauma has considerable potential to impact the epigenome in a stable manner that may help explain the long-term effects of trauma on later health, including risk for psychiatric disorders [80, 81] and more specifically, PTSD.

The epigenome varies across cells and tissues, and rodent models have therefore been invaluable for investigating how trauma influences epigenetic patterns in the brain, given its central role in PTSD aetiology. Such studies have examined the effects of exposure to early life stress (e.g. prenatal stress, maternal behaviour/care, separation) and stress in adulthood (e.g. fear conditioning, social defeat stress, chronic stress, and immobilization) on DNA methylation and/or histone modifications. Genes involved in neurogenesis and neuronal plasticity (*Bdnf* and Glial cell-derived neurotrophic factor, *Gdnf*) [82, 83], as well as HPA-axis signalling (*Crh; Crhr1; Nr3c1*; arginine vasopressin gene, *Avp*) [82, 84-87], have been implicated. Effects are however, brain region dependent [86].

The findings from experimental trauma studies in animals cannot readily be extrapolated to the human context, and access to human brain tissue is of course limited. Despite this, there has

been an exponential increase over the last decade of human PTSD studies, thus far investigating DNA methylation patterns in peripheral tissue.

DNA methylation patterns in PTSD

Epigenome-wide association studies (EWAS) are similar to GWAS, in that they simultaneously investigate thousands of genetic loci throughout the genome. The primary aim of EWAS has been to identify DNA methylation differences at individual CpG sites or gene regions across individuals. The first EWAS of PTSD used the Infinium HumanMethylation27 BeadChip (HM27K) Array (Illumina) to measure blood DNA methylation of more than 14,000 genes from 100 individuals in the Detroit Neighborhood Health Study [88]. All individuals had been exposed to at least one past traumatic event and 23 were diagnosed with lifetime PTSD. An overrepresentation of loci found to be differentially methylated between cases and controls were annotated to genes involved in immune system functions. Differential methylation at two genes encoding DNA methyltransferases, *DNMT3B* and *DNMT3L*, were also identified [88]. Only two subsequent EWAS studies have been undertaken, both from the Grady Trauma Project, although the analysed sub-samples varied. One study measured blood methylation with the HM27K array in 50 individuals with PTSD and 50 controls matched for childhood trauma [89]. PTSD was associated with increased average methylation across all probes, and specific sites in five genes (translocated promoter region, *TPR*; C-type lectin domain family 9 member A, *CLEC9A;* acid phosphatase 5 tartrate resistant, *ACP5;* annexin A2, *ANXA2;* and toll-like receptor 8, *TLR8*) were found to be differentially methylated [89]. A number of identified genes have been linked with inflammation, supporting the initial EWAS findings, as well as prior observations that disrupted immune function is a feature of PTSD [90]. The second EWAS used the more recent Infinium HumanMethylation450K BeadChip (HM450K) array (Illumina) measuring DNA methylation at over 485,000 loci throughout the genome. Blood methylation profiles were compared between 32 current PTSD cases with a history of moderate to severe childhood abuse, 29 current PTSD cases with other lifetime traumas, and controls free of lifetime PTSD but matched for trauma exposure [91]. A number of differentially methylated loci were identified, particularly in the Spondin-1 (*SPON1)* and the Tetraspanin-32 (*TSPAN32)* genes that almost completely distinguished the three groups. PTSD cases with a history of childhood abuse, in particular, showed the most distinct methylation patterns. Of note, these EWAS studies have all involved predominantly high risk African-American populations, with low socioeconomic status and high rates of exposure to assaultive trauma. Sample sizes have almost universally been inadequate to reliably detect small effect sizes.

