Preclinical and clinical evidence of DNA methylation changes in response to trauma and chronic stress

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Review, *Chronic Stress*

Preclinical and clinical evidence of DNA methylation changes in response to trauma and chronic stress

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Abstract

Exposure to chronic stress, either repeated severe acute or moderate sustained stress, is one of the strongest risk factors for the development of psychopathologies such as post-traumatic stress disorder (PTSD) and depression. Chronic stress is linked with several lasting biological consequences, particularly to the stress endocrine system but also affecting intermediate phenotypes such as brain structure and function, immune function, and behaviour. Although genetic predisposition confers a proportion of the risk, the most relevant molecular mechanisms determining those susceptible and resilient to the effects of stress and trauma may be epigenetic. Epigenetics refers to the mechanisms that regulate genomic information by dynamically changing the patterns of transcription and translation of genes. Mounting evidence from preclinical rodent and clinical population studies strongly support that epigenetic modifications can occur in response to traumatic and chronic stress. Here, we discuss this literature examining stress-induced epigenetic changes in preclinical models and clinical cohorts of stress and trauma occurring early in life or in adulthood. We highlight that a complex relationship between the timing of environmental stressors and genetic predispositions likely mediate the response to chronic stress over time, and that a better understanding of epigenetic changes is needed by further investigations in longitudinal and postmortem brain clinical cohorts.

1. Introduction: defining chronic stress

Exposure to chronic stressors across the human lifespan can multiply an individual's susceptibility to adverse medical conditions.[1,2] In this article, chronic stress refers to (1) a series of intense traumatic events such as accidents, physical assault, sexual assault, natural disasters or combat exposure, leading to psychopathologies such as complex PTSD,[3] or (2) non-traumatic but major events in life, whereby an individual is exposed to sustained periods of stress, for example, caregiving, difficult divorce, or a stressful work environment leading to burnout.[4] Chronic stress is a robust risk factor for many medical conditions including cardiovascular disease, obesity, cancer and immune disorders.[5] It is also highly linked with the development of a broad range of psychiatric illnesses, including post-traumatic stress disorder (PTSD),[6] major depressive disorder,[6] bipolar disorder,[7] schizophrenia [7], anxiety disorders,[8] substance abuse disorders[9] and addiction[10].

Chronic stress and trauma are associated with several lasting biological changes, particularly of the stress endocrine systems. For example, enduring stress and trauma can lead to permanent changes in the structure of several brain regions important for processing traumatic information (e.g. decreased somatosensory field of genital region after childhood sexual abuse[11]), which might be an adaptive response to protect an individual from future adversity.[12,13] Interestingly, such adaptive mechanisms appear to occur in all individuals exposed to similar levels of stress or trauma, regardless of diagnostic outcome. Yet, only a fraction (~20%) of people who have experienced trauma go on to develop PTSD, while some even experience posttraumatic growth.[14] Additionally, some individuals may not develop PTSD but other psychotic, anxiety or affective disorders – or a combination of these – highlighting the heterogeneity of responses to stress.[15,16] Considering that the outcomes following chronic stress exposure differ greatly between individuals, susceptibility to adversity resulting in psychopathology might be determined by how efficiently an individual is able to compensate for adversity at the molecular level.

In addition to genetic factors, some of the most relevant molecular mechanisms that may contribute to risk or resilience to the effects of stress and trauma are epigenetic. For example, DNA methylation profiles in monozygotic twin pairs diverge as twins grow older, with the differences between these genetically identical individuals at least partially explained by differing environmental exposures such as psychological stress.[17] Further, in genome-wide association studies (GWAS) of stress-related psychiatric disorders, single genetic variants explain only a small proportion of disease risk and overall do not completely explain heritability.

Epigenetics refers to the mechanisms that regulate genomic information by dynamically changing the patterns of transcription and translation of genes without altering the underlying DNA sequence. This occurs by altering the accessibility of DNA to transcriptional regulators by: (i) post-translational modifications of histone proteins, (ii) the addition of chemical groups most commonly at cytosine-phosphate-guanine (CpG) sites (e.g. methylation, hydroxymethylation or other modification), or (iii) the binding of non-coding RNAs to specific sequences in DNA. A growing body of evidence convincingly shows that exposure to adversity can cause long-lasting epigenetic changes that lead to widespread alterations of gene expression. Such alterations are observed in both brain and peripheral tissues, indicating the widespread effects of stress throughout the whole organism. Epigenetics thus provides a mechanism by which the environment can have a global and lasting influence on the expression of genes in an individual following stress exposure, with epigenetic processes believed to underlie associations of disease burden, environmental risk and individual phenotype.[18] Elucidating the interface of gene and environment interactions can help lead to a better understanding of the development of psychopathologies, and how they may be prevented or treated. In this review, we summarise preclinical and clinical research findings that support the existence of epigenetic modifications, particularly DNA methylation, in response to conditions of chronic stress, highlighting the effects of chronic and traumatic

stressors occurring at different developmental time points and how this may lead to vulnerability to severe psychiatric disorders.

2. Epigenetics: overview and relevance

For environmental exposures to have lasting consequences on biological systems, alterations in gene transcription are required. This can occur by epigenetic modifications that influence chromatin state, and thus the accessibility of transcriptional machinery to regulatory regions such as gene promoters. Epigenetic modifications can be long lasting, and can elicit either targeted or genome-wide changes in gene expression by processes including DNA methylation, post-translational histone modifications, and non-coding RNA regulation. One of the best understood epigenetic mechanisms involves the methylation of cytosine (5-methylcytosine or 5mC) on DNA, which occurs most abundantly at cytosines followed by guanine residues.[19,20] In most instances the presence of methylated cytosine recruits repressor complexes that modulate gene transcription by altering chromatin and inhibiting accessibility of the promoter to transcription factors and enhancers.[21] Opposite effects have been reported in a site-specific manner, with inhibition of repressor binding or enhancement of methylation-specific enhancers.[22,23] DNA methylation is considered to be a stable epigenetic mark in post-mitotic cells, although increasing evidence indicates that 5mC and 5-hydroxymethylcytosine (5hmC; the stable intermediary between methylation and de-methylation) are dynamic, reversible and modifiable under certain conditions.[20,21]

Patterns of gene expression are also regulated by histone modifications,[24] which involve the covalent modification of histone amino-terminal tails by, for instance, methylation, acetylation, phosphorylation, or ubiquination among others. Histone modifications can either alter the level of chromatin condensation by modifying electrostatic charge, or by recruiting epigenetic “readers” or “erasers” that bind to histones to respectively increase or decrease histone modifications. Epigenetic modifications to transcription and translation also extend to the effects of non-coding RNAs. These are short RNA species that act post-transcriptionally to repress protein synthesis, often by inducing degradation of mRNA targets.[25] Patterns of

epigenetic modifications are able to elicit functional effects that result in either gene expression repression or enhancement.

