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Review

Understanding posttraumatic stress disorder: insights from the methylome

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Genome-wide association studies (GWAS) have identified numerous disease-associated variants; however, these variants have a minor effect on disease and explain only a small amount of the heritability of complex disorders. The search for the missing heritability has shifted attention to rare variants, copy number variants, copy neutral variants and epigenetic modifications. The central role of epigenetics, and specifically DNA methylation, in disease susceptibility and progression has become more apparent in recent years. Epigenetic mechanisms facilitate the response to environmental changes and challenges by regulating gene expression. This makes the study of DNA methylation in psychiatric disorders such as posttraumatic stress disorder (PTSD) highly salient, as the environment plays such a vital role in disease aetiology. The epigenome is dynamic and can be modulated by numerous factors, including learning and memory, which are important in the context of PTSD. Numerous studies have shown the effects of early life events, such as maternal separation and traumas during adulthood, on DNA methylation patterns and subsequent gene expression profiles. Aberrations in adaptive DNA methylation contribute to disease susceptibility when an organism is unable to effectively respond to environmental demands. Epigenetic mechanisms are also involved in higher order brain functions. Dysregulation of methylation is associated with neurodevelopmental and neurodegenerative cognitive disorders, affective disorders, addictive behaviours and altered stress responses. A thorough understanding of how the environment, methylome and transcriptome interact and influence each other in the context of fear and anxiety is integral to our understanding and treatment of stress-related disorders such as PTSD.

Keywords: Anxiety, CNS methylation, DNA hydroxymethylation, DNA methylation, epigenetics, posttraumatic stress disorder, PTSD animal models, trauma-associated methylation changes

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Genetic research in PTSD

Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating trauma-related disorder that significantly impairs normal functioning and quality of life (DSM-5, American Psychiatric Association 2013). It is characterized by the presence of four symptom clusters: re-experiencing, avoidance, hyperarousal and negative alterations in cognition and mood (DSM-5, American Psychiatric Association 2013). The disorder occurs in about 7% of the general population (Kessler *et al.* 2005). The development of PTSD is associated with learned fear-conditioned responses, which serve as reminders of traumatic events, and which can persist for several years after traumatic exposure (Blechert *et al.* 2007; Orr *et al.* 2000).

Single nucleotide polymorphisms (SNPs) are one of the most commonly investigated polymorphisms in case–control candidate gene association studies of PTSD (Koenen 2007; Risch & Merikangas 1996). These studies rely on the selection of candidate genes based on the current knowledge regarding the neurobiology of the disorder. In PTSD research, such genes typically include those involved in hypothalamic–pituitary–adrenal (HPA) axis regulation, the noradrenergic system and limbic–frontal brain systems (especially genes that are involved in fear conditioning) (see the review by Cornelis *et al.* 2010 for more details).

Genome-wide association studies (GWAS) represent an alternative, more robust and hypothesis-neutral approach that can be applied to case–control studies. In GWAS, SNPs (frequencies of SNPs) across the entire genome of cases are compared to controls (Hirschhorn & Daly 2005). However, to date, very few GWAS have been conducted in anxiety disorders and in PTSD in particular. In a PTSD GWAS by Logue *et al.* (2013) the sample comprised trauma-exposed Caucasian (non-Hispanic) military veterans and their intimate partners. Although several SNPs were found to be associated with PTSD, only one withstood correction for multiple testing; rs8042149, which is located in the retinoid-related orphan receptor alpha gene (*RORA*) was significantly associated with a lifetime diagnosis of PTSD. Recently, Xie *et al.* (2013) conducted a GWAS in a sample of European Americans and African Americans in order to find novel common risk alleles for PTSD. They identified a SNP on chromosome 7p12, rs406001, which exceeded genome-wide significance.

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Furthermore, a SNP that maps to the first intron of the Tolloid-Like 1 gene (TLL1) also showed strong evidence of association but did not reach genome-wide significance. However, further analysis of two SNPs in the first intron of TLL1, rs6812849 and rs7691872, in 2000 European Americans replicated the association findings from the GWAS.

As PTSD, by definition, requires exposure to a traumatic event and only a subset of individuals develop PTSD after trauma, studies of gene–environment (G × E) interactions might be better suited to elucidate the genetic underpinnings of the disorder. These studies have provided evidence that PTSD is influenced by interactive effects from both environmental and genetic factors (for more information, refer to the review article by Mehta & Binder 2012).

Transcriptional perturbations in PTSD

An alternative approach to understanding the genetic underpinnings of complex disorders, such as PTSD, includes gene expression profiling studies. Distinct differences in gene expression patterns between PTSD-affected and unaffected individuals have been observed in genes involved in the HPA axis, immune function and genes that transcribe neural and endocrine proteins (Segman *et al.* 2005; Uddin *et al.* 2010; Weaver *et al.* 2002; Yehuda *et al.* 2010; Zieker *et al.* 2007). Identification of differentially expressed genes involved in the aetiology of PTSD could aid in the identification of pathways involved in the development of the disorder. In addition, factors that contribute to altered gene expression patterns hold promising clues to the complex biological underpinnings of PTSD. A number of genes have been reported to be differentially expressed in either human or animal PTSD models (reviewed by Skelton *et al.* 2012).

Regulatory gene regions, such as epigenetic elements, have recently received attention as major contributors to phenotypic diversity and disease, especially in complex disorders. Several researchers have hypothesized that epigenetic perturbations, such as DNA methylation, may facilitate the process whereby life experiences alter gene expression patterns (Fraga *et al.* 2005). Epigenetics provides a link between the environment and the transcriptome – the effect of environmental influences, such as maternal separation and childhood trauma, on DNA methylation and subsequent gene expression profiles – has been well reported in the literature (Binder *et al.* 2008; Champagne 2008; Franklin *et al.* 2010; Koenen & Uddin 2010). Epigenetic modifications may explain the interindividual variation in disease susceptibility as well as the long-lasting effects elicited by trauma exposure (Yehuda & Bierer 2009). This review will focus on the main findings of DNA methylation studies in PTSD and how this may shape the development of new treatment strategies.

Epigenetics

Epigenetics is the study of mitotically and/or meiotically heritable changes in gene function that are not attributable to DNA sequence changes (Russo *et al.* 1996). These epigenetic changes are heritable and potentially reversible (Jaenisch & Bird 2003), and provide an additional layer of transcriptional control that may mediate the interaction between genetic predisposition, changes in neural functioning and environmental factors (Bjornsson *et al.* 2004). Such epigenetic mechanisms include DNA methylation, posttranslational modifications of histone proteins (acetylation, methylation, phosphorylation, ubiquitination and sumoylation) and non-coding RNA-mediated alterations (such as microRNAs and small interfering RNAs).

Epigenetic remodelling has been found to be a crucial component of the neuronal changes that underlie learning and memory processes (Bredy *et al.* 2007; Chwang *et al.* 2006; Miller & Sweatt 2007). It has been postulated that epigenetic factors play an important role in the regulation of activity-dependent neuronal gene expression (Chen *et al.* 2003; Martinowich *et al.* 2003). Epigenetic regulation may be particularly important in shaping the effect of early environment on the development of dysfunctional fear extinction given that epigenetic regulation of gene expression may underlie neural plasticity in the event of early-life adversity. For example, early life experience in the form of maternal care has been shown to result in stable epigenetic markings that contribute to an anxiety-like phenotype in adult rats (Weaver *et al.* 2004, 2005, 2006). These results have recently been extrapolated to human subjects (McGowan *et al.* 2009).

DNA methylation (5mC)

In mammals, DNA methylation occurs mainly at the C-5 position of cytosine residues within CpG dinucleotides (Fig. 1). Globally, about 70–80% of all CpG dinucleotides in the human genome are methylated (Ehrlich *et al.* 1982); however, numerous temporal and spatial variations are evident, especially during early development (Reik & Walter 2001). DNA methylation regulates developmental genes and is vital for genomic imprinting. During specific stages of mammalian development CpG methylation undergoes dramatic global changes. New methylation patterns are acquired during early development; primordial germ cells are characterized by genome-wide removal of DNA methylation marks and, following fertilization, the sperm-derived genome is stripped of DNA methylation (Sasaki & Matsui 2008). DNA methylation patterns are maintained after cell division and are consequently passed from parent to daughter cells (Taylor & Jones 1985; Turner 2002; Razin 1998). Dysregulation of methylation can lead to aberrant transcriptional control and subsequent alterations in gene expression (Yehuda & LeDoux 2007). Another essential role of DNA methylation is the repression of retrotransposons and other foreign elements (Sasaki & Matsui 2008).

The process of DNA methylation is strongly dependent on DNA methyltransferases (DNMTs), namely DNMT1 and *de novo* DNMT enzymes, DNMT3A and DNMT3B (essential for DNA methylation patterns in early development). DNMT1 acts as a maintenance DNMT which, in turn, acts on

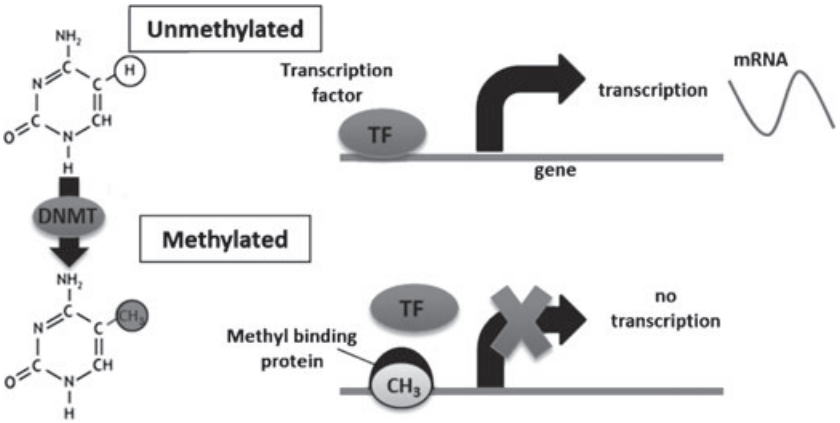


Figure 1: Graphical representation of unmethylated and methylated cytosine residues and their respective effects on mRNA transcription. The process of methylation, whereby a methyl group (CH_3) is added to the C-5 position of cytosine residues within CpG dinucleotides, is strongly dependent on the DNA methyltransferase (DNMT) enzymes. The methyl group, together with the methyl-binding protein, prevents transcription factors from binding to TSS or promoters and hinders transcription of the gene.