The most frequently investigated candidate gene is *NR3C1*, primarily as differential *NR3C1* methylation has been reported following early-life stress and linked to stress sensitivity [92] (**Table 1**). Among 122 combat veterans, *NR3C1* blood methylation was negatively correlated with PTSD symptoms [93] and decreased *NR3C1* methylation in blood has also been found in 30 individuals with current or past PTSD [94]. Similarly, increased *NR3C1* methylation in saliva was associated with less intrusive traumatic memory and a decreased risk of PTSD in 83 male survivors of the Rwandan genocide [95]. It is hypothesised that decreased *NR3C1* methylation is linked with lower circulating cortisol levels, the end product of HPA-axis signalling (Figure 1), but this has not yet been adequately investigated. Another study of maternal PTSD severity (n=45) also found a negative correlation with *NR3C1* methylation [96]. Likewise, maternal prefrontal cortical activation in response to video-stimuli which was negatively correlated with PTSD severity, was positively correlated with DNA methylation [96].

A number of other significant associations have also been reported, but none yet replicated. Complementing their findings of an association between *ADCYAP1R1* genetic variation and PTSD in women, Ressler and colleagues found a positive correlation between *ADCYAP1R1* blood methylation and total PTSD symptoms, although this finding was not sex specific [48]. In the same cohort, increased *COMT* methylation was observed in individuals with fear inhibition, an intermediate phenotype in PTSD [56]. Among 200 trauma exposed war veterans, PTSD symptom severity has been positively associated with blood methylation levels of the spindle and kinetochore-associated complex subunit 2 (*SKA2*) gene, which was identified as a promising biomarker of suicide risk [97]. Furthermore, methylation was shown to mediate in part the association between PTSD and reduced cortical thickness. Although not yet specifically replicated, another study found that individual increase in SKA2 methylation corresponded with the emergence in PTSD symptoms in a Dutch military sample [98]. Interestingly, increased methylation of this gene has also been associated with lower cortisol stress reactivity [98], which is a common feature of PTSD [99].

Other studies have reported a lack of independent associations between candidate gene methylation and PTSD, but have found that methylation levels modified the association between traumatic events and PTSD risk. For example, a positive association was found between the number of traumatic events and PTSD risk (diagnosis, severity and symptoms) in 100 individuals from the Detroit Neighborhood Health Study, but only for those with lower *5HTT* promoter methylation levels [30]. Likewise in the same study, higher mannosidase alpha class 2C member 1 (*MAN2C1*) methylation levels augmented the association between cumulative traumatic burden and lifetime PTSD [100]. Interestingly, while animal models of PTSD have implicated differential *Bdnf* methylation in the hippocampus [101], and hypermethylation of *BDNF* has been found in the brain of suicide victims compared to non-suicide controls [102], only one human study has yet investigated epigenetic regulation of this gene in PTSD. This small study (n=48) which focused on interpersonal violence-related PTSD, found that *BDNF* exon IV methylation was positively associated with maternal anxiety and brain activation, but not with PTSD [103].

Evidence is beginning to emerge of genetic and epigenetic interactions influencing PTSD risk. A study of 16 PTSD cases and 67 controls that investigated both genetic variation and DNA methylation of the *DAT1* dopamine transporter gene found that the 9-repeat allele previously reported as a risk factor for PTSD, only increased risk when *DAT1* promoter methylation levels were high [25]. Individuals with the 9-repeat allele and low methylation levels had no increased risk. DNA methylation patterns of *5-HTTLPR* have also been associated with an increased risk of unresolved loss or trauma, but only for individuals with the L allele [104] and *5-HTTLPR* genetic-epigenetic interactions influence circulating cortisol levels in response to stress [105]. Recent evidence suggests that DNA methylation may be a mechanism by which specific genetic variants can influence the risk of PTSD. A functional polymorphism in the *FKBP5* gene for example, resulted in DNA demethylation of specific glucocorticoid response elements in this gene, and this led to an increased risk of developing PTSD following childhood trauma [106].