Several genetic variants are consistently shown to interact with stress and trauma, with some of these effects long-lasting. For example, in rodents exposed to stress early in life – such as stress caused by changes in maternal behaviour – the epigenetic landscape of prominent "stress genes" such as *NR3C1* and *CRH* can be altered, with these effects lasting into adulthood.[26,27] Similar epigenetic patterns have been observed in humans exposed to childhood maltreatment who develop a mood disorder or PTSD later in life,[28] and epigenetic alterations are found in adults exposed to periods of chronic stress including combat exposure,[29] employment in a high-stress environment[30] or a high lifetime stress load.[31] Thus, chronic stress and trauma are environmental exposures that can cause lasting epigenetic changes and lead to the development of psychiatric pathology.

A number of studies, ranging from twin to molecular genetic studies in large longitudinal cohorts, indicate that the impact of adverse life events is likely moderated by genetic variants, in the context gene x environment (GxE) interactions.[32,33] The studies describe, however, only statistical interactions by nature. Epigenetic marks could provide a mechanism that may link how effects of genetic variants and environmental stimuli converge to regulate gene transcription and in consequences changes in brain structure, endocrine responses or behaviour that have been reported following stress exposure. Control of gene expression by epigenetic modifications modulates important cellular processes including neurogenesis, neurotransmission and synaptic plasticity, which are all disrupted in conditions of sustained stress.[34,35] Such effects may influence an organism at not only a molecular level but also at circuitry and systemic levels.

Some evidence suggests that epigenetic modifications may be sustained during cell division, and possibly transmitted across generations.[32,33] This is important in the context of

chronic stress exposure, as progressive but sustained epigenetic changes may be cumulative and could even be a result of prior chronic stress exposure in the parent. Several studies based on periods of deprivation, such as the Swedish Famine,[36] Dutch Hunger Winter,[37] Montreal Ice Storm[38,39] and the Holocaust[40] provide some support for intergenerational transmission of traits through epigenetics, through investigating the offspring of parents who were subject to this trauma. However the strongest evidence for epigenetic transgenerational inheritance comes from animal studies of paternal transmission, which show that the offspring of chronically stressed adult male mice are more vulnerable to stressful stimuli, have dysregulated stress responses and altered HPA axis activity compared to control mice.[41,42] It has been hypothesized that these apparently heritable effects may be underpinned by an epigenetic mechanism transmitted via sperm.[43] However, these studies remain highly controversial in light of currently accepted models that epigenetic modifications are erased during meiosis.[44,45] Further, it remains unclear whether transgenerational stress-related behavioural alterations are truly epigenetic imprinting or a post-conception consequence of change in the uterine milieu or parental behaviour.

Several caveats of epigenetic studies should be mentioned here. Firstly, human and animal studies investigating stress-induced epigenetic modifications for the most part report associative or correlational effects, rather than causal effects. They also often do not elucidate the mechanism by which the epigenetic change is induced nor its functional consequence. Secondly, while DNA methylation levels for some CpGs are correlated across brain and blood or saliva and blood,[46–48] most are not. However, specific genetic variants or environments that cause the release of hormones with system-wide effects may have common effects on DNA methylation across tissues that may only differ in magnitude.[49,50] There is also some evidence that overall, DNA methylation derived from buccal cells may be more closely related to brain, as both tissues are derived from the ectoderm. However, compared to the low relatedness between brain and whole blood, the improvements are small.[48]

Additionally, in complex tissues such as the brain, epigenetic mechanisms also differ according to brain regions, sub-brain regions and cell types.[51] Thus results from epigenetic studies in peripheral blood or saliva may have to be interpreted with great care with regards to their reflection of causal effects on disease risk.

Methylation-wide approaches have the advantage of giving a broad picture of the methylome, but there are some important considerations for each method. While array-based methods are cost-efficient and technically robust, data pre-processing – including normalization and batch-correction – can have strong influences on the data, especially if small changes are expected. Several publications thus advocate that studies using array technologies should focus on results that survive multiple correction methods.[52–54] Further, currently used methylation arrays focus on promoter regions, with lower coverage in enhancers that appear critical for stress-related impact. Sequencing approaches may therefore seem optimal, however, these methods are also subject to limitations. Firstly, DNA methylation does not vary at the majority of CpGs in a given tissue and so untargeted sequencing-based methods may result in lower sensitivity with less reads per CpG generated, reducing cost-efficiency. Importantly, a bias can also be observed with bisulfite conversion.[55] Direct sequencing of unmodified DNA using single molecule real time sequencing methods might be a superior approach and is being developed by a number of companies. In addition there is a push for the development of arrays that focus on additional CpGs and other bases not covered by current array technologies.[56]

An important requirement of the field is now to thoroughly catalogue which epigenetic modifications occur in response to which types of stress (e.g. nature vs. timing vs. length of stress) in which different cell-types and tissues, and how this characterises risk profiles and psychopathologies. The development of targeted, highly sensitive DNA methylation detection methods will propel this effort.