hemimethylated CpG sites (Turek-Plewa & Jagodzinski 2005), whereas DNMT3A and 3B are responsible for *de novo* DNA methylation by acting on hemimethylated and unmethylated CpG sites (Xie *et al.* 1999). DNMT1 and DNMT3A are abundant in the mature brain (Feng *et al.* 2010), whereas DNMT3B and DNMT3L are almost undetectable in the mature brain. DNMT3L is an accessory protein; it is catalytically inactive and is required to stimulate the DNA methylation activity of DNMT3A and 3B in embryonic stem (ES) cells (Turek-Plewa & Jagodzinski 2005). *De novo* methylation in cells that express DNMT3L requires a tetrameric complex of two DNMT3A2 and DNMT3L molecules as well as the nucleosome. The nucleosome forms the fundamental unit of eukaryotic chromatin and consists of DNA wound around eight histone protein cores (McGhee & Felsenfeld 1980). Active transcription start sites (TSSs) lack nucleosomes and, as a result, do not contain this substrate for *de novo* methylation (Ooi *et al.* 2007). A family of methyl CpG-binding domain (MBD) proteins [including methyl CpG-binding protein 2 (MeCP2) and MBD 1–4 (MBD1-4)] interpret DNA methylation by interacting with histone deacetylases (HDACs) and DNMTs to induce gene silencing. In addition, the binding of these proteins to methylated DNA seems to be important in maintaining the DNA methylation status because site-specific demethylation is associated with the dissociation of this complex (specifically MeCP2) (Chen *et al.* 2003; Martinowich *et al.* 2003; Murgatroyd *et al.* 2009). The process of active demethylation requires a mechanism that involves cell division or DNA repair and the removal of the base rather than the methyl group directly from the 5mC unit (Bhutani *et al.* 2010; Popp *et al.* 2010). Recent studies indicate the involvement of enzymes such as ten-eleven translocation (TET) methylcytosine dioxygenases, thymine DNA glycosylase and activation-induced cytidine deaminase in active and passive demethylation as well as in gene activation (Bhutani *et al.* 2010; Inoue & Zhang 2011; Iqbal *et al.* 2011).

It has been hypothesized that DNA methylation and histone deacetylation may function along a common pathway to induce transcriptional repression (Cameron *et al.* 1999; Jones *et al.* 1998; Nan *et al.* 1998). Proteins that contain MBDs recognize methylated DNA and recruit an HDAC complex to remodel the chromatin (Jones *et al.* 1998; Nan *et al.* 1998; Zhang *et al.* 1999). The association between DNA methylation and histone deacetylation was shown to be more direct than originally anticipated, when results from Fuks *et al.* (2000) indicated that DNMT1 was directly associated with HDAC activity *in vivo*. Results showed that HDAC1 has the ability to bind DNMT1 and to purify methyltransferase activity from nuclear extracts. Furthermore, a transcriptional repression domain in DNMT1, which functions partly by recruiting HDAC activity, was identified in this study (Fuks *et al.* 2000). The authors suggested that DNMT1-mediated DNA methylation may generate or depend on a transformed chromatin state through HDAC activity.

Methylation, in close proximity to the TSS, prevents transcription factors and RNA polymerase from accessing the DNA and results in silencing of the gene (Fig. 1). In addition to gene silencing, these methyl groups also attract other protein complexes that promote histone deacetylation, further inhibiting gene expression (Strathdee & Brown 2002; Turner 2002). The bond between the methyl group and the cytosine nucleotide is very strong, resulting in stable, yet potentially reversible, changes in gene expression. It has been well established that transcription cannot be initiated at methylated CpG islands (CGIs) of TSSs after the DNA has been assembled into nucleosomes (Hashimshony *et al.* 2003; Kass *et al.* 1997; Venolia & Gartler 1983). The question of which comes first, silencing or methylation, has been the subject of much discussion. In 1987, Lock *et al.* showed that methylation of the hypoxanthine phosphoribosyltransferase (*Hprt*) gene (on the inactive X chromosome) occurred only after inactivation of the chromosome. Consequently, it was postulated that methylation serves as a lock that reinforces a previously silenced state of X-linked genes (Lock *et al.* 1987). However, results from a study that investigated the role of DNMT3A in haematopoietic stem cell differentiation have raised questions about the universality of the long-term locking model (Challen *et al.* 2012). The aforementioned study indicated that methylase was vital for differentiation of a short-lived cell type. It is likely that DNA methylation instructs rather than reinforces gene silencing and that there is a general mechanism whereby silencing precedes methylation, although more data are required to confirm this. The process of DNA methylation is, therefore, more complex than was initially thought and requires in-depth

research to address a number of unanswered questions. It is also important to note that the position of methylation affects gene expression. Methylation in the TSS prevents initiation of transcription (as discussed above), whereas methylation in the gene body does not necessarily block transcription, and may even stimulate transcription elongation. It has been suggested that gene body methylation may play a role in splicing (Moarefi and Chedin 2011). Gene body methylation is a feature of transcribed genes (Wolf *et al.* 1984); the majority of gene bodies contain a limited number of CpG dinucleotides, numerous repetitive and transposable elements, and they are extensively methylated. One of the main causes of C → T transition mutations is CpG methylation in gene exons, which could result in disease-causing mutations in the germline and cancer-causing mutations in somatic cells (Jones 2012; Rideout *et al.* 1990). A 'methylation paradox' thus exists, whereby promoter methylation is inversely correlated with gene expression, and gene body methylation is positively correlated with gene expression (Jones 1999). Thus, initiation of transcription, and not transcription elongation, appears to be sensitive to DNA methylation silencing in mammals. The presence of a 5mC does not, of itself, elicit a transcriptional effect; this effect is elicited by the interpretation of the 5mC in a particular genomic and cellular context (Jones 2012).

As most genes have at least two TSSs, it has also been suggested that methylation could help regulate the process of alternative promoter usage (Maunakea *et al.* 2010). CpG-rich sequences are abundant in the genome and are referred to as CGIs, most often situated in promoter regions. These CGIs are usually protected from methylation (Yehuda & LeDoux 2007). A fraction of these CGIs, present in certain tissues during ageing (Issa 2000) or in abnormal cells (such as cancer cells) (Baylin & Herman 2000), are susceptible to progressive methylation. In mammals, the glucocorticoid (GC) content of CGIs is roughly 65% compared to 40% for the entire genome (Suzuki & Bird 2008). CpG island shores and shelves are regions outside CGIs. Shores are 0–2000 bp outside CGIs, whereas CpG shelves flank CpG shores and are 2000–4000 bp adjacent to CGIs (Pastor *et al.* 2011). Methylation mostly occurs a short distance from the CGIs at the CGI shores.

Although gene promoters contain many CGIs, CGIs also exist within the gene bodies and within gene deserts (long stretches of the genome devoid of protein-coding genes) (Jones 1999; Venter *et al.* 2001). In the human brain up to 34% of all intragenic CGIs are methylated (Maunakea *et al.* 2010); however, the exact function of CGI methylation at these intragenic locations remains to be fully elucidated. One hypothesis is that these regions may represent 'orphan promoters' that have escaped methylation in the germline, thus maintaining their high CpG density. It is therefore plausible that they play a functional role during development (Illingworth *et al.* 2010). The function of gene body methylation outside CGIs was initially assumed to be a mechanism for silencing repetitive DNA elements, such as retroviruses, LINE1 and *Alu* elements (Yoder *et al.* 1997). Whole-genome studies have recently revealed possible alternative functions for DNA methylation in gene bodies. For example, exons show a higher level of methylation than introns and changes in the degree of methylation occur at exon–intron boundaries, suggesting a role for methylation in regulating splicing (Laurent *et al.* 2010).

Initially, it was believed that cytosine methylation in mammalian DNA was limited to both strands of the symmetrical CpG sequence; however, research has shown that sequences other than CpG may also be methylated (Grafstrom *et al.* 1985; Ramsahoye *et al.* 2000; Salomon & Kaye 1970). Approximately 25% of all the ES cell methylation is in a non-CpG context (Lister *et al.* 2009). In addition to human and mouse ES cells and human induced pluripotent stem (iPS) cells, non-CpG methylation has also been observed in mouse brain and mouse germinal vesicle oocytes, human somatic tissue and brain tissue (Kobayashi *et al.* 2012; Shirane *et al.* 2013; Stadler *et al.* 2011; Xie *et al.* 2012). In human ES cells and mouse brain, CA methylation sites are most common, while lower levels of methylation are present in the CT and CC sites (Laurent *et al.* 2010; Lister *et al.* 2009; Xie *et al.* 2012). In ES cells non-CpG methylation is enriched in gene bodies and mostly absent in protein-binding sites and enhancers. Following induced differentiation of the ES cells, non-CpG methylation disappears and in iPS cells, non-CpG methylation is restored (Lister *et al.* 2009). These findings suggest that different methylation mechanisms may be used by ES cells to control gene regulation. Recently, non-CpG methylation was also found to be present in male germ cells among B1 retrotransposon sequences scattered in the mouse genome (Ichiyanagi *et al.* 2013). Accumulating levels are evident in mitotically arrested foetal prospermatogonia, with the highest levels of non-CpG methylation reached by the time of birth, occurring in a DNMT3L-dependent manner. CpA is the most common form of non-CpG methylation site in male germ cells (Ichiyanagi *et al.* 2013). Although DNMT3A is mainly a CpG methylase, it is also capable of inducing methylation at CpA and at CpT sites (Lister *et al.* 2009; Ramsahoye *et al.* 2000).

In addition to epigenetic mechanisms themselves, the various enzymes that regulate these mechanisms have also been linked to memory formation (Day & Sweatt 2010). One such example is the regulation of active DNA demethylation, with focus on the Gadd45 (growth arrest and DNA-damage-inducible, beta) family (Leach *et al.* 2012; Sultan *et al.* 2012). Gadd45b in particular has been found to be involved in activity-dependent demethylation in the adult central nervous system (CNS). The deletion of *GADD45B* (*GADD45B*$^{-/-}$) (the gene that encodes the growth arrest and DNA-damage-inducible, beta protein) leads to the abolishment of neuronal activity-induced DNA demethylation in the adult mouse dentate gyrus at specific genomic loci, including the promoters of the brain-derived neurotrophic factor (*BDNF*) gene and fibroblast growth factor 1 (*FGF1*). This reduces activity-induced adult hippocampal neurogenesis (Ma *et al.* 2008). In addition, studies have shown that pharmacological inhibition of changes in DNA methylation also affects synaptic plasticity, learning and memory (Day & Sweatt 2010).