Longitudinal studies

While these studies have provided important information on the epigenetic profile of PTSD, the vast majority have been cross-sectional studies, making it impossible to determine whether the methylation marks proceed the development of PTSD or are a result of the disorder. Studies with longitudinally collect biospecimens are crucial, enabling the investigation of temporal changes in DNA methylation. In a cohort of US military personal deployed to the Middle East,

blood DNA methylation of immune system-related genes was measured in 75 individuals pre- and post- PTSD diagnosis, and in 75 military controls [107]. The degree of DNA methylation change was different between cases and controls for the long non-coding RNA transcript (*H19*) and interleukin-8 (*IL8*). A similar study of 96 Dutch military measured DNA methylation pre- and post- deployment in Afghanistan using the HM450K array, and compared groups according to their level of trauma exposure, as well as the severity of PTSD symptoms [108]. Trauma was associated with increased DNA methylation age (a marker of accelerated epigenetic ageing [109]), but interestingly, development of PTSD symptoms appeared to reverse this. Methylation data for individual loci was not reported. A small longitudinal study of psychotherapy in combat veterans with PTSD showed that *NR3C1* blood methylation pre-treatment, predicted treatment outcome but itself was not significantly altered post-treatment, in either responders (n=8) or non-responders (n=8) [110]. Conversely, *FKBP5* promoter methylation decreased with recovery, but pre-treatment levels did not predict response [110], but clearly this sample size is too small to be conclusive. One of the only civilian samples to investigate longitudinal changes in DNA methylation, examined genes coding for DNA methyltransferease genes (*DNMT*) pre- and post-trauma in 30 PTSD cases and 30 matched controls [111]. The investigators identified distinct DNA methylation marks following trauma in PTSD cases (*DNMT1*), but also potentially resilient marks present prior to trauma (*DNMT3B*) and differentiated cases from controls [111]. Further work is clearly needed in larger sample sizes to determine the possibility of identifying a unique epigenetic signature which could help predict individuals at greatest risk of PTSD.

Trauma exposure is associated with epigenetic modifications

In addition to PTSD, trauma itself has been shown to alter DNA methylation patterns and early-life has been highlighted as a particularly sensitive period of biological vulnerability. Indeed, trauma during critical periods of development *in utero* and early childhood is thought to be particularly important as epigenetic patterns are established, and the effects of trauma are more likely to become embedded, with long-term consequences [112]. Early-life trauma is also a major risk factor for later psychiatric disorders [113], including PTSD.

A couple of studies have investigated the effect of early-life trauma on DNA methylation in central tissue, focusing on the hippocampus given its direct involvement in stress signalling. In their study of post-mortem brain tissue from 36 adult suicide victims, McGowan and colleagues found that a history of severe childhood abuse (ascertained with proxy-based interviews) was associated with increased hippocampal *NR3C1* methylation [114]. An EWAS (HM450K array) also identified numerous genes that were differently methylated in the hippocampus of 25 men with a history of severe childhood abuse, compared to 16 non-abused controls [115]. The most significant genes mapped to pathways involved in neuronal plasticity, rather than HPA-axis signalling, with the top hit being the Alsin gene (*ALS2*) that regulates small GTPase activity [115]. This finding complements the results of neurobiology studies which have shown that epigenetic mechanisms play an important role in synaptic plasticity and the formation of memories [116], and intrusive memories are one of the hallmark features of PTSD.

The finding from McGowan, combined with the observation that prenatal stress was also associated with increased *NR3C1* methylation in peripheral tissue [117], lead to a wave of subsequent candidate gene studies investigating epigenetic regulation of this gene in early-life trauma. Although the studies have been quite heterogeneous in terms of the type and timing of exposure (e.g. prenatal *in utero* stress exposure, trauma or adversity in childhood), the delay between exposure and the methylation measure, as well as the tissue investigated (i.e. cord blood, peripheral blood, placenta, buccal cells) there is now evidence for a relatively weak but

consistent association between early-life trauma and increased *NR3C1* methylation [92, 118] [119]. These findings are of particular relevance given that they align with the earlier observations in brain tissue, and suggest that, at least for some genes, peripheral epigenetic patterns may be a good reflection of changes occurring in relevant brain regions. Interestingly however, the increased *NR3C1* methylation following early-life trauma, contrasts with decreased levels observed in PTSD [93, 94]. This could fit with observations that trauma results in HPA-axis hyperactivation, but low circulating levels have been observed in PTSD.