3. Epigenetic mechanisms early in life and lasting effects of early life adversity

Stress occurring early in development can cause dramatic effects on physiology and behaviour.[57] In this context, early epigenetic changes in the brain are important factors. Changing methylation patterns have been observed in promoter regions of many genes across healthy brain development, especially in the human dorsolateral prefrontal cortex, a brain region which is late to mature and highly implicated in neuropsychiatric disorders.[58] Specifically, a switch from de-methylation to increased methylation of many genes at the genome level characterise the transition from foetal to childhood time points.[59] It is likely that interruption of normal developmental epigenetic patterns by stress or trauma early in life may alter epigenetic trajectories and lead to the development of psychopathology later in life.

A growing number of associations support the link between stress or trauma exposure and the occurrence of widespread epigenetic changes. For example, common genetic variants in *DNMT* genes appear to moderate the effects of daily life stressors and perinatal adversity on paranoid ideation.[60] DNMTs are enzymes is required for the methylation of neurogenic genes, also suggesting that exposure to chronic stressors early in life might disturb brain function in adulthood.[61] Large methylation changes at the beginning of the lifespan, particularly on genes implicated in developing severe psychiatric conditions (e.g. *DLG4, DRD2, NOS1, NRXN1,* and *SOX10*) may also indicate vulnerability to the effects of stress and psychiatric disorders by epigenetic mechanisms.[59]

3.2.1 Prenatal adversity

Even from the first possible window of stress exposure – *in utero* – organisms may be susceptible to epigenetic programming which persists into adult life (Table 1A).[62] In particular, epigenetic differences in key genes that regulate the HPA axis are reported in people exposed to prenatal adversity.[62] These effects are hypothesized to last into adulthood

by epigenetic re-programming of the hypothalamic-pituitary-adrenal (HPA) axis.[57] For example, women and their children exposed to trauma or chronic stress from the war in the Democratic Republic of Congo reportedly had differential patterns of methylation on genes associated with stress; these included hyper-methylation of *CRH* and *NR3C1* in cord blood and *CRH, FKBP5, CRHBP* in the placenta.[63,64] Yehuda and colleagues additionally showed that *FKBP5*, a gene involved in glucocorticoid receptor and stress sensitivity, was hyper-methylated in Holocaust survivors, while hypo-methylation of the same CpGs occurred in the offspring.[40] An additional cohort examining pregnant mothers exposed to the Quebec Icestorm of 1998, prenatal stress caused a widespread change to the epigenetic landscape of offspring, particularly in genes related to immune function.[38,39]

Considering the unequivocal role of the glucocorticoid receptor in stress responsivity, several studies have focused on epigenetic modifications of *NR3C1* in response to various types of prenatal stress. For example, in those exposed to intimate partner violence during the prenatal period, DNA methylation was increased in the *NR3C1* 1F promoter region.[65] Additionally, in offspring of mothers pregnant during the Holocaust genocide, hyper-methylation of the *NR3C1* exon 1F promoter methylation was observed much later in life, specifically in offspring who had one parent suffering from PTSD.[66] Methylation was however decreased for offspring with two parents who had PTSD, presenting what could be a complex mechanism of potential maternal and paternal effects of PTSD on epigenetic regulation of *NR3C1*, although it is possible that epigenetic changes occurring in offspring were an effect of living with a parent with PTSD rather than genocide exposure. Together with evidence from animal studies,[67] hyper-methylation of the *NR3C1* promoter appears to be a robust epigenetic modification occurring in response to stress early in life. Altogether, there appear to be strong and widespread effects of prenatal stress on the epigenetic regulation of the stress signalling system that lasts into adulthood, although the intergenerational

mechanisms for some genes (e.g. *FKBP5* and *CRH*) need to be further explored perhaps using animal models.

3.2.2 Childhood adversity assessed during childhood and adolescence

Considering that certain epigenetic markers occurring during development may set an individual on a path for developing psychopathology later in life, some studies have aimed to examine methylation patterns in the brains of those exposed to chronic stress during childhood (Table 1B). Some of the effects seen with prenatal stress are also seen with stress during childhood. In suicide completers exposed to childhood abuse, hyper-methylated sites were observed in the *NR3C1* variant 1 promoter region at a non-canonical binding site for nerve growth factor–induced protein A. This was further associated with down-regulated expression of the *NR3C1* 1F splice variant in the hippocampus.[68] While these localized findings for epigenetic regulation of the GR are quite interesting, the effects of childhood adversity is likely epigenome-wide, as methylation profiles in the hippocampus of suicide completers with a history of childhood abuse were reportedly bidirectional and dynamic, with both methylation and de-methylation of numerous genes observed.[69] Hyper-methylation at promoters of genes that encode ribosomal RNAs were also reported in abused suicide victims compared to controls, possibly indicating widespread effects as ribosomal RNA methylation and transcription directly affect cellular function and synaptic plasticity associated with fear memory and cognitive dysfunction.[70]

Additional studies have examined epigenome-wide methylation differences in children exposed to childhood maltreatment or trauma (e.g. physical abuse, sexual abuse, neglect, deprivation, emotional abuse, witnessed domestic violence) compared to non-traumatized individuals.[71–73] Weder and colleagues reported that methylation patterns at the genes *ID3*, *GRIN1*, and *TPPP* were genome-wide significant predictors of depression later in life. Maltreated children relative to control children had significantly altered methylation profiles

at the *BDNF*, *NR3C1* and *FKBP5* genes, which are all genes that respond to stress exposure and implicated in psychopathology.[72] In a sample of English and Romanian adoptees, individuals chronically exposed to severe deprivation before 43 months of age showed hyper-methylation across 9 CpGs spanning the promoter of the *CYP2E1* gene at the age of 15 years. Hyper-methylation of *CYP2E1* was also associated with impaired social cognition, a clinical marker of deprivation.[73] In addition, Houtepen *et al.*[71] reported that methylation of the KIT ligand gene *KITLG*, which is involved in haematopoiesis and the cortisol response, strongly mediated the relationship between childhood trauma and cortisol stress responsivity.[71] This would suggest a link between stress occurring early in life and epigenetic modifications lasting into adulthood that may increase risk to psychopathology in adulthood by altering the responsivity of the stress hormone system.