Two research groups have investigated the effects of deletion of the *GADD45B* gene on fear conditioning and memory. Both studies found that *GADD45B* transcription is regulated in an experience-dependent manner and suggested its involvement in regulating memory capacity

(Leach *et al.* 2012; Sultan *et al.* 2012). However, conflicting data have emerged from these two studies with regard to the involvement of *GADD45B* in fear conditioning. Sultan *et al.* (2012) observed enhanced contextual fear conditioning in *GADD45B*$^{-/-}$, whereas Leach *et al.* (2012) observed a deficit in contextual fear conditioning. Although there is no clear explanation for these contradictory findings, Sultan *et al.* (2012) hypothesized that a loss of such a potent epigenomic regulator could be sensitive to the background genome where strain differences may have arisen during backcrossing. Different training facilities or housing environments could have augmented background genome or epigenome differences in the mutant mice (Crews 2011). Another factor that could have contributed to the discrepant results is the difference in training paradigms; Leach *et al.* (2012) utilized a foreground training paradigm, whereas Sultan *et al.* (2012) used background training for contextual memory assessment. Irrespective of these differences, both studies have emphasized the importance of epigenetic DNA modification mechanisms in the adult nervous system. They showed that the transcription of *GADD45B* is regulated by experience and that *GADD45B* may play an important role in long-term hippocampus-dependent memory. However, it is not only a methyl group that occurs on the C-5 position of cytosine residues but also 5-hydroxymethylcytosine (5hmC), and although these two groups are very similar, they may have distinct effects on gene expression.

DNA hydroxymethylation (5hmC)

DNA hydroxymethylation, another modified form of cytosine, has recently become a focus in epigenetic research. It is thought to play an important role in regulating gene transcription (Li & Liu 2011). 5-Methylcytosine (5mC) can be enzymatically oxidized to 5hmC by the TET 1 enzyme, one of the three enzymes of the TET family of enzymes [group of Fe(II)/2-oxoglutarate-dependent dioxygenases] (Branco *et al.* 2012). 5-Hydroxymethylcytosine was first detected in mammalian DNA in 1972 (Penn *et al.* 1972). The exact biological function of 5hmC has not yet been fully elucidated, but owing to its identification in mouse ES and neuronal cells (Davis & Vaisvila 2011), it has generated interest as a potential biomarker. It has been postulated to play an important role in the process of demethylation (Guo *et al.* 2011), where 5hmC facilitates passive demethylation, in turn promoting gene transcription. This effect, yet to be confirmed, is thought to be achieved when 5hmC prevents DNMTs from maintaining DNA methylation status (Tahiliani *et al.* 2009). The two types of cytosine modification, 5mC and 5hmC, therefore seem to have distinct, often opposite, roles in gene expression. This is illustrated by the fact that 5hmC-specific factors are recruited by hydroxymethylation of DNA, which prevents the association of certain 5mC-specific enzymes or transcription factors in DNA methylation assays and cancer cell lines (Ko *et al.* 2010; Mifsud *et al.* 2011; Tahiliani *et al.* 2009; Valinluck & Sowers 2007).

As 5hmC is present in mammalian DNA at levels that suggest physiological importance, and owing to the fact that 5hmC is expressed in a tissue-specific manner (Kinney *et al.* 2011), it is important to determine the genomic location of 5hmC. 5-Hydroxymethylcytosine has been found to be more targeted to genes compared to 5mC and is especially enriched in intragenic regions (gene bodies) and promoters, while mostly absent from non-gene regions. Peak levels of 5hmC were detected at TSSs but this was not correlated with gene expression levels for promoters with intermediate to high CpG levels. The presence of 5hmC in gene bodies, however, has been found to be more positively correlated with gene expression levels (Jin *et al.* 2011).

The levels of 5hmC in the genome are about 10% that of 5mC and 0.4% of all cytosines, suggesting that they may be somewhat short-lived. Genome-wide 5hmC profiling has unveiled a distribution that is distinct to that of 5mC; 5hmC profiles can be associated with promoters, gene expression and polycomb-mediated silencing, thus adding to the complexity of epigenetic regulators (Branco *et al.* 2012). Furthermore, research into 5hmC expression profiles across different tissues has revealed high levels of 5hmC in the brain, liver, kidney and colorectal tissues (0.40–0.65%), whereas relatively low levels were detected in the lung (0.18%) and the placenta, with heart and breast containing very low levels (0.05–0.06%) (Li & Liu 2011). The role of 5hmC in gene expression regulation together with its distinct expression patterns in different tissues and high expression levels in the brain suggests that 5hmC is a stable epigenetic mark that may have important implications for normal neuronal functioning and disease.

Most research techniques aimed at investigating methylation, including the current gold standard bisulfite sequencing, are unable to accurately distinguish between 5mC and 5hmC. This is because bisulfite conversion only converts unmethylated cytosines to uracil; thus, both 5mC and 5hmC remain unaffected and cannot be distinguished. This could present some difficulties in identifying which methyl group is present and determining the effect it has on gene expression as these two methylation states can have opposite effects on gene expression (Davis & Vaisvila 2011). It is imperative to accurately discriminate between these methylation states, particularly if gene expression studies are to be correlated with methylation status. Although a discussion of the methodologies that can be used to distinguish between 5hmC and 5mc is beyond the scope of this review, the reader is directed to a review by Branco *et al.* (2012), which discusses methods that can be used to investigate 5hmC, such as thin layer chromatography, liquid chromatography and mass spectrometry, glucosylation, antibody detection and chemical labelling.

Neuronal DNA methylation in PTSD: animal studies

A key clinical feature of PTSD is dysfunctional fear extinction which, among other factors, results from dysregulation of the HPA axis. The HPA axis, arguably the key stress response system, interacts with the immune system in order to maintain homeostasis (Wong 2002). Studies have shown

that maternal care in rodents influences the development of HPA responses to stress in the pups. Adult offspring of mothers exhibiting increased levels of licking/grooming and arched back nursing (high LG-ABN mothers) had more modest HPA responses to stress (Weaver *et al.* 2002). Little is known, however, about the molecular mechanisms by which early environmental influences alter anxiety circuits in the brain. However, researchers have found that these alterations are, in part, mediated by changes in hippocampal GC receptor (GR) expression, which mediates the negative feedback regulation of corticotropin-releasing factor (CRF) expression. The effects on GR expression were found to be associated with increased expression of the transcription factor, growth factor-inducible protein A gene (*NGFI-A*) and increased activation of GR gene expression via a promoter on exon 1 (exon IZ) of the GR gene. Adult offspring of the high LG-ABN mothers had reduced methylation levels of exon IZ, associated with increased NGFI-A (transcription factor) binding to the GR promoter. Therefore, better maternal care increases *NGFI-A* expression in the offspring and results in differential methylation of specific DNA sequences with subsequent stable, long-term alterations in gene expression (Weaver *et al.* 2002).

Several studies have confirmed the abovementioned results; researchers have found reduced expression of GRs in the hippocampi of pups raised by dams exhibiting low rates of maternal licking and grooming compared to the offspring of mothers exhibiting high rates of maternal care (Szyf *et al.* 2005; Weaver *et al.* 2004). The reduced expression of GRs was attributed to increased methylation of the GR gene promoter. Long-term transcriptional alteration is established within the first week of life and may persist and be passed to the next generation (Champagne 2008). To this end, these alterations are effectively reversed by cross-fostering the rats with dams that exhibit high maternal care or by infusion of trichostatin A (TSA), an HDAC inhibitor (HDACi) (Weaver *et al.* 2004).

Lee *et al.* (2010) investigated GC-induced epigenetic changes in candidate HPA axis genes. The FK506-binding protein 5 (FKBP5) mediates GR translocation. This GR co-chaperone protein is associated with heat shock protein 90 (HSP90) and together they form a chaperone complex that regulates GR dynamics (Hubler & Scammell 2004). Lee *et al.* found decreased DNA methylation levels in the *FKBP5* gene in brain and blood samples following GC administration; these alterations persisted for up to 4 weeks following GC withdrawal. In addition, these DNA methylation changes were associated with behavioural deficits (such as anxiety-like behaviour in the elevated plus maze task) in an animal model of Cushing's syndrome (Lee *et al.* 2010). *FKBP5* genotype and methylation profiles have recently been found to be associated with GR sensitivity and exposure to early childhood trauma (Klengel *et al.* 2013). A functional polymorphism in *FKBP5* altered the chromatin interaction between the TSS and long-range enhancers. This resulted in an increased risk of developing stress-related psychiatric disorders during adulthood through early-life trauma-dependent DNA demethylation in *FKBP5* functional GC response elements (Klengel *et al.* 2013). These findings underscore the intricate interplay between genetic polymorphisms, the epigenome and the environment, and their interaction in contributing to stress-related disorders.

Yang *et al.* (2012) found that the intronic enhancer region of *FKBP5* undergoes demethylation in response to dexamethasone treatment (in a dose-dependent manner) in a pituitary adenoma cell line AtT-20. They focused their investigation on the mouse hippocampal dentate gyrus (a vital region in the HPA axis stress response) to determine if epigenetic alterations are enriched in this region compared to the entire hippocampus. They observed an overall greater decrease in DNA methylation in the dentate gyrus compared to the entire hippocampal region. Moreover, they assessed whether DNMT1 was involved in these epigenetic alterations. They found that dexamethasone treatment resulted in a dose-dependent decrease in *DNMT1* expression in a pituitary adenoma cell line and corticosterone-treated mouse hippocampus. Their research identified methylation as a potential epigenetic mediator of the stress response. In addition, they illustrated that GC-induced loss of methylation in pituitary cells can occur (Yang *et al.* 2012). A thorough understanding of the molecular mechanisms of GC-induced changes in gene function is thus crucial for improved therapeutic strategies for mood and trauma-related disorders.

Another early life stress study in mice has suggested that vasopressin-induced gene hyperactivity could be involved in the aetiology of PTSD (Murgatroyd *et al.* 2009). In this study of maternally separated mice, a stable increase in GCs, vasopressin and depressive behaviour was observed in the separated pups. This behaviour was reversed by administration of a vasopressin receptor antagonist. Further investigation revealed that this effect was attributable to a reduction in DNA methylation of the transcription factor that increases vasopressin gene activity. Increased release of vasopressin into brain regions involved in anxiety and fear induces increased anxiety-like behaviour. DNA methylation could, therefore, act as an additional putative neurobiological marker for vulnerability to PTSD development in the context of early life stress (Murgatroyd *et al.* 2009).

It is clear that early life stress has a profound impact on gene expression profiles and subsequent behavioural abnormalities. This is further emphasized by the fact that some of these effects are heritable. Franklin *et al.* (2010) have investigated the transgenerational effects of early stress on behavioural traits and the modes of inheritance in mice. They found that only when maternal separation was unpredictable and combined with unpredictable maternal stress, did it induce long-lasting behavioural effects in the offspring and in subsequent generations. Chronic and unpredictable maternal separation induced depressive-like behaviours as well as altered behavioural responses to aversive environments during adulthood in separated animals. The male offspring of males subjected to maternal separation also exhibited most of these behavioural alterations, even though they were reared normally. In addition, chronic and unpredictable maternal separation modified the DNA methylation profile (in the germline) of the separated males in promoter regions of several candidate genes [MeCP2, cannabinoid receptor-1 (*CB1*) and CRF receptor 2 (*CRFR2*)]. Comparable DNA methylation changes were also evident in the brains of

their offspring and were associated with changes in gene expression (Franklin *et al.* 2010).