A number of other studies provide preliminary evidence of an association between early-life trauma and candidate genes in peripheral tissue, such as *5-HTT* [120], *BDNF* [121] and insulin-like growth factor 2 (*IGF2*) [122], as well as genes involved in immune response [123]. EWAS however, have provided the opportunity to identify novel genes. Parental stress predicted differences in buccal DNA methylation patterns (HM27K array) of genes involved in biosynthetic and metabolic processes in offspring adolescences [124]. These methylation differences varied depending on the sex of the child, and timing of stress exposure, with maternal stress having a greater effect in early infancy, while paternal stress was more predictive in the preschool years. A study of 96 maltreated children removed from their parents care, and 96 matched controls, identified 2868 loci which were differentially methylated across the genome (using the HM450K array) [125]. Many of the loci were intragenic and localized to genes previously associated with childhood diseases, for example asthma (*FANK1*), cancer (*WNT3A*), diabetes (*PTPRN2*) and cortical development (*CCDC85C*).

DNA methylation has also been shown to play a role in stress regulation following exposure to traumatic events. A very recent EWAS identified a locus in the Kit ligand gene (*KITLG)* as being differentially methylated following childhood trauma, and methylation of this gene mediated 32% of the association between early-life trauma and later stress reactivity [126]. This study initially involved 85 healthy adults and measured methylation in whole blood, followed by a replication sample of 45 individuals from an independent cohort and then a cross-tissue validation using buccal swabs from adolescents. Interestingly, this gene which is involved in cellular developmental processes has previously been linked with HPA-axis activity and has been shown to regulate the expression of *NR3C1* in erythroblasts [127].

Outside of the "critical" early-life period, a number of studies have also reported associations between trauma and epigenetic marks. An EWAS of civilian at-risk African Americans, failed to identify any genes which were differentially methylated between individuals exposed to childhood trauma and controls, but identified one loci, near the neuropeptide FF receptor 2 (*NPFFR2*) that distinguished individuals based on the total number of stressful events experienced over a lifetime [89]. This neurotransmitter has previously been implicated in PTSD [63]. In the same population, cumulative life stress was a stronger predictor of accelerated epigenetic ageing compared to childhood trauma or current stress [128].

Again genes involved in HPA-axis signaling (**Figure 1**) have been strong candidates for studies in this area. In keeping with the earlier findings concerning *NR3C1,* stressful life events in adolescence have also been associated with increased *NR3C1* blood methylation, independently of childhood trauma [129]. Further, a study of 32 Holocaust survivors and their adult offspring, as well as 8 control parent-child dyads, found that Holocaust exposure was associated with differentially *FKBP5* methylation. Interestingly, while lower *FKBP5* methylation was observed in those exposed individuals, compared to the controls, the offspring of Holocaust survivors actually had higher methylation levels than the offspring of non-exposed individuals [130]. These findings may represent an adaptation of the next, but further work is needed to investigate the underlying mechanisms for this intergenerational transmission.

Limitations of genetic and epigenetic association studies of PTSD

Over the last decade there has been considerable increase in research aimed at advancing our knowledge of the genetic and epigenetic underpinnings of trauma and PTSD. While the findings presented here provide evidence for the involvement of specific gene and gene pathways, many of which are supported by their known involvement in key biological systems disrupted in PTSD, there are also a number of limitations to this research and caution must also be taken in the interpretation of findings to date. Overall, there has been a general lack of clearly replicable findings, which may be partly accounted for by the inherent statistical limitations of the generally small studies. Differences are also likely to relate to the heterogeneity of the populations (ethnicity, gender, age, clinical versus community samples), and the trauma experienced (type, number, severity and timing). These cumulative differences across studies may also make it impossible to observe the generally small effect sizes in subsequent studies. Of note, the vast majority of studies presented here involved either Caucasian males in the military with combat exposure, or African-American heavily traumatized populations in the USA. While these studies have contributed crucial insights in this area, whether or not these findings can be extrapolated to other populations and contexts remains to be determined. Of further note, although women are less likely to experience a traumatic event (overall) than men, but have higher rates of PTSD. Sex-specific epigenetic changes in the brain, driven by steroid hormones, may help account for this differing vulnerability. Accumulating findings from animal studies suggest that the effects of various exposures on the epigenome can vary between males and females, but this has not yet been sufficiently investigated in human epigenetic studies.