Another group of studies suggest epigenetic changes in peripheral tissue at the *SLC6A4* locus and the consequences of stress in older children.[74] For example, in a study by Beach and colleagues,[75] children 11-13 years old with cumulative exposure to low socio-economic status (SES) showed hyper-methylation at the *SLC6A4* locus. A similar effect was observed in a second study, with increased methylation of *SLC6A4* in bullied twins at age 10 compared to their non-bullied monozygotic twins.[76]

3.2.2 Effects of childhood adversity lasting into adulthood

The studies assessing epigenetic modifications that occur in response to prenatal and childhood adversity strongly support evidence that stress during development leads to epigenetic modifications that likely influence gene expression later in life,[77] especially in genes involved in stress responsivity. During these critical developmental time points, changes in expression of "stress genes" might alter an individual's ability to cope with stress and set the stage for vulnerability to psychopathology later in life. Some groups have assessed clinical populations of individuals who were exposed to childhood adversity and who

developed PTSD in adulthood (Table 1C). These investigations collectively support that childhood adversity is associated with specific epigenetic modifications in those that develop a psychiatric disorder.

Other groups assessing childhood abuse in adults with severe psychiatric disorders have focused on stress candidate genes. In one report, the severity and number of exposures to childhood sexual abuse was positively correlated with *NR3C1* hyper-methylation in subjects with severe psychiatric disorders including PTSD and comorbid major depressive disorder.[28] In another cohort of subjects with PTSD exposed to childhood adversity, DNA demethylation was found decreased in a glucocorticoid response element in *FKBP5*, specifically in the presence of an *FKBP5* functional polymorphism that increased risk to psychiatric disorder later in life.[78] These data mirror the findings from prenatal adversity studies, supporting the inference that such epigenetic modifications to the stress system occur in response to early life adversity and may last into adulthood to increase development of a severe psychiatric condition.

4. Stress and trauma during adulthood

In addition to early life adversity, experiencing extreme levels of stress or trauma during adulthood can also increase the risk to development of severe psychiatric conditions. However, the mechanism is likely distinct between these two scenarios. Firstly, early life experiences occur during a particularly sensitive time, where environmental influences can have strong effects on the brain and body, causing molecular, structural and behavioural disturbances that stay with the individual into adulthood. While severe stress or trauma occurring exclusively in adulthood can also result in detrimental biological consequences, body systems are already matured thus possibly causing a different cascade of events. In fact, we could show that patients suffering from PTSD with either only adult trauma or a combination of child abuse and adult trauma had distinct gene expression and DNA methylation signatures in peripheral blood.[79]

4.1 Epigenetic changes in preclinical stress models

Adult animals exposed to severe acute or non-traumatic chronic stress have shown various epigenetic changes attributable to stress exposure. Considering a hallmark of PTSD is the formation of fear memories, many studies have used fear learning paradigms for exploring the consequences of trauma exposure.[80] These studies have shown that fear conditioning induces the expression of DNA methyl-transferases type 3A and 3B (*Dnmt3A/3B*, leading to altered DNA methylation at genes that are important for memory formation and plasticity (e.g. *Reelin, Pp1, Bdnf*).[20,81–83] Others have highlighted a role for histone acetylation in long-term fear recall and spatial memory. For example, environmental enrichment was shown to improve memory deficits in the brain; this was associated with up-regulation of histone acetylation and activity of histone deacetylases, which modulate synaptic plasticity via altering the expression of genes including glutamate receptor subunits and *Bdnf*.[84–86] These

findings have linked stress to brain plasticity and behaviour, by long-lasting epigenetic modifications.

Clear epigenetic changes have also been observed in animal models of chronic stress.[87] One of the primary findings from genome-wide epigenetic studies in rodent models is that chronic stress induces widespread alterations in histone modifications, particularly in the hippocampus.[88] For example, chronic variable stress caused alterations to the expression of transcripts that encode histones or recruit histone writers and erasers.[89,90] With regards to methylation, associations between chronic defeat stress and *Dnmt3A* expression was shown in the nucleus accumbens, which is a brain region that plays a significant role in processing of aversion, motivation and rewards.[91] Interventions (mainly pharmacological such as antidepressants), which induce H3K9 di- or tri- methylation, have been shown to promote resilience to stress and depressive-like behaviour.[92,93] Chronic stress in adult animals also exerts epigenetic modifications to key genes involved in HPA-axis signalling,[94] such as *Crh*,[95,96] *Hsp90*[97] and *Bdnf*.[98,99]

Epigenetic changes in response to severe and chronic stress can clearly be associated with stress exposure in animal models. While this is more complex in human studies, clinical studies complement these findings, supporting the existence of lasting epigenetic effects in response to trauma or prolonged stress within both the central and peripheral nervous systems. In the following sections, we will discuss examples of epigenetic changes observed in humans exposed to acute traumatic events or non-traumatic chronic stress in adulthood.

4.2 Epigenetic changes due to acute traumatic events in adults

Studies examining the epigenetic effects of traumatic events in adults have either used genome-wide methylation approaches or focused on known "stress genes". Most of these studies are cross-sectional studies of individuals examined post-trauma, derived from

epidemiological cohorts. Additionally, most examine epigenetic profiles in peripheral tissues such as blood and saliva, which may be useful for biomarker identification (Table 2).

Genome-wide methylation studies support the existence of epigenetic differences between trauma-exposed individuals with PTSD compared to psychiatrically healthy controls, with cross-sectional differences in DNA methylation have been observed in cohorts from Atlanta[100] and Detroit.[101] These studies have shown epigenetic differences were able to differentiate those who have developed PTSD compared to those who did not. Later studies from longitudinal cohorts of US military personnel exposed to combat-trauma further suggested significant differences in global methylation in PTSD patients relative to controls,[102,103] particularly in genes involved in immune[101,103] and nervous system function.[104] Such longitudinal studies allow the dissection of separate contributions of trauma exposure vs. disease development which is not readily possible in cross-sectional studies.