A study by Miller and Sweatt (2007) focused on DNMT and its function in DNA methylation and memory. The transcription of *DNMT*s was found to be upregulated in the rat hippocampus during contextual fear conditioning (using electric shock), whereas inhibition of DNMT blocked memory formation. Furthermore, fear conditioning was found to be associated with methylation and subsequent transcriptional repression of the protein phosphatase 1 (*PP1*) gene, the memory suppressor gene, and demethylation and transcriptional activation of reelin (*RELN*), a synaptic plasticity gene. Thus, methyltransferase and demethylase are both involved in the memory consolidation process. In addition, pharmacological inhibition of DNMT activity blocked normal memory consolidation. This study highlighted the dynamic regulation of DNA methylation in the adult nervous system and its critical function in memory formation (Miller & Sweatt 2007).

A number of studies have shown that brain DNA methylation is integral to PTSD disease aetiology. It is important to note, though, that DNA methylation patterns differ across brain regions (Gibbs *et al.* 2010; Ladd-Acosta *et al.* 2007). A study investigating the association between *BDNF* DNA methylation and PTSD-like behaviour in an adult rat model of PTSD (psychosocial stress consisted of two acute cat exposures in conjunction with 31 days of daily social instability) compared methylation levels in the dorsal and ventral hippocampi, medial prefrontal cortex and basolateral amygdala (Roth *et al.* 2011). The researchers evaluated DNA methylation patterns of exon IV of *BDNF* and performed subsequent gene expression analysis. They found that psychosocial stress in adulthood resulted in a significant increase in *BDNF* methylation in the dorsal CA1 subregion. However, in the ventral hippocampus (CA3), stress significantly decreased methylation. Furthermore, decreased expression levels of *BDNF* were evident in both the dorsal and ventral CA1 regions. The medial prefrontal cortex and basolateral amygdala exhibited no changes in *BDNF* methylation. These results indicate that traumatic stress can induce DNA methylation in certain parts of the CNS and that hippocampal dysfunction in response to traumatic stress might be induced by *BDNF* methylation. Furthermore, these results also suggest that altered hippocampal *BDNF* methylation is one mechanism underlying the cognitive deficits typical of PTSD pathophysiology (Roth *et al.* 2011).

Another study that focused specifically on DNA methylation patterns in the hippocampus in a rat PTSD model (using predator scent stress) revealed that maladaptation to traumatic stress is associated with various changes in the methylation pattern of the hippocampus. One of the differentially methylated genes identified by this global screening is disks large homolog-associated protein 2 (*DLGAP2*). *DLGAP2* had increased methylation levels in a specific site associated with a reduction in *DLGAP2* expression in rats with a PTSD-like (maladapted) phenotype compared to non-PTSD-like (well adapted) rats (Chertkow-Deutsher *et al.* 2010). Proteins of the DLGAP family are enriched in the postsynaptic density (PSD) zone. This is regarded as the main region underlying synaptic plasticity seeing as the main PSD scaffolding protein, PSD-95, regulates the development, maintenance and plasticity of synapses and spines (Han & Kim 2008), and has furthermore been associated with long-term potentiation (LTP) (Migaud *et al.* 1998). The LTP is a model of synaptic plasticity, which is proposed to be similar to the plasticity underlying learning and memory (Bliss & Collingridge 1993; Holscher 1999) – the two cognitive processes that are impaired in PTSD (Friedman 1997; Vermetten & Bremner 2002). Alterations in methylation patterns could thus be involved in behavioural adaptation to environmental stress and could aid in the identification of possible treatment targets for PTSD (Chertkow-Deutsher *et al.* 2010).

DNA methylation studies in human subjects

Individuals who suffer from child abuse have a greater risk of developing PTSD and depression in later life (MacMillan *et al.* 2001; Mullen *et al.* 1996). These individuals are also prone to exacerbated physiological responses to stress (Heim & Nemeroff 2001; Weiss *et al.* 1999) and corresponding alterations in CNS functioning (Liu *et al.* 1997; Weiss *et al.* 1999). The link between an environmental stressor and disease pathogenesis was investigated by Beach *et al.* (2010) in an adoptee sample from Iowa. The authors found that the level of methylation of the CGI upstream from the serotonin transporter gene (solute carrier family 6, member 4), *SLC6A4*, was associated with self-reported childhood trauma in both males and females. Increased levels of methylation were evident in abused males compared to non-abused males across the entire promoter region. Two loci (CpG1 and CpG3) were significantly hypermethylated in women who experienced child abuse. These results suggest that methylation could be an intermediary for gene–environment interactions and potentially modulate risk for psychiatric disorders (Beach *et al.* 2010).

Similar to the animal studies discussed previously, studies in human subjects have demonstrated alterations in the HPA stress response and an increased risk of suicide following childhood trauma. McGowan *et al.* (2009) examined epigenetic differences in the promoter of a neuron-specific GR (*NR3C1*) between post-mortem hippocampi obtained from suicide victims with a history of childhood abuse and those from suicide victims with no childhood abuse, and controls. They found reduced levels of *NR3C1* mRNA and mRNA transcripts containing the GR 1F splice variant, as well as increased cytosine methylation of the *NR3C1* promoter in suicide victims with a history of childhood abuse compared to suicide victims without childhood trauma and controls. These findings are consistent with rat studies and suggest a common effect of early adverse experiences on the epigenetic regulation of hippocampal GR expression (McGowan *et al.* 2009).

DNA methylation thus provides a mechanism whereby the activity of genes is programmed to regulate HPA activity through early life events. Early life events have been found to be associated with the development of PTSD as well as the changes in the HPA axis described in PTSD. Epigenetic changes could provide a molecular link

between early environmental contexts and gene expression and function (Yehuda & LeDoux 2007). Caution should be exercised when interpreting findings from post-mortem brain samples. First, the cause of death, in this case suicide, may affect methylation results, for example, an overdose of medication or carbon monoxide poisoning could potentially alter methylation levels. Second, in the case of suicide, these individuals may have suffered from depression, which raises the question of whether these methylation profiles are more specific to depression or to PTSD. Third, differentiating methylation profiles associated with PTSD pathologies from those related to the physiological changes resulting from death is difficult. These confounding variables must be kept in mind in the planning, and interpretation of results, of post-mortem DNA methylation studies.

Researchers have further speculated on whether childhood trauma affects a limited number of candidate genes or whether the effects on the epigenome and various functional downstream pathways are more extensive. In a study that investigated genome-wide promoter methylation in the hippocampus of individuals who suffered severe childhood abuse, the authors identified 362 differentially methylated promoters in abused individuals compared with controls (Labonte *et al.* 2012). Of these promoters, 248 were hypermethylated and 114 were hypomethylated. Methylation differences occurred mostly in the neuronal cellular fraction and the most significantly differentially methylated genes were those involved in cellular or neuronal plasticity. One of these hypermethylated genes, Alsin (*ALS2*), is specifically expressed in neurons and has two major predicted transcripts encoding two protein isoforms, which are postulated to be involved in behavioural fear responses. Functional assays (methylated *ALS2* constructs that mimicked the methylation state in samples) revealed a decrease in promoter transcription. This was associated with decreased expression of hippocampal *ALS2* variants. These results demonstrate how childhood adversity affects DNA methylation patterns on a genome-wide scale and how these alterations may be associated with altered transcriptional patterns (Labonte *et al.* 2012).

DNA methylation patterns in other traumas and nervous system disorders

It is not only childhood trauma that alters methylation and gene expression patterns; for example, prenatal exposure to maternal stress and adult exposure in the form of intimate partner violence (IPV) have also been found to induce lasting methylation changes that could affect psychological function in later life. To this end, researchers have shown that a mother's experience of IPV during pregnancy could have a long-term influence on the methylation status of *NR3C1* in the child that is still detectable during the child's adolescent years (10–19 years after birth). These sustained epigenetic changes that are established *in utero* provide a potential mechanism whereby prenatal stress programs adult psychological function (Radtke *et al.* 2011).

The relationship between genotype and/or methylation status and the association between the number of traumatic events and PTSD were investigated in the Detroit Neighbourhood Health Study (Koenen *et al.* 2011). Genotype and methylation status of the serotonin transporter (*SLC6A4* or *5HTT*) promoter were investigated in 100 individuals. The results indicated that the number of traumatic events was associated with PTSD outcome; this association was modified by *SLC6A4* methylation level at cg22584138. This CpG site is located within intron 1 of *SLC6A4*, located upstream of the gene's start codon and downstream of the *5-HTTLPR* variable number of tandem repeat (VNTR) locus and TSSs. There was a robust association between the number of traumatic events and the risk for PTSD, specifically at lower levels of methylation. Higher methylation levels had a protective effect in individuals who experienced a higher number of traumatic events. The authors deduced that *SLC6A4* methylation levels can modify the effects of traumatic events in the context of PTSD aetiology, acting as a molecular marker for PTSD risk profiling. Their results provided a novel site for further investigation of anxiety-related outcomes (Koenen *et al.* 2011).

A study of genetic susceptibility to PTSD in military soldiers investigated the possible association between DNA methylation patterns in the repetitive elements LINE-1 and *Alu* and PTSD. Numerous factors play a role in chromosomal stability, including methylation in repeat regions such as centromeres. This suppresses the expression of transposable elements (such as LINE-1 and *Alu*), thereby contributing to genome stability (Moarefi & Chedin 2011). The study cohort consisted of US military soldiers recently deployed to Afghanistan or Iraq. Serum samples were collected pre- and post-deployment from individuals diagnosed with PTSD post-deployment (cases) and randomly selected service members with no PTSD diagnosis (controls). When comparing post- and pre-deployment controls, they found that LINE-1 was hypermethylated in the post-deployment group. When comparing cases to controls post-deployment, LINE-1 was hypomethylated in cases, while pre-deployment *Alu* was found to be hypermethylated. Their results indicate that hypermethylation of LINE1 in controls post-deployment and of *Alu* in cases post-deployment should be further investigated as potential resilience or vulnerability factors (Rusiecki *et al.* 2012).

Earlier gene expression studies revealed distinct expression patterns in genes involved in immune activation between PTSD-affected and -unaffected individuals (Segman *et al.* 2005; Zieker *et al.* 2007). This prompted researchers to investigate the mechanisms whereby the experience of a traumatic event could alter gene expression profiles, in turn affecting immune function and inducing physiological changes. Uddin *et al.* (2010) investigated epigenetic changes in immune system-related gene clusters in PTSD-affected and -unaffected individuals. They found a distinct signature of immune activation among PTSD-affected individuals. Lower levels of methylation were observed in genes with immune-related functions in PTSD-affected individuals, while methylation profiles among the PTSD-unaffected individuals were distinguished by hypomethylation of genes with neurogenesis-related functions [such as contactin 2 (*CNTN2*)

and tubulin, beta 2B class IIb (*TUBB2B*)]. The authors hypothesized that immune dysfunction among persons with PTSD may be influenced by epigenetic effects (resulting in immune activation) as well as by an absence of epigenetic profiles associated with the development of normal neural–immune interactions (Uddin *et al.* 2010).