Genetic and epigenetic association studies of PTSD also have unique complexities which must be considered in the study design and interpretation of findings. PTSD is a highly heterogeneous condition which can present itself differently across individuals. Cases can be defined based on PTSD diagnosis or the presence of specific PTSD symptoms, current or lifetime diagnosis and first episodes or recurrent PTSD, thus contributing to large heterogeneity across studies. The selection of the most appropriate controls for these studies also remains a difficult yet important consideration, as they cannot be easily sampled from the wider population. By definition a PTSD diagnosis requires a previous trauma to have occurred, and controls must therefore be sampled from those having experienced a similar trauma but without developing PTSD, normally within a given time-frame since the event. This selection strategy also helps minimize risk factors (including genetic) which are associated with the likelihood of exposure, rather than or as well as the risk of PTSD. Natural disasters provide a perfect platform for this type of study, but are not without their own challenges. In addition to the obvious difficulties arising in these environments, ensuring adequate matching on the severity and duration of trauma can be problematic, as well as other environmental factors such as socio-support and economic status, which may themselves influence PTSD risk. Prospective exposed cohort designs can be very useful, where for example, participants are recruited from hospital emergency wards after a trauma exposure. However recruiting samples large enough for genetic analysis remains an issue. Another difficulty concerns the considerable overlap in the heritability of PTSD with other psychiatric disorders [9] that could share genetic risk factors.

Finally, when investigating the possible involvement of epigenetic processes involved in trauma and PTSD, human post-mortem studies of brain tissue are particular relevant. Unlike genetic marks, epigenetic patterns are tissue and cell specific, and the brain would thus be the most appropriate tissue for disorders involving central processes. However post-mortem studies are not without their own limitations, which includes confounding related to the cause

of death (including possible comorbid psychiatric disorders like depression), the timing and condition under which the sample is obtained, the lack of detailed patient history including information on the trauma itself and diagnosis of other psychiatric conditions, and the fact that findings from such studies will only ever be correlational (i.e. lacking a prospective design to help ascertain causality). Conversely, the usefulness of peripheral epigenetic markers in complex neurobiological phenotypes and brain disorders is now well recognized [80]. This may be particularly relevant for trauma which is known to impact on a range of biological systems involving humoral mechanisms, including stress reactivity and HPA axis, or associated inflammatory response.

Conclusion

Individuals differ in their vulnerability for PTSD and through a growing number of GWAS, several novel genetic risk factors have now been identified. There is now consistent evidence that early-life trauma can result in changes in DNA methylation patterns in both central and peripheral tissue. Individuals with PTSD have also been found to carry a unique DNA methylation signature, however whether this can be used to predict risk of PTSD or is a consequence of the disease process, remains to be determined. Furthering our knowledge of the genetic and epigenetic architecture underlying response to trauma and risk for PTSD will enhance our understanding of disease aetiology and could enable the early identification of vulnerable individuals, with the future possibility of targeted preventative interventions. Given that epigenetic processes are dynamic in nature and highly sensitive to environmental cues, there is great promise that appropriate interventions could help counteract or reverse the negative effects of trauma, building resilience through changes in gene activity.

FUTURE PERSPECTIVE

Over the next five to ten years there will be a greater shift away from individual candidate gene analysis to studies focused on groups of genes in common biological pathways, and larger genome-wide analysis. GWAS and EWAS investigate hundreds of thousands of genetic loci which are analyzed individually and stringent criteria must therefore be used to account for the multiple testing. The predominantly small studies to date have been underpowered to detect the likely small risk ratios and effect sizes, and much larger samples are therefore essential. Current estimates based on other psychiatric disorders suggest that future GWAS studies will need to involve 10,000 or more participants if variants are to be identified at the necessary corrected levels of significance [131]. EWAS are more favourably powered owing to the continuous nature of methylation data (compared to gene frequencies), but other data complexities (e.g. large variances, uneven distributions) ensure that large samples are also needed to generate robust findings. This is where consortium, such as the Psychiatric Genetics Consortium (PGC) PTSD group [132], will become essential to advance research in this area. Such consortium will also provide a platform to integrate GWAS and EWAS data, which is a crucial next step for research in this field.