A larger number of studies have investigated DNA methylation of specific candidate genes, including genes involved in the regulation of the stress hormone axis. Here studies have focused on *NR3C1* (exon 1F) and *FKBP5*. In one study from saliva, *NR3C1* methylation at CpG site 42 was increased in men with PTSD,[105] however in another study from blood, methylation at CpG site 28 was reduced in PTSD.[29] The differences may represent tissue- or CpG site- specific differences, variability in the trauma type or its timing, or sex and age.[106] In an earlier study, veterans exposed to severe war trauma underwent prolonged exposure therapy and were assessed pre- and post- exposure treatment.[107] The study showed that methylation of *NR3C1* exon 1F locus pre-treatment predicted treatment outcome, but methylation was not significantly different in responders or non-responders to the exposure therapy at post-treatment or follow-up.[107] Methylation of the *FKBP5* exon 1 promoter also did not predict treatment response, but was decreased in association with recovery.[107] This study provides preliminary evidence that these methylation markers may underlie the severity of

PTSD symptoms, and that reversal of the methylation changes may tract with effective therapeutic intervention.

The immune system is one of the main effectors of HPA axis activity, and in turn, changes in methylation are associated with total life stress, PTSD diagnosis and a history of childhood abuse.[100] Epigenetic effects to genes involved in immune function are a consistent finding in PTSD, supporting that sustained sympathetic nervous system activity in response to trauma stimulates immune system activation. For example, DNA methylation of genes associated with immune function were found to be largely negatively correlated with traumatic burden in PTSD.[101] Others consistently reported altered methylation of pro-inflammatory cytokines, such as interleukin 12 and 18, in individuals with PTSD compared to those without.[100,103,108] Analysis of histone and DNA methylation markers suggest that widespread epigenetic changes in PTSD, including those in immune genes, might occur by alterations to DNMT1[108] or miRNAs,[109] which are molecules that induce and maintain global methylation patterns.

In addition to identification of epigenetic markers associated with diagnostic outcomes, some studies have begun to additionally assess associations of DNA methylation to endophenotypes of psychiatric disease, including neuroimaging phenotypes. For example, a genome-wide significant variant, rs717947 (positioned in an inter-genic locus at chromosome 4p15) was identified as a methylation quantitative trait locus associated with altered cortical activation in response to fearful faces.[110] Another study showed associations between the methylation status a polymorphic site in the 3'UTR of the *SKA2* gene - involved in mitosis- with reduced thickness of several cortical areas and symptom severity in PTSD.[111] Lastly, methylation in peripheral blood samples of *ADCYAP1R1,* a gene involved in regulating the cellular stress response, was associated with PTSD diagnosis and symptom severity,

specifically in females.[112] Collectively, these studies support that epigenetic changes in response to stress may "mediate" particular stress phenotypes.

Several studies have now investigated DNA methylation in the context of GxE including candidate genes involved in neurotransmitter turn-over such as *SLC6A4* (encoding the serotonin transporter SERT), *SLC6A3* (encoding the dopamine transporter), and *COMT* (encoding enzymes that degrade neurotransmitters such as dopamine). In one study, reduced *SLC6A4* methylation levels were associated with more traumatic events and increased risk for PTSD, only in those carrying a specific *SLC6A4* risk allele genotype, while higher methylation appeared protective against the development of PTSD.[113] In an independent cohort, individuals with PTSD carrying the *SLC6A3* 9-repeat allele were at higher risk to PTSD when also having higher methylation in the *SLC6A3* promoter locus.[114] Lastly, increased *COMT* promoter methylation was associated with impaired fear inhibition in individuals with PTSD carrying the *COMT* met/met genotype; this genotype has been extensively associated with a range of psychiatric conditions and phenotypes.[115] These studies suggest that epigenetic changes following trauma often only occur in the context of specific genetic variation and that genetic risk may only manifest with concomitant epigenetic changes.

4.3 Epigenetic changes due to sustained non-traumatic stress in adults

It is well established that chronic stress results in adverse health outcomes, but only a handful of studies exist that explore the association of non-traumatic chronic stress (e.g. work and lifestyle-related stresses) with epigenetic changes (Table 3). To our knowledge there are no genome-wide analyses examining differences in epigenetic marks in populations exposed to non-traumatic chronic stress. One study used *LINE-1* as a proxy for global methylation, but no differences in *LINE-1* methylation were found in subjects exposed to recent life stressors compared to controls.[116] A few other studies have examined epigenetic changes to candidate genes within populations of individuals exposed to occupational chronic stress.

For example, in workers employed in the manufacturing industry in environments of high or low stress, Myaki and colleagues examined DNA methylation of the tyrosine hydroxylase (*TH*) gene.[117] *TH* encodes an enzyme involved in synthesis of catecholamines including serotonin, dopamine, epinephrine and norepinephrine, which are all altered in rodent models of chronic stress.[118,119] Methylation of CpG sites in the *TH* gene and the flanking 5' region was significantly higher in individuals exposed to high occupational stress compared to individuals working in low stress environments.[117] The same group also explored altered methylation in the *BDNF* gene based on *a priori* hypothesis that this gene is also epigenetically affected in a preclinical model of PTSD.[98] Slightly increased methylation was observed across the whole gene in subjects exposed to high-stress compared to low-stress environments, although the groups showed no difference in promoter-specific methylation.[120] Work-related stress in this study was assessed using a self-administered questionnaire, with methylation levels of groups from the lowest and highest job-strain quartiles compared. [120]

Another group focused on methylation of the *SLC6A4* gene due to some evidence that methylation differences in this gene occur in stress-induced psychopathologies such as PTSD (discussed in section 4.2) and depression.[121] In shift-working nurses in high-stress work

environments compared to those in low-stress work environments, five CpG sites in the promoter region of *SLC6A4* were examined.[30] Higher methylation across the tested *SLC6A4* CpGs was found in those working in a high compared to low stress environment, and this was associated with burnout and the perception of stress.[30] Further investigation into the effects of non-traumatic chronic stressors, such as work related stress or caregiving, is limited. This is likely as the length and severity of non-traumatic stress is often not as well defined as traumatic stress, and many confounding factors make the results of these studies less clear. These studies might also be confounded by the number of stressors, which might have an additive effect, and further studies are needed to disentangle this.