Many of the differentially methylated genes identified by Uddin *et al.* (2010) were confirmed in an independent cohort of traumatized individuals from Atlanta (Smith *et al.* 2011). Researchers investigated whether DNA methylation, following chronic stress, could mediate altered gene function. Their cohort consisted of African American subjects grouped according to PTSD diagnosis and a history of child abuse. They investigated both global and site-specific methylation. Associations between methylation and PTSD, history of child abuse and total life stress (TLS) were assessed. They found increased levels of global methylation in subjects with PTSD and CpG sites in five genes that were differentially methylated in PTSD subjects. These genes were translocated promoter region (*TPR*), C-type lectin domain family 9, member A (*CLEC9A*), acid phosphatase 5 (*APC5*), annexin A2 (*ANXA2*) and toll-like receptor 8 (*TLR8*). Furthermore, the authors found an association between a CpG site in neuropeptide FF receptor 2 (*NPFFR2*) and TLS. Most of these genes are associated with inflammation. In light of these findings, and previously described impaired immune function associated with trauma history, the authors compared plasma cytokine levels in this cohort. They found that the proinflammatory cytokines, interleukin (IL)-2 and tumour necrosis factor-α (TNF-α), were elevated, whereas the anti-inflammatory cytokine, IL-4, was decreased in PTSD patients. Levels of IL-4 were associated with PTSD and increased TNF-α plasma levels were associated with child abuse and higher TLS. These results provide evidence that genes involved in immune function may be altered by psychosocial stress through altered DNA methylation patterns (global and gene-specific). In addition, these results suggest that cytokines are dysregulated in such a manner that anti-inflammatory responses are decreased while proinflammatory responses are augmented to a state of heightened inflammation. This may result in comorbid immune-related symptoms such as fatigue, malaise and altered patterns in sleep and appetite, as was shown following the brain–blood barrier crossing of TNF-α (Dunn 2006; Silverman *et al.* 2005; Sternberg 2006). These are important factors that should be taken into consideration in the holistic treatment of PTSD.

The pituitary adenylate cyclase-activating polypeptide (PACAP) is another important role player in cellular stress response regulation and neurotrophic function. The PACAP–PAC1 receptor protein is encoded by the adenylate cyclase-activating polypeptide 1 (pituitary) receptor type I (*ADCYAP1R1*) gene. A sex-specific association of *PACAP* blood levels with fear responses, PTSD diagnosis and symptoms was evident in severely traumatized female participants (Ressler *et al.* 2011). Posttraumatic stress disorder [based on the PTSD symptom scale-interview version (PSS-I) measures] was found to be associated with methylation levels; furthermore, methylation at the first site within the *ADCYAP1R1* CGI (in peripheral blood DNA) was significantly associated with total PTSD symptoms in a sex-independent manner. Ressler *et al.* (2011) attributed the differential function of the PAC1 receptor in PTSD to the epigenetic regulation of *ADCYAP1R1*. Also, in rodent models they found that fear conditioning or oestrogen replacement induced *ADCYAP1R1* mRNA expression. The authors hypothesized that the PACAP–PAC1 pathway is involved in regulating the psychological and physiological responses to traumatic stress and that variations in this pathway could affect stress responses underlying PTSD.

In PTSD research, as with other research fields, an important goal is delineation of the functional effects of thousands of SNPs identified by GWAS. Smith *et al.* (2008) showed that the minor *A*-allele of the promoter polymorphism in the serotonin receptor 2A gene (*HTR2A*), rs6311 (−1438G/A), is associated with chronic fatigue syndrome (CFS) and measures of disability and fatigue. This SNP, which results in the creation of a binding site for E47, has also been associated with other complex disorders such as depression, PTSD and schizophrenia (Smith *et al.* 2008). The neurotransmission of serotonin plays an integral role in the pathophysiology of a number of neuropsychiatric disorders. This polymorphism results in the loss of a CpG methylation site at −1439 (Falkenberg *et al.* 2011). Falkenberg *et al.* (2011) found that this sequence variation at the promoter resulted in differential methylation at two CpG sites, −1224 and −1420 between CFS and non-fatigued (NF) subjects (Falkenberg *et al.* 2011). Altered regulation of *HTR2A* expression, via differential DNA methylation, may be relevant in PTSD susceptibility. It is thus important to correlate GWAS findings to that of the methylome in order to assess their individual and combined contributions in the molecular pathophysiology of neuropsychiatric disease.

Norrholm *et al.* (2013) investigated the interaction of the catechol-*O*-methyltransferase (*COMT*) genotype, *COMT* DNA methylation levels (in whole blood) and PTSD characteristics (specifically fear-potentiated startle during fear conditioning and extinction) in a community study of African American individuals from Atlanta. The *COMT* Val158Met polymorphism has previously been implicated in PTSD, with the Met/Met homozygous genotype being associated with increased susceptibility to PTSD development. Individuals with the Met/Met genotype, compared to Val/Met and Val/Val genotypes, exhibited increased fear-potentiated startle to a non-reinforced conditioned stimulus (CS−) (safety signal) and during extinction of the reinforced conditioned stimulus (CS+) (danger signal). Individuals diagnosed with PTSD who had the Met/Met genotype had the greatest impairment in fear inhibition to the CS− compared to Val carriers. Moreover, the Met/Met genotype was associated with DNA methylation at four CpG sites; two of these sites were found to be associated with impaired fear inhibition to the CS−. These results illustrate the multiple differential mechanisms that regulate COMT function (genotype and/or DNA methylation levels) associated with impaired fear inhibition in PTSD (Norrholm *et al.* 2013).

DNA methylation was found to play an important role in memory and synaptic plasticity in a study of Rett syndrome (RS), an X-linked neurodevelopmental disorder. Missense and truncating mutations in *MECP2*, a MBD protein that

interprets DNA methylation, lead to MECP2 deficiency and reduced binding to methylated DNA; this has been shown to contribute, in part, to the disease phenotype (Amir *et al.* 1999; Ellaway & Christodoulou 2001; Sirianni *et al.* 1998). Further, analyses in animal models have shown that MECP2 overexpression results in enhanced long-term memory formation and induction of hippocampal LTP. These results suggest that MECP2 is a modulator of memory formation and an inducer of synaptic plasticity, which could have implications for PTSD (Collins *et al.* 2004). Table 1 provides a summary of the DNA methylation studies in human subjects that have found associations between trauma and/or PTSD and DNA methylation and gene expression profiles.

DNA methylation and personalized medicine in PTSD

Since the advent of the phrase 'personalized medicine' there have been high expectations that patient-specific pharmacogenetic data will improve treatment outcomes in neuropsychiatric disorders. However, owing to the complexity of transcriptional regulation and the influence of environmental factors and the epigenome, simple translation of individual genetic information into personalized treatment has not proved to be enough. How could pharmacogenetics explain the fact that monozygotic twins, who are both treated for major depression with the same drug, exhibit different clinical responses? Why do some patients who suffer from recurrent major depression not show an efficacious response to a drug as they did during a previous episode? The answers might lie in epigenetics seeing that the dynamic nature of DNA methylation patterns and histone acetylation provide plausible explanations for some of these puzzling pharmacogenetic questions (Holsboer 2008).

DNA and histone methylation in the brain can be compromised when neurons are starved of methyl donors. The depletion of methyl donors may be due to inherited metabolism errors, folate deficiency or methyl donor deficiencies of folate, L-methylfolate and *S*-adenosylmethionine (SAM) in the diet or depletion due to pregnancy, gastrointestinal disease, smoking, alcohol or drug addiction (Stahl 2007, 2010). The depletion of methyl donors can occur to such an extent that it induces elevated homocysteine levels, psychosis and developmental delay (Freeman *et al.* 1975; Regland *et al.* 1994). Where hypomethylation causes susceptibility to a disease, methyl donors or drugs that target methyl metabolism may have utility as therapeutic agents.

Another possible epigenetic biomarker is the enzyme enolase phosphatase. Different isoforms of this enzyme were found to be present in high-anxiety compared to low-anxiety mice. Differences in enolase phosphatase protein levels are attributable to SNPs that alter the amino acid sequence (Weaver *et al.* 2004). Enolase phosphatase is involved in the methionine recovery pathway that reconstitutes methionine after its conversion into SAM (a methyl donor). Variations in the methionine/SAM ratio may affect DNA methylation levels. Indeed, methionine infusion was found to reverse the effect of poor maternal care on DNA methylation profiles (detected in the rats during adulthood) as well as behavioural responses to stress (Kagan *et al.* 1990); SAM has been reported to have antidepressant effects (Kagan *et al.* 1990). More research is required, however, to determine whether a mutation in the enolase phosphatase gene is a potential biomarker of treatment response to SAM (Holsboer 2008).

Epigenetic markings, such as DNA methylation, may also be transmitted transgenerationally (Mill & Petronis 2007); thus, the level of gene expression, as well as its timing and location, could be heritable and subsequently influence an individual's phenotype, disease susceptibility and drug response. Epigenetic therapies have enabled researchers to correct some of these aberrant expression profiles (Simonini *et al.* 2006; Tremolizzo *et al.* 2002). Most of the epigenetic therapies target DNA methylation and histone deacetylation enzymes and several drugs (mainly developed to treat cancer) have been tested in clinical trials (Szyf 2009). Some DNMT inhibitors have been approved for clinical therapy [such as azacytidine (AZA) and decitabine (DAC)], others are in phase 1 (e.g. 5-fluoro-2′-deoxycytidine) or still in preclinical development (e.g. zebularine) (Amatori *et al.* 2010).

A more thorough understanding of the genes and epigenetic events associated with a specific disease is a necessary step in pursuing targeted approaches (Graff & Mansuy 2009). The role of epigenetics in antidepressant response was demonstrated in a study that found that in chronically stressed mice, there was a decrease in the activity of the HDAC5 enzyme, leading to the removal of acetyl groups from histones and subsequently inhibited gene activity (Renthal *et al.* 2007). Upon chronic administration of an antidepressant, this effect on HDAC5 was effectively reversed (Renthal *et al.* 2007). Furthermore, increased stress-induced depression-like behaviour was evident in HDAC5-knockout mice. In a similar fashion, stress-induced downregulation of *BDNF* expression was associated with increased methylation of the *BDNF* promoter. Antidepressants were able to reverse this effect and activate *BDNF* gene expression by increasing histone acetylation at the *BDNF* promoter (Tsankova *et al.* 2006). Subsequently, one of the strategies to achieve demethylation in the brain involves the use of HDACis. Indeed, in animal models pharmacological treatment with HDACi effectively reversed hypermethylation of *RELN* (in the context of schizophrenia) (Simonini *et al.* 2006; Tremolizzo *et al.* 2002). In addition, treatment with valproate (Dong *et al.* 2007), TSA (Weaver *et al.* 2004) and a benzamide HDACi, *N*-(2-aminophenyl)-4-[*N*-(pyridin-3-ylmethoxycarbonyl) aminomethyl] benzamide derivative (MS-275) (Simonini *et al.* 2006) has all been shown to effectively induce demethylation in the brain. Development of DNMT antagonists using nanotechnology could enable the development of DNA methylation inhibitors that are effective in postmitotic tissues, such as the brain, and provide exciting new directions in psychiatric drug development (Szyf 2009).