Large prospective studies which can adequately control for the various bias inherent in these types of studies (as discussed previously), are now needed. There have been some promising findings from the few longitudinal epigenetic studies so far undertaken, and it is likely that an increasing number of prospective cohorts will collect biological samples at multiple time-points, enabling temporal epigenetic changes to be examined. This is particularly important to differentiate between DNA methylation marks which proceed PTSD, rather than those which are a consequence. Early biomarkers of at-risk individuals are particularly interesting, given

their potential clinical utility. This could include administering early pharmacological treatments or cognitive-behavioural interventions to the most vulnerable individuals following major trauma exposure, which could reduce their risk of subsequently developing PTSD. For example, medication records showed that U.S. military personnel who received morphine in post-trauma care had lower rates of PTSD than personnel who did not receive post trauma treatment [63]. It's possible that such treatment would be particularly beneficial in individuals with biomarkers indicating that are already at an increased risk of PTSD. Biomarkers could also be used to monitor the effectiveness of treatment interventions. In the broader field of epigenetics, a lot of attention is also being paid to new epigenetic therapies. Histone deacetylase (HDAC) inhibitors for example, have been shown to reverse deficits in stress-related behaviours, as well as synaptic plasticity, learning and memory. Again, these treatments may prove to most beneficial in certain individuals, such as those at-risk of PTSD [133]. However, more work is firstly required to establish the biological relevance of differences in peripheral methylation patterns observed in PTSD or trauma, by investigating whether they are associated with functional changes in gene expression and protein levels. For example, although some recent findings are starting to emerge [98, 126], there is a lack of studies investigating how epigenetic factors can be linked directly with cortisol levels or stress reactivity in PTSD. Further, determining how peripheral epigenetic marks correlate with methylation and expression patterns in brain tissue will be essential to understand their possible involvement in disease etiology.

EXECUTIVE SUMMARY

Heritability of trauma and post-traumatic stress disorder (PTSD)

- The heritability of PTSD is estimated at up to 35%, but varies widely when individual PTSD symptoms are examined.
- The risk of experiencing certain types of traumas may also have a substantial genetic component.

Genetic risk variants for PTSD

- More than 25 genes have been identified for their involvement in PTSD.
- The majority of genetic studies have been candidate gene, focusing on genes involved in neurotransmitter systems and stress signalling.

Genome-wide association studies (GWAS) of PTSD

- Six GWAS of PTSD have so far been undertaken and a number of novel loci have been identified.
- None of the top hits have been found in more than one GWAS, although the retinoid-related orphan receptor alpha (RORA) is a biologically plausible gene, which has also been associated with closely-related phenotypes in other candidate studies

Gene-environment (trauma) interactions

- PTSD is unique in that its aetiology is directly linked to having experienced a trauma.
- The effects of trauma on other health outcomes can also be influenced by genetic susceptibility.

The epigenome is responsive to trauma

- Trauma is likely to have an impact on the epigenome and could help explain the long lasting effects on later health.
- Animal studies provide good experimental support for a direct link between epigenetic modifications and PTSD.

DNA methylation patterns in PTSD

- A number of predominantly small studies have compared epigenome-wide methylation patterns between PTSD cases and controls, and genes involved with immune system function have been implicated.
- Numerous candidate genes have been found to be differentially methylated in PTSD, but to date, have only been investigated in a few studies.
- Genetic variation in combination with specific DNA methylation patterns, can influence the risk of PTSD.

Longitudinal studies

- Recent longitudinal studies have begun to investigate how DNA methylation changes with the development of PTSD.

Trauma exposure is associated with epigenetic modifications

- Early-life trauma influences both central and peripheral DNA methylation, with the most consistent evidence implicating the glucocorticoid receptor NR3C1.
- Trauma occurring outside of the early-life period, has also been associated with differential methylation.