7. Future directions and conclusions

Studies examining epigenetic changes in response to stress are providing a potential opportunity to identify vulnerable and resilient populations (e.g. based on combined prior exposure, other environmental factors and genetics) and biomarkers of risk for those exposed to chronic stress. However, additional studies of how different types and lengths of stress lead to epigenetic changes are required as well as of combined effects of positive and negative environments. The field of epigenetics is in fact relatively young, and studies examining epigenetic modifications in conditions of chronic stress are limited. One example is that, to the best of our knowledge, no studies have examined epigenetic modifications in postmortem brain samples derived from individuals with PTSD. Additionally, human studies are also limited in that there are few longitudinal studies with repetitive measurements from the same individual at different time points relative to the stress exposure, and currently, many studies published are cross-sectional and underpowered. Changes to gene expression by epigenetic modifications are prominent gene and environment interactions that are an important aspect of future chronic stress and trauma research. Particularly, epigenetic patterns in peripheral tissues may act as biomarkers that may serve as sentinels of when genetic predisposition followed by environmental exposure leads to trajectories of risk to develop psychiatric symptoms and syndromes. For mechanistic understanding, tissue specificity of these epigenetic changes must be addressed and understood. Overall, more research in this area will lead to a better understanding of stress-induced epigenetic effects and mechanisms as well as their potential moderation, allowing for their therapeutic exploitation and the development of new interventions and treatments.

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Table 1. Summary of studies examining epigenetic modifications in response to early life adversity during (A) prenatal time points (B) childhood time points or (C) in adults with a history of early life adversity.

References	Type of trauma	Sample Size/Relevant Diagnosis	Tissue Type	Method	Findings	Genome-wide methylation study or target genes
(A) PRENATAL STRESS						
[65]	Intimate partner domestic violence	24 mothers and children: 8 prenatal exposure to maternal stress, 16 no prenatal exposure to maternal stress	Peripheral blood	Sodium bisulfite sequencing	Increased DNA methylation of the NR3C1 promoter.	NR3C1 exon 1F promoter
[64]	Pregnant mothers exposed to chronic stress and/or traumatic war-related stress	25 mother-newborn dyads	Maternal peripheral blood and umbilical cord blood	Sodium bisulfite sequencing	Significant correlation between maternal prenatal stress, newborn birth weight and newborn methylation in the *NR3C1* promoter.	NR3C1 exon 1F promotor
[66]	Pregnant mothers during genocide	80	Peripheral blood	Bisulfite sequencing	Increased DNA methylation for one PTSD parent; decreased for two PTSD parents.	NR3C1 exon 1F promoter
[122]	Pregnant mothers during genocide	32 Holocaust survivors and 22 adult offspring; 8 comparable parents and 9 comparable offspring	Peripheral blood	Bisulfite sequencing	Hyper-methylation of the FKBP5 CpGs close to intron 7 glucocorticoid response elements in Holocaust survivors, hypo-methylation in Holocaust offspring.	FKBP5 intron 7
[123]	Pregnant mothers during a severe ice storm	34 children (assessed twice, age 8 and 13) whose mothers were exposed to the ice storm while pregnant.	T cells from peripheral blood mononuclear cells and saliva	Illumina Infinium HumanMethylation450K BeadChip Array; Validation by bisulfite pyro-sequencing	Objective hardship, but not subjective distress, was correlated with DNA methylation in 957 genes, predominately in pathways related to immune function.	Genome-wide methylation study
[124]	Pregnant mothers during a severe ice storm	36 children of mothers who subjectively rated the consequences of the ice storm as positive or negative.	T cells from peripheral blood mononuclear cells	Illumina Infinium HumanMethylation450K BeadChip Array	CpGs in 1564 different genes and 408 biological pathways (prominently associated with immune function) were differentially methylated in adolescents from positive and negative maternal groups. A large proportion of differentially methylated CpGs and affected pathways overlapped with those associated with objective measures of prenatal maternal stress, as published in Cao-Lei et al., 2014.	Genome-wide methylation study
[63]	Pregnant mothers exposed to chronic stress and/or traumatic war-related stress	24 mother-newborn dyads	Neonatal cord blood, placenta, maternal blood	Illumina Infinium HumanMethylation450K BeadChip Array	*Cord blood*: increased methylation in CRH was associated with chronic stress and war trauma. *Placenta*: reduced methylation of NR3C1 associated with war trauma and chronic stress. War-trauma was also associated wiht methylation of CRH, FKBP5, CRHBP. *Maternal blood*: NR3C1 methylation was associated with war trauma. NR3C1, CRH, CRHBP, NR3C1, FKBP5 were nominally associated with war trauma and/or stress.	Targeted analysis CRH, CRHBP, MR3C1, FKBP5