An alternate approach to generic epigenetic inhibitors is the development of drugs designed for gene-specific epigenetic targeting. With regard to DNA methylation, this has recently been achieved both *in vitro* and *in vivo* in a study that used specific zinc finger peptides that confer *de novo* methylation

Table 1: DNA methylation studies in human subjects that describe associations between trauma, DNA methylation profiles, gene expression profiles and PTSD

Association of DNA methylation with trauma, gene expression or PTSD	Sample group	Reference
Increased *SLC6A4* promoter methylation in abused males vs. non-abused males	Iowa adoptee sample (EBV-transformed lymphoblast cell lines)	Beach *et al.* (2010)
Hypermethylation of CpG1 and CpG3 regions of *SLC6A4* promoter in women who experienced child abuse	Iowa adoptee sample (EBV-transformed lymphoblast cell lines)	Beach *et al.* (2010)
Increased methylation of *NR3C1* promoter in suicide victims with childhood abuse history vs. no abuse history and controls	Post-mortem suicide victims (hippocampi)	McGowan *et al.* (2009)
Hypermethylation of *ALS2* in abused individuals	Individuals who suffered severe childhood abuse (hippocampi)	Labonte *et al.* (2012)
Hypermethylation of *SLC6A4* at cg22584138 had a protective effect in individuals who experienced a higher number of traumatic events	Detroit Neighbourhood Health Study (whole blood)	Koenen *et al.* (2011)
Hypermethylation of LINE-1 in post-deployment controls vs. pre-deployment controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki *et al.* (2012)
Hypomethylation of LINE-1 in post-deployment cases vs. controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki *et al.* (2012)
Hypermethylation of *Alu* in pre-deployment cases vs. controls	US military soldiers deployed to Afghanistan or Iraq (serum samples)	Rusiecki *et al.* (2012)
Hypermethylation of *BDNF p*romoter (exon IV) in Wernicke's area of the brain in suicide victims compared with non-suicide controls resulting in decreased *BDNF* expression	Suicide victims and non-suicide controls (brain samples)	Keller *et al.* (2010)
Hypomethylation of genes with immune-related functions in PTSD-affected individuals	PTSD-affected and -unaffected individuals (blood samples)	Uddin *et al.* (2010)
Hypomethylation of genes with neurogenesis-related functions in PTSD-unaffected individuals	PTSD-affected and -unaffected individuals (blood samples)	Uddin *et al.* (2010)
Increased global methylation in subjects with PTSD	Traumatized African American individuals from Atlanta (PBMCs)	Smith *et al.* (2011)
Differential methylation of *TPR CLEC9A*, *APC5*, *ANXA2* and *TLR8* in PTSD subjects	Traumatized African American individuals from Atlanta (PBMCs)	Smith *et al.* (2011)
ADCYAP1R1 CpG island methylation directly associated with total PTSD symptoms	Traumatized African American individuals from Atlanta	Ressler *et al.* (2011)
PACAP methylation levels associated with PTSD in females	Traumatized African American individuals from Atlanta (saliva and blood samples)	Ressler *et al.* (2011)
Total PTSD symptoms associated with methylation at *ADCYAP1R1* CpG island in females	Traumatized African American individuals from Atlanta (saliva and blood samples)	Ressler *et al.* (2011)
HTR2A minor A-allele (resulting in loss of CpG methylation site at −1439) associated with disorders including PTSD	Chronic fatigue syndrome patients and non-fatigued controls (PBMCs)	Smith *et al.* (2008)
COMT Met/Met genotype associated with increased susceptibility to PTSD development and DNA methylation at four CpG sites (two sites found associated with impaired fear inhibition)	Community study in African American individuals from Atlanta (whole blood)	Norrholm *et al.* (2013)

EBV, Epstein–Barr virus.

to specific loci (Smith & Ford 2007). However, these therapies are not without counter-implications or side effects. Azanucleosides, such as AZA and DAC, function by silencing DNMT and are one of the only demethylating strategies approved for clinical therapy. One of the side effects is that AZA and DAC could be incorporated directly into centromeric DNA sequences, which could lead to decondensation of the heterochromatin and altered centromeric structure, ultimately resulting in destabilization of the genome and impaired kinetochore formation. Consequently, the whole mitotic process could malfunction (Amatori *et al.* 2010). Furthermore, AZA is not very stable, has quite a short half-life of 1.5 ± 2.3 h and is dependent on the cell cycle for its activity; thus, prolonged administration schedules are required. Decitabine, however, is more stable, has a half-life of 20 ± 5 h in aqueous solutions (Momparler 2005; Rudek *et al.* 2005) and effectively incorporates into DNA, making it more effective than AZA in inducing DNA demethylation. Moreover, it can be administered at lower doses (Appleton *et al.* 2007; Kantarjian *et al.* 2006, 2007; Kornblith *et al.* 2002; Silverman *et al.* 2002, 2006).

Conclusion

The study of methylomes and the environment hold great promise for understanding the aetiology and treatment of PTSD. Epigenetic mechanisms represent an exciting frontier because of their ability to define specific molecular pathways by which environmental risk factors directly change the expression of a gene, thus contributing to individual differences in gene function and vulnerability to a specific disorder. An exemplar of this is PTSD where DNA methylation patterns could help explain gene expression alterations associated with PTSD and PTSD risk (Yehuda & LeDoux 2007). A growing body of literature describes the interactions between DNA methylation, traumatic experience and physiologic manifestations of PTSD. The interactions could be simple, such as differential methylation of exon 1 of the neuron-specific GR (*NTRK3*) (in both humans and rats) as a function of variations in maternal care (McGowan *et al.* 2009; Szyf *et al.* 2005; Weaver *et al.* 2004). In this instance, higher levels of childhood abuse or maternal neglect may be associated with higher levels of methylation and in turn lower expression of *NTRK3*. Alternatively, these interactions could be more complex, such as hypermethylation of intron 1 of *SLC6A4* providing a protective effect in individuals who have experienced a higher number of traumatic events (Koenen *et al.* 2011).

In recent years, significant technological advances have been made with the advent of microarrays and next-generation sequencing (NGS) technologies, enabling researchers to comprehensively profile the entire methylome. However, the study of DNA methylation is not without its challenges. First, brain material is seldom available for human methylation studies; surrogate tissues such as peripheral blood mononuclear cells (PBMCs) are often used. In the context of PTSD, it is important to determine to what extent methylation profiles (and subsequent gene expression patterns) of peripheral tissues correlate with brain regions implicated in PTSD aetiology. Second, owing to the highly compartmentalized nature and cellular heterogeneity of the brain, studies have investigated DNA methylation in different brain regions. More recently, the focus has moved to DNA methylation analyses at a single-cell level (Cipriany *et al.* 2012; Flusberg *et al.* 2010; Kantlehner *et al.* 2011; Meissner *et al.* 2008). The rationale for this is the high variability in DNA methylation profiles across individual cells (even within the same organ) that are dependent on gene function, disease state, environmental influences and various other factors (Kantlehner *et al.* 2011). Third, the use of bisulfite-treated DNA for downstream applications such as NGS, microarrays or polymerase chain reactions for methylation studies is challenging. As bisulfite treatment can only discriminate between unmethylated DNA and either 5mC or 5hmC (and cannot discriminate between 5mC and 5hmC), additional analyses are needed to establish whether 5mC or 5hmC is present at a particular site. Methyl-sensitive enzymes can be used to discriminate between 5mC and 5hmC (Song *et al.* 2011).

Despite these challenges, DNA methylation analyses have yielded insights into the influence of the environment on the transcriptome and proteome in PTSD. Methylation studies have also shown how changes in DNA methylation contribute to phenotypic diversity and disease susceptibility. In a study performed on hurricane survivors, Perilla *et al.* (2002) found significant differences in the prevalence of PTSD between Hispanics, non-Hispanic blacks and Caucasians groups. Furthermore, Roberts *et al.* (2011) also found ethnic differences between Caucasian, Black, Hispanic and Asian individuals in PTSD prevalence, risk for trauma exposure, risk of developing PTSD and treatment-seeking behaviours. Significant differences in DNA methylation levels at birth between African Americans and Caucasians have also been documented for a specific subset of CpG dinucleotides. If these differences in methylation correlate with differences in gene expression, this could ultimately lead to disease progression and may, in part, explain the racial differences in incidence rates of disorders such as PTSD (Adkins *et al.* 2011).

In the context of PTSD, DNA methylation is an epigenetic mediator of the stress response. The study of DNA methylation and methylation machinery is an essential step in the development of epigenetic drugs that target DNA methylation or histone deacetylation enzymes or DNA methyl donors such as folate. Yet, in order to fully harvest the benefits of epigenetic treatments for PTSD, and embrace treatments that are truly personalized, the exact epigenetic mechanisms underlying the disorder first need to be fully understood.

References

Adkins, R.M., Krushkal, J., Tylavsky, F.A. & Thomas, F. (2011) Racial differences in gene-specific DNA methylation levels are present at birth. *Birth Defects Res A Clin Mol Teratol* **91**, 728–736.

Amatori, S., Bagaloni, I., Donati, B. & Fanelli, M. (2010) DNA demethylating antineoplastic strategies: a comparative point of view. *Genes Cancer* **1**, 197–209.

American Psychiatric Association. (2013) *Diagnostic and statistical manual of mental disorders*, 5th edn. American Psychiatric Publishing, Arlington, VA.

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U. & Zoghbi, H.Y. (1999) Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* **23**, 185–188.

Appleton, K., Mackay, H.J., Judson, I., Plumb, J.A., McCormick, C., Strathdee, G., Lee, C., Barrett, S., Reade, S., Jadayel, D., Tang, A., Bellenger, K., Mackay, L., Setanoians, A., Schatzlein, A., Twelves, C., Kaye, S.B. & Brown, R. (2007) Phase I and pharmacodynamic trial of the DNA methyltransferase inhibitor decitabine and carboplatin in solid tumors. *J Clin Oncol* **25**, 4603–4609.

Baylin, S.B. & Herman, J.G. (2000) DNA hypermethylation in tumorigenesis: epigenetics joins genetics. *Trends Genet* **16**, 168–174.