Limitations of genetic and epigenetic association studies of PTSD

- The vast majority of findings have come from studies of at-risk African-American populations, with high PTSD rates, or white Caucasian males exposed to war trauma.
- The unique complexities of PTSD must be considered in the design of future studies and when interpreting the results to date.
- DNA methylation patterns in peripheral tissue have potential utility as biomarkers of PTSD risk, however they are unlikely to inform knowledge of disease aetiology.

FINANCIAL DISCLOSURE/ACKNOWLEDGEMENTS

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Table 1. Candidate genes investigated in genetic or epigenetic studies of PTSD.

Pathway and gene	Gene association studies			DNA methylation studies	
	Symbol	**Region**	**Risk of PTSD**	**Tissue and sites**	**Risk of PTSD**[1]
Dopaminergic signalling					
- Dopamine receptor D2	*DRD2*	various SNPs	some associations [22, 24]	NI	
- Ankyrin repeat and kinase domain containing 1	*ANKK1*	*rs1800497*	↑ with T allele; others no association [19-21]	NI	
- Dopamine receptor D4	*DRD4*	exon 3 VNTR	↑ with L allele[1] [23]	NI	
- Dopamine transporter	*DAT1; SLC6A3*	VNTR in 3'UTR	↑ with 9-repeat [25, 26]	Blood, 2 loci in promoter (using HM27K array data)	No independent association. Gene-methylation interaction. [25]
Serotonin signalling					
- Serotonin Transporter	*5-HTT; SLC6A4*	*5-HTTLPR,* VNTR	↑ risk with S allele (predominantly) [30-32, 36-39]	Blood, 2 loci in promoter (using HM27K array data)	No independent association. Trauma-methylation interaction. [104, 105]
- Serotonin receptor 2A	*HTR2A*	*rs6311*	↑ with G allele[1] [31]	NI	
HPA-axis signalling					
- Corticotrophin-releasing hormone receptor 1	*CRHR1*	various SNPs	some associations[1] [40, 41]	NI	
- Glucocorticoid receptor	*NR3C1*	various SNPs	no associations[1] [45]	Various, regions in exon 1 promoter	↓ methylation [93-96]

- FK506 binding protein 5	*FKBP5*	various SNPs	↑ risk with *rs9470080* T allele; others mixed findings [42, 43]		↓ methylation and longitudinal change [106]
Other genes					
- Pituitary adenylate cyclase-activating polypeptide type I receptor	*ADCYAP1R1*	*rs2267735*	↑ risk with C allele in females [48-50]	Blood, 1 loci in promoter (using HM27K array data)	↑ methylation [48]
- Apolipoprotein E	*APOE*	*rs7412, rs429358*	mixed findings[1] [60]		NI
- Brain-derived neurotrophic factor	*BDNF*	*rs6265*	↑ with A allele; others no association [53-55]	Saliva, exon IV promoter	No association [103]
- Cannabinoid receptor	*CNR1*	Various SNPs	No association[1] [59]		NI
- Catechol-o-methyltransferase	*COMT*	*rs4680*	↑ with A allele; others no association [56]	Blood, 41 loci across gene (using HM450K array data)	↑ methylation with fear inhibition in PTSD [56]
- C-reactive protein	*CRP*	*rs1130864*	↑ with T allele[1] [61]		NI
- Mannosidase alpha class 2C member 1	*MAN2*	NI			No independent association. Trauma-methylation interaction [100]
- Opioid receptor-like 1 gene	OPRL1	*rs6010719*	↑ with G allele[1] [62]		NI
- Protein kinase C alpha	*PRKCA*	*rs4790904*	mixed findings [57-58]		NI
- Spindle and kinetochore-associated complex subunit 2	*SKA2*	*rs7208505*	No independent association[1] [98]	Blood, 1 loci	↑ methylation [98]

[1]based on only a couple of studies. HPA-axis: Hypothalamic Pituitary Adrenal Axis; NI: not investigated; SNP: Single nucleotide polymorphism; UTR: untranslated region; VNTR: Variable Number Tandem Repeat; HM27K array: Methylation data obtained from the Infinium HumanMethylation27 BeadChip Array; HM450K array: Methylation data obtained from the Infinium HumanMethylation450K BeadChip array.