(B) CHILDHOOD STRESS						
[76]	Children exposed to bullying	28 10 year old bullied children and 28 of their non-bullied monozygotic twins	Buccal cells	Sequenom EpiTYPER platform	Increased SERT DNA methylation observed in bullied children compared to their non-bullied twin.	SLC6A4
[72]	Childhood abuse (physical, sexual, emotional), neglect, witnessed domestic violence)	94 maltreated and 96 healthy non-traumatized children	Saliva	Illumina Infinium HumanMethylation450K BeadChip Array	Methylation in ID3, GRIN1, and TPPP were genome-wide significant predictors of depression. Maltreated and control children had significantly different methylation profiles of BDNF, NR3C1 and FKBP5	Genome-wide methylation study
[75]	Cumulative exposure to low socioeconomic status	388 pre-adolescent children (11 to 13 years) of African American ethnicity.	Peripheral blood	Illumina Infinium HumanMethylation450K BeadChip Array	No effect on SLC6A4 methylation across 16 CpG residues, and no effect of the 5-HTTLPR genotype. In females only, one cpG site showed increased methylation in assoication with cumulative low socioeconomic status.	SLC6A4
[71]	Childhood trauma before age 13	45 individuals with/without diagnosis of MDD and/or a history of childhood adversity	Peripheral blood	Illumina Infinium HumanMethylation450K BeadChip Array	Methylation of Kit ligand gene (KITLG) strongly mediated the relationship between childhood trauma and cortisol stress reactivity.	Genome-wide methylation study
[31]	Various types of stress (perinatal stress, stressful life events and traumatic youth experiences)	468 adolescents (mean age 16 years)	Peripheral blood	Bisulfite conversion and quantitative mass spectrometry	Increased DNA methylation of 11 CpGs in the promoter of NR3C1 exon 1D after stressful life events and after exposure to traumatic youth experiences.	NR3C1
[73]	Children exposed to extended severe adversity before the age of 43 months	49 individuals assessed at ages 15 years compared to adoptees exposed to stress <6 months or no early life stress.	Buccal cell samples	Illumina Infinium HumanMethylation450K BeadChip Array	A differentially methylated region spanning 9 CpG sites in the promotor-regulatory region of the CYP2E1 gene was identified, and associated with deprivation related clinical markers of impaired social cognition.	CYP2E1
C) ADULTS WITH A HISTORY OF EARLY LIFE ADVERSITY						
[74]	Healthy young adults with recent life stress/trauma and/or a childhood and prenatal trauma	133	Peripheral blood	Bisulfite pyrosequencing	No effects of prenatal, early or recent life stress/trauma on the mean SLC6A4 methylation levels and no correlation between methylation and SERT mRNA expression. Hyper-methylation at 2 CpGs were significantly associated with mRNA expression and prenatal stress/trauma, but not recent life stress/trauma.	SLC6A4

125	Adults adopted in the first few months after birth into middle class families, with psychological dysfunction due to unresolved loss or trauma	143 adoptees	EBV transformed lymphoblast cell lines	Bisulfite conversion and quantitative mass spectrometry	Higher methylation of the SLC6A4 promoter was associated with increased risk of unresolved responses to loss or other trauma in participants carrying a SNP in SLC6A4, usually with protective associations. The risk allele predicted more unresolved loss or trauma in participants with lower levels of methylation.	SLC6A4
100	Childhood abuse leading to PTSD	110 (25 PTSD + childhood abuse, 25 PTSD no childhood abuse, 26 conrols with childhood abuse, 34 controls wihtout childhood abuse)	Peripheral blood	Illumina Human Methylation27 BeadChip	Increased global methylation PTSD vs controls. Five gene promoters were differentially methylated (TPR, CLEC9A, APC5, ANXA2, and TLR8) in PTSD compared to controls, but no CpGs were associated with childhood abuse.	Genome-wide methylation study
28	Severe psychiatric disorders with history of childhood adversity	101 Borderline personality disorder, 99 major depression, 15 major depression with PTSD	Peripheral blood	Bisulfite pyrosequencing	Severity and number of type of childhood sexual abuse correlated with NR3C1 methylation	NR3C1
69	Male suicide completers with a history of childhood adversity	25 with history of childhood abuse, 16 control subjects	Hippocampal tissue	Custom 400K eArray (Agilent Technologies)	Methylation patterns of gene promoters between those with childhood adversity and those without were distinct.	Genome-wide methylation study
78	PTSD, physical and sexual abuse during childhood (CTQ)	76 (30≥2 childhood trauma, 46 no childhood trauma)	Peripheral blood	Bisulfite pyrosequencing	DNA demethylation in a functional glucocorticoid response element in FKBP5 in the presence of FKBP5 functional polymoprhism increased risk to psychiatric disorder later in life.	FKBP5
79	Individuals with history of childhood abuse, with and without PTSD	169 (32 PTSD affected, 77 PTSD unaffected)	Peripheral blood	Illumina Human Methylation 450K array	Distinct (95%) gene expression profiles seen in PTSD groups with a history of childhood abuse compared to those with no history of childhood abuse, which appeared to be mediated by changes of DNA methylation.	Genome-wide methylation study

Table 2. Summary of studies examining epigenetic changes in response to trauma in adulthood, with a focus on genome-wide methylation studies and candidate gene studies in post-traumatic stress disorder (PTSD).

References	Type of trauma	Sample Size	Tissue Type	Method	Findings	Target (genome-wide methylation study or genes)
[101]	PTSD due to traumatic event(s)	23 PTSD affected, 77 PTSD unaffected	Peripheral blood	Illumina Human Methylation27 BeadChip	Genes associated with immune function were un-methylated and negatively correlated with traumatic burden in PTSD	Genome-wide methylation study
[100]	PTSD due to traumatic event(s)	25 PTSD with history of childhood trauma 25 PTSD without history of childhood trauma, 26 controls with and 24 controls without childhood truama	Peripheral blood	Illumina Human Methylation27 BeadChip	Global increase in methylation levels in PTSD vs controls. CpG sites in TPR, CLEC9A, APC5, ANXA2, and TLR8 were differentially methylated in subjects with PTSD. A CpG site in NPFFR2 was associated with total life stress after adjustment for multiple testing.	Genome-wide methylation study
[112]	PTSD due to traumatic event(s)	107 PTSD	Peripheral blood	Illumina Human Methylation27 BeadChip	A SNP in a putative oestrogen response element within ADCYAP1R1, rs2267735, predicted PTSD and symptoms in females; the SNP also associated with fear discrimination and ADCYAP1R1 expression in human brain; methylation of ADCYAP1R1 was associated with PTSD in peripheral blood	ADCYAP1R1
[126]	PTSD due to traumatic event(s)	23 PTSD affected, 77 PTSD unaffected	Peripheral blood	Illumina Human Methylation27 BeadChip	Risk of lifetime PTSD was markedly higher in patients with higher MAN2C1 methylation with greater exposure to PTEs	33 candidate genes associated with PTSD (based on RNA expression)
[113]	PTSD: accumulative exposure to traumatic effects after initial trauma	23 PTSD-affected, 77 PTSD-unaffected	Peripheral blood	Pyrosequencing	Increased risk for PTSD in cases with reduced DNA methylation levels	SLC6A4
[102]	PTSD due to combat exposure	75 with post-deployment PTSD, 75 without post-deployment PTSD	Peripheral blood	Pyrosequencing	Hyper-methylation of Line1 in non-PTSD group pre- vs. post-deployment; Hypo-methylated Line1 in PTSD vs. non-PTSD post-deployment. Alu was hyper-methylated in cases vs. controls pre-deployment	LINE1 and Alu
[114]	PTSD due to traumatic event(s)	62 PTSD, 258 controls	Peripheral blood	Illumina Human Methylation27 BeadChip	Risk allele carriers (9R) showed increased risk of lifetime PTSD only in conjunction with high promoter methylation of SLC6A3	SLC6A3
[127]	PTSD due to traumatic event(s)	98 PTSD, 172 no PTSD	Peripheral blood	Illumina Human Methylation27 BeadChip	Increased COMT promoter methylation associated with impaired fear inhibition	COMT
[103]	PTSD due to combat exposure	74 post-deployment PTSD, 74 no PTSD	Peripheral blood	Pyrosequencing	Reduced methylation of H19 and IL18 in those resilient to PTSD after deployment. Post-deployment PTSD individuals increased IL8 methylation after deployment.	IGF2, H19, IL8, IL16, IL18