Beach, S.R., Brody, G.H., Todorov, A.A., Gunter, T.D. & Philibert, R.A. (2010) Methylation at SLC6A4 is linked to family history of child abuse: an examination of the Iowa Adoptee sample. *Am J Med Genet B Neuropsychiatr Genet* **153B**, 710–713.

Bhutani, N., Brady, J.J., Damian, M., Sacco, A., Corbel, S.Y. & Blau, H.M. (2010) Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* **463**, 1042–1057.

Binder, E.B., Bradley, R.G., Liu, W., Epstein, M.P., Deveau, T.C., Mercer, K.B., Tang, Y., Gillespie, C.F., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F. & Ressler, K.J. (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* **299**, 1291–1305.

Bjornsson, H.T., Fallin, M.D. & Feinberg, A.P. (2004) An integrated epigenetic and genetic approach to common human disease. *Trends Genet* **20**, 350–358.

Blechert, J., Michael, T., Vriends, N., Margraf, J. & Wilhelm, F.H. (2007) Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behav Res Ther* **45**, 2019–2033.

Bliss, T.V. & Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39.

Branco, M.R., Ficz, G. & Reik, W. (2012) Uncovering the role of 5-hydroxymethylcytosine in the epigenome. *Nat Rev Genet* **13**, 7–13.

Bredy, T.W., Wu, H., Crego, C., Zellhoefer, J., Sun, Y.E. & Barad, M. (2007) Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. *Learn Mem* **14**, 268–276.

Cameron, E.E., Bachman, K.E., Myohanen, S., Herman, J.G. & Baylin, S.B. (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet* **21**, 103–107.

Challen, G.A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J.S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y., Liang, S., Lu, Y., Darlington, G.J., Meissner, A., Issa, J.P., Godley, L.A., Li, W. & Goodell, M.A. (2012) Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* **44**, 23–31.

Champagne, F.A. (2008) Epigenetic mechanisms and the trans-generational effects of maternal care. *Front Neuroendocrinol* **29**, 386–397.

Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R. & Greenberg, M.E. (2003) Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* **302**, 885–889.

Chertkow-Deutsher, Y., Cohen, H., Klein, E. & Ben-Shachar, D. (2010) DNA methylation in vulnerability to post-traumatic stress in rats: evidence for the role of the post-synaptic density protein Dlgap2. *Int J Neuropsychopharmacol* **13**, 347–359.

Chwang, W.B., O'Riordan, K.J., Levenson, J.M. & Sweatt, J.D. (2006) ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. *Learn Mem* **13**, 322–328.

Cipriany, B.R., Murphy, P.J., Hagarman, J.A., Cerf, A., Latulippe, D., Levy, S.L., Benitez, J.J., Tan, C.P., Topolancik, J., Soloway, P.D. & Craighead, H.G. (2012) Real-time analysis and selection of methylated DNA by fluorescence-activated single molecule sorting in a nanofluidic channel. *Proc Natl Acad Sci U S A* **109**, 8477–8482.

Collins, A.L., Levenson, J.M., Vilaythong, A.P., Richman, R., Armstrong, D.L., Noebels, J.L., David, S.J. & Zoghbi, H.Y. (2004) Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. *Hum Mol Genet* **13**, 2679–2689.

Cornelis, M.C., Nugent, N.R., Amstadter, A.B. & Koenen, K.C. (2010) Genetics of post-traumatic stress disorder: review and recommendations for genome-wide association studies. *Curr Psychiatry Rep* **12**, 313–326.

Crews, D. (2011) Epigenetic modifications of brain and behavior: theory and practice. *Horm Behav* **59**, 393–398.

Davis, T. & Vaisvila, R. (2011) High sensitivity 5-hydroxymethyl cytosine detection in Balb/C brain tissue. *J Vis Exp* **48**, e2661.

Day, J.J. & Sweatt, J.D. (2010) DNA methylation and memory formation. *Nat Neurosci* **13**, 1319–1323.

Dong, E., Guidotti, A., Grayson, D.R. & Costa, E. (2007) Histone hyperacetylation induces demethylation of reelin and 67-kDa glutamic acid decarboxylase promoters. *Proc Natl Acad Sci U S A* **104**, 4676–4681.

Dunn, A.J. (2006) Effects of cytokines and infections on brain neurochemistry. *Clin Neurosci Res* **6**, 52–68.

Ehrlich, M., Gama-Sosa, M.A., Huang, L.H., Midgett, R.M., Kuo, K.C., McCune, R.A. & Gehrke, C. (1982) Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res* **10**, 2709–2721.

Ellaway, C. & Christodoulou, J. (2001) Rett syndrome: clinical characteristics and recent genetic advances. *Disabil Rehabil* **23**, 98–106.

Falkenberg, V.R., Gurbaxani, B.M., Unger, E.R. & Rajeevan, M.S. (2011) Functional genomics of serotonin receptor 2A (HTR2A): interaction of polymorphism, methylation, expression and disease association. *Neuromolecular Med* **13**, 66–76.

Feng, J., Zhou, Y., Campbell, S.L., Le, T., Li, E., Sweatt, J.D., Silva, A.J. & Fan, G. (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat Neurosci* **13**, 423–430.

Flusberg, B.A., Webster, D.R., Lee, J.H., Travers, K.J., Olivares, E.C., Clark, T.A., Korlach, J. & Turner, S.W. (2010) Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nat Methods* **7**, 461–465.

Fraga, M.F., Ballestar, E., Paz, M.F. *et al.* (2005) Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A* **102**, 10604–10609.

Franklin, T.B., Russig, H., Weiss, I.C., Graff, J., Linder, N., Michalon, A., Vizi, S. & Mansuy, I.M. (2010) Epigenetic transmission of the impact of early stress across generations. *Biol Psychiatry* **68**, 408–415.

Freeman, J.M., Finkelstein, J.D. & Mudd, S.H. (1975) Folate-responsive homocystinuria and "schizophrenia". A defect in methylation due to deficient 5,10-methylenetetrahydrofolate reductase activity. *N Engl J Med* **292**, 491–496.

Friedman, M.J. (1997) Posttraumatic stress disorder. *J Clin Psychiatry* **58** (Suppl. 9), 33–36.

Fuks, F., Burgers, W.A., Brehm, A., Hughes-Davies, L. & Kouzarides, T. (2000) DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat Genet* **24**, 88–91.

Gibbs, J.R., van der Brug, M.P., Hernandez, D.G., Traynor, B.J., Nalls, M.A., Lai, S.L., Arepalli, S., Dillman, A., Rafferty, I.P., Troncoso, J., Johnson, R., Zielke, H.R., Ferrucci, L., Longo, D.L., Cookson, M.R. & Singleton, A.B. (2010) Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet* **6**, e1000952.

Graff, J. & Mansuy, I.M. (2009) Epigenetic dysregulation in cognitive disorders. *Eur J Neurosci* **30**, 1–8.

Grafstrom, R.H., Yuan, R. & Hamilton, D.L. (1985) The characteristics of DNA methylation in an in vitro DNA synthesizing system from mouse fibroblasts. *Nucleic Acids Res* **13**, 2827–2842.

Guo, J.U., Su, Y., Zhong, C., Ming, G.L. & Song, H. (2011) Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. *Cell* **145**, 423–434.

Han, K. & Kim, E. (2008) Synaptic adhesion molecules and PSD-95. *Prog Neurobiol* **84**, 263–283.

Hashimshony, T., Zhang, J., Keshet, I., Bustin, M. & Cedar, H. (2003) The role of DNA methylation in setting up chromatin structure during development. *Nat Genet* **34**, 187–192.

Heim, C. & Nemeroff, C.B. (2001) The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* **49**, 1023–1039.

Hirschhorn, J.N. & Daly, M.J. (2005) Genome-wide association studies for common diseases and complex traits. *Nat Rev Genet* **6**, 95–108.

Holsboer, F. (2008) How can we realize the promise of personalized antidepressant medicines? *Nat Rev Neurosci* **9**, 638–646.

Holscher, C. (1999) Synaptic plasticity and learning and memory: LTP and beyond. *J Neurosci Res* **58**, 62–75.

Hubler, T.R. & Scammell, J.G. (2004) Intronic hormone response elements mediate regulation of FKBP5 by progestins and glucocorticoids. *Cell Stress Chaperones* **9**, 243–252.

Ichiyanagi, T., Ichiyanagi, K., Miyake, M. & Sasaki, H. (2013) Accumulation and loss of asymmetric non-CpG methylation during male germ-cell development. *Nucleic Acids Res* **41**, 738–745.

Illingworth, R.S., Gruenewald-Schneider, U., Webb, S., Kerr, A.R., James, K.D., Turner, D.J., Smith, C., Harrison, D.J., Andrews, R. & Bird, A.P. (2010) Orphan CpG islands identify numerous conserved promoters in the mammalian genome. *PLoS Genet* **6**, e1001134.

Inoue, A. & Zhang, Y. (2011) Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. *Science* **334**, 194.

Iqbal, K., Jin, S.G., Pfeifer, G.P. & Szabo, P.E. (2011) Reprogramming of the paternal genome upon fertilization involves genome-wide oxidation of 5-methylcytosine. *Proc Natl Acad Sci U S A* **108**, 3642–3647.

Issa, J.P. (2000) CpG-island methylation in aging and cancer. *Curr Top Microbiol Immunol* **249**, 101–118.

Jaenisch, R. & Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* **33** (Suppl.), 245–254.

Jin, S.G., Wu, X., Li, A.X. & Pfeifer, G.P. (2011) Genomic mapping of 5-hydroxymethylcytosine in the human brain. *Nucleic Acids Res* **39**, 5015–5024.

Jones, P.A. (1999) The DNA methylation paradox. *Trends Genet* **15**, 34–37.

Jones, P.A. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* **13**, 484–492.

Jones, P.L., Veenstra, G.J., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J. & Wolffe, A.P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* **19**, 187–191.

Kagan, B.L., Sultzer, D.L., Rosenlicht, N. & Gerner, R.H. (1990) Oral S-adenosylmethionine in depression: a randomized, double-blind, placebo-controlled trial. *Am J Psychiatry* **147**, 591–595.

Kantarjian, H., Issa, J.P., Rosenfeld, C.S., Bennett, J.M., Albitar, M., DiPersio, J., Klimek, V., Slack, J., de Castro, C., Ravandi, F., Helmer, R. III, Shen, L., Nimer, S.D., Leavitt, R., Raza, A. & Saba, H. (2006) Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. *Cancer* **106**, 1794–1803.

Kantarjian, H., Oki, Y., Garcia-Manero, G., Huang, X., O'Brien, S., Cortes, J., Faderl, S., Bueso-Ramos, C., Ravandi, F., Estrov, Z., Ferrajoli, A., Wierda, W., Shan, J., Davis, J., Giles, F., Saba, H.I. & Issa, J.P. (2007) Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood* **109**, 52–57.