104	PTSD due to traumatic event(s)	23 PTSD affected, 77 PTSD unaffected	Peripheral blood	Illumina Human Methylation27 BeadChip	Methylation in genes predominately related to nervous system function and risk to develop PTSD was modified by low socioeconomic status.	Genome-wide methylation study
107	Veterans with PTSD received who prolonged exposure (PE) psychotherapy	PTSD PE responders (n = 8), defined by no longer meeting diagnostic criteria for PTSD, and non-responders (n = 8)	Peripheral blood lymphocytes	Targeted bisulfite sequencing	Methylation NR3C1 exon 1F assessed at pre-treatment predicted treatment outcome, but was not significantly altered in responders or non-responders at post-treatment or follow-up. Methylation of the FKBP5 gene (FKBP51) exon 1 promoter region did not predict treatment response, but decreased in association with recovery.	NR3C1 and FKBP5 promoters
105	PTSD due to combat exposure	152 PTSD	Saliva	Pyrosequencing	Increased DNA methylation in men	NR3C1 exon 1F promoter
128	Subjects with pre and post incidental-trauma with and without PTSD	30 PTSD and 30 controls	Peripheral blood	Pyrosequencing	Differential DNA methylation levels of DNMTs in cases vs controls	DNMT1, DNMT3A, DNMT3B, DNMT3L
29	PTSD due to combat exposure	122 PTSD	Peripheral blood	Pyrosequencing	Reduced DNA methylation of the NR3C1 promoter in PTSD	NR3C1 exon 1F promoter
129	PTSD due to combat exposure (high exposure)	63 with severe PTSD and 84 without PTSD	Peripheral blood	Illumina Human Methylation 450K Beadchip	A genome-wide significant SNP (rs717947) was identified and associated with PTSD symptoms. The SNP was then discovered to be as a methylation quantitative trait loci which was also associated with altered DLPFC activation in response to fearful faces.	Genome-wide methylation study
108	PTSD due to combat exposure	76 controls and 23 PTSD	Peripheral blood mononuclear cells (PBMCs)	Illumina HumanMethylation27 BeadChip	Global DNA methylation level did not differ significantly between control and PTSD. The promoters of several individual immune function genes were differentially methylated and/or were more highly expressed.	Genome-wide methylation study
109	PTSD due to combat exposure	76 controls and 23 PTSD	Peripheral blood mononuclear cells (PBMCs)	Illumina HumanMethylation27 BeadChip	326 genes and 190 miRNAs were had altered expression levels in PTSD. DNA methylation of differentially expressed genes showed a trend toward differences between control and PTSD.	Genome-wide methylation study
130	PTSD due to traumatic event(s)	12 PTSD, 12 without PTSD but with the similar level of trauma exposure	Peripheral blood	Affymetrix Gene Chip Human Exon 1.0 ST Array	No differences with adjusted significance for DNA methylation were found.	Genome-wide methylation study
111	PTSD due to combat exposure	200 trauma exposed veterans (+145 with structural brain image information)	Peripheral blood	Illumina Human Methylation 450K Beadchip	DNA methylation of rs7208505 was associated with reduced thickness in of several cortical areas; methylation was also positively associated with PTSD symptom severity.	SKA2 methylation at the rs7208505 locus

Table 3. Summary of studies examining epigenetic changes in response to non-traumatic chronic stress exposure in adulthood.

References	Type of trauma	Sample Size	Tissue Type	Method	Findings	Target genes
[30]	Shift-working nurses with burnout or depression	24 high stress, 25 low stress	Peripheral blood leukocytes	Direct sanger bisulfite sequencing and Methylation 450k Beadchip	Nurses with stress had lower promoter methylation at all CpG residues compared to those with low stress. Methylation levels were not associated with burnout/depression.	SLC6A4
[120]	Manufacturing workers	180 (quartiles of 90 with lowest and highest job strain with bottom and top quartiles compared)	Saliva leukocytes	Illumina Human Methylation27 BeadChip	Slightly increased methylation across the all tested CpGs in BDNF in high stress compared to low stress occupational environments. No group-specific differences in promoter methylation.	BDNF
[117]	Japanese manufacturers	180 (quartiles of 90 with lowest and highest job strain with bottom and top quartiles compared)	Saliva leukocytes	Illumina Human Methylation27 BeadChip	Methylation of TH CpG sites and the flanking 5' region were higher in individuals exposed to high occupational stress compared to individuals working in low stress environments.	TH
[116]	Healthy adult males chronic lifestyle stressors	105 (18 to 77 years old)	Peripheral blood mononuclear cells	Global methylation (LINE-1; PyroMark) and Sequenom EpiTyper MassArray of SLC6A4 locus	Chronic stress correlated with global DNA methylation, which appeared to be driven by a 5-HTTLPR genotype effect.	LINE-1 and SLC6A4