Kantlehner, M., Kirchner, R., Hartmann, P., Ellwart, J.W., Alunni-Fabbroni, M. & Schumacher, A. (2011) A high-throughput DNA methylation analysis of a single cell. *Nucleic Acids Res* **39**, e44.

Kass, S.U., Landsberger, N. & Wolffe, A.P. (1997) DNA methylation directs a time-dependent repression of transcription initiation. *Curr Biol* **7**, 157–165.

Keller, S., Sarchiapone, M., Zarrilli, F. *et al.* (2010) Increased BDNF promoter methylation in the Wernicke area of suicide subjects. *Arch Gen Psychiatry* **67**, 258–267.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R. & Walters, E.E. (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* **62**, 593–602.

Kinney, S.M., Chin, H.G., Vaisvila, R., Bitinaite, J., Zheng, Y., Esteve, P.O., Feng, S., Stroud, H., Jacobsen, S.E. & Pradhan, S. (2011) Tissue-specific distribution and dynamic changes of 5-hydroxymethylcytosine in mammalian genomes. *J Biol Chem* **286**, 24685–24693.

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J.C., Pariante, C.M., Pace, T.W., Mercer, K.B., Mayberg, H.S., Bradley, B., Nemeroff, C.B., Holsboer, F., Heim, C.M., Ressler, K.J., Rein, T. & Binder, E.B. (2013) Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci* **16**, 33–41.

Ko, M., Huang, Y., Jankowska, A.M., Pape, U.J., Tahiliani, M., Bandukwala, H.S., An, J., Lamperti, E.D., Koh, K.P., Ganetzky, R., Liu, X.S., Aravind, L., Agarwal, S., Maciejewski, J.P. & Rao, A. (2010) Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* **468**, 839–843.

Kobayashi, H., Sakurai, T., Imai, M., Takahashi, N., Fukuda, A., Yayoi, O., Sato, S., Nakabayashi, K., Hata, K., Sotomaru, Y., Suzuki, Y. & Kono, T. (2012) Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. *PLoS Genet* **8**, e1002440.

Koenen, K.C. (2007) Genetics of posttraumatic stress disorder: review and recommendations for future studies. *J Trauma Stress* **20**, 737–750.

Koenen, K.C. & Uddin, M. (2010) FKBP5 polymorphisms modify the effects of childhood trauma. *Neuropsychopharmacology* **35**, 1623–1624.

Koenen, K.C., Uddin, M., Chang, S.C., Aiello, A.E., Wildman, D.E., Goldmann, E. & Galea, S. (2011) SLC6A4 methylation modifies the effect of the number of traumatic events on risk for posttraumatic stress disorder. *Depress Anxiety* **28**, 639–647.

Kornblith, A.B., Herndon, J.E., Silverman, L.R., Demakos, E.P., Odchimar-Reissig, R., Holland, J.F., Powell, B.L., DeCastro, C., Ellerton, J., Larson, R.A., Schiffer, C.A. & Holland, J.C. (2002) Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. *J Clin Oncol* **20**, 2441–2452.

Labonte, B., Suderman, M., Maussion, G., Navaro, L., Yerko, V., Mahar, I., Bureau, A., Mechawar, N., Szyf, M., Meaney, M.J. & Turecki, G. (2012) Genome-wide epigenetic regulation by early-life trauma. *Arch Gen Psychiatry* **69**, 722–731.

Ladd-Acosta, C., Pevsner, J., Sabunciyan, S., Yolken, R.H., Webster, M.J., Dinkins, T., Callinan, P.A., Fan, J.B., Potash, J.B. & Feinberg, A.P. (2007) DNA methylation signatures within the human brain. *Am J Hum Genet* **81**, 1304–1315.

Laurent, L., Wong, E., Li, G., Huynh, T., Tsirigos, A., Ong, C.T., Low, H.M., Kin Sung, K.W., Rigoutsos, I., Loring, J. & Wei, C.L. (2010) Dynamic changes in the human methylome during differentiation. *Genome Res* **20**, 320–331.

Leach, P.T., Poplawski, S.G., Kenney, J.W., Hoffman, B., Liebermann, D.A., Abel, T. & Gould, T.J. (2012) Gadd45b knockout mice exhibit selective deficits in hippocampus-dependent long-term memory. *Learn Mem* **19**, 319–324.

Lee, R.S., Tamashiro, K.L., Yang, X., Purcell, R.H., Harvey, A., Willour, V.L., Huo, Y., Rongione, M., Wand, G.S. & Potash, J.B. (2010) Chronic corticosterone exposure increases expression and decreases deoxyribonucleic acid methylation of Fkbp5 in mice. *Endocrinology* **151**, 4332–4343.

Li, W. & Liu, M. (2011) Distribution of 5-hydroxymethylcytosine in different human tissues. *J Nucleic Acids* **2011**, 870726.

Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., Nery, J.R., Lee, L., Ye, Z., Ngo, Q.M., Edsall, L., Antosiewicz-Bourget, J., Stewart, R., Ruotti, V., Millar, A.H., Thomson, J.A., Ren, B. & Ecker, J.R. (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315–322.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M. & Meaney, M.J. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277**, 1659–1662.

Lock, L.F., Takagi, N. & Martin, G.R. (1987) Methylation of the Hprt gene on the inactive X occurs after chromosome inactivation. *Cell* **48**, 39–46.

Logue, M.W., Baldwin, C., Guffanti, G., Melista, E., Wolf, E.J., Reardon, A.F., Uddin, M., Wildman, D., Galea, S., Koenen, K.C. & Miller, M.W. (2013) A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha (RORA) gene as a significant risk locus. *Mol Psychiatry* **18**, 937–942.

Ma, D.K., Chiang, C.H., Ponnusamy, K., Ming, G.L. & Song, H. (2008) G9a and Jhdm2a regulate embryonic stem cell fusion-induced reprogramming of adult neural stem cells. *Stem Cells* **26**, 2131–2141.

MacMillan, H.L., Fleming, J.E., Streiner, D.L., Lin, E., Boyle, M.H., Jamieson, E., Duku, E.K., Walsh, C.A., Wong, M.Y. & Beardslee, W.R. (2001) Childhood abuse and lifetime psychopathology in a community sample. *Am J Psychiatry* **158**, 1878–1883.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G. & Sun, Y.E. (2003) DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* **302**, 890–893.

Maunakea, A.K., Nagarajan, R.P., Bilenky, M. *et al.* (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* **466**, 253–257.

McGhee, J.D. & Felsenfeld, G. (1980) Nucleosome structure. *Annu Rev Biochem* **49**, 1115–1156.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonte, B., Szyf, M., Turecki, G. & Meaney, M.J. (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* **12**, 342–348.

Mehta, D. & Binder, E.B. (2012) Gene x environment vulnerability factors for PTSD: the HPA-axis. *Neuropharmacology* **62**, 654–662.

Meissner, A., Mikkelsen, T.S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., Zhang, X., Bernstein, B.E., Nusbaum, C., Jaffe, D.B., Gnirke, A., Jaenisch, R. & Lander, E.S. (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**, 766–770.

Mifsud, K.R., Gutierrez-Mecinas, M., Trollope, A.F., Collins, A., Saunderson, E.A. & Reul, J.M. (2011) Epigenetic mechanisms in stress and adaptation. *Brain Behav Immun* **25**, 1305–1315.

Migaud, M., Charlesworth, P., Dempster, M., Webster, L.C., Watabe, A.M., Makhinson, M., He, Y., Ramsay, M.F., Morris, R.G., Morrison, J.H., O'Dell, T.J. & Grant, S.G. (1998) Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature* **396**, 433–439.

Mill, J. & Petronis, A. (2007) Molecular studies of major depressive disorder: the epigenetic perspective. *Mol Psychiatry* **12**, 799–814.

Miller, C.A. & Sweatt, J.D. (2007) Covalent modification of DNA regulates memory formation. *Neuron* **53**, 857–869.

Moarefi, A.H. & Chedin, F. (2011) ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. *J Mol Biol* **409**, 758–772.

Momparler, R.L. (2005) Pharmacology of 5-aza-2′-deoxycytidine (decitabine). *Semin Hematol* **42**, S9–S16.

Mullen, P.E., Martin, J.L., Anderson, J.C., Romans, S.E. & Herbison, G.P. (1996) The long-term impact of the physical, emotional, and sexual abuse of children: a community study. *Child Abuse Negl* **20**, 7–21.

Murgatroyd, C., Patchev, A.V., Wu, Y., Micale, V., Bockmuhl, Y., Fischer, D., Holsboer, F., Wotjak, C.T., Almeida, O.F. & Spengler, D. (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci* **12**, 1559–1566.

Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N. & Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386–389.

Norrholm, S.D., Jovanovic, T., Smith, A.K., Binder, E., Klengel, T., Conneely, K., Mercer, K.B., Davis, J.S., Kerley, K., Winkler, J., Gillespie, C.F., Bradley, B. & Ressler, K.J. (2013) Differential genetic and epigenetic regulation of catechol-O-methyltransferase is associated with impaired fear inhibition in posttraumatic stress disorder. *Front Behav Neurosci* **7**, 30.

Ooi, S.K., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z., Erdjument-Bromage, H., Tempst, P., Lin, S.P., Allis, C.D., Cheng, X. & Bestor, T.H. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**, 714–717.

Orr, S.P., Metzger, L.J., Lasko, N.B., Macklin, M.L., Peri, T. & Pitman, R.K. (2000) De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. *J Abnorm Psychol* **109**, 290–298.

Pastor, W.A., Pape, U.J., Huang, Y., Henderson, H.R., Lister, R., Ko, M., McLoughlin, E.M., Brudno, Y., Mahapatra, S., Kapranov, P., Tahiliani, M., Daley, G.Q., Liu, X.S., Ecker, J.R., Milos, P.M., Agarwal, S. & Rao, A. (2011) Genome-wide mapping of 5-hydroxymethylcytosine in embryonic stem cells. *Nature* **473**, 394–397.

Penn, N.W., Suwalski, R., O'Riley, C., Bojanowski, K. & Yura, R. (1972) The presence of 5-hydroxymethylcytosine in animal deoxyribonucleic acid. *Biochem J* **126**, 781–790.

Perilla, J.L., Norris, F.H. & Lavizzo, E.A. (2002) Ethnicity, culture, and disaster response: identifying and explaining ethnic differences in PTSD six months after Hurricane Andrew. *J Soc Clin Psychol* **21**, 20–45.

Popp, C., Dean, W., Feng, S.H., Cokus, S.J., Andrews, S., Pellegrini, M., Jacobsen, S.E. & Reik, W. (2010) Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency. *Nature* **463**, 1101–1105.

Radtke, K.M., Ruf, M., Gunter, H.M., Dohrmann, K., Schauer, M., Meyer, A. & Elbert, T. (2011) Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Transl Psychiatry* **1**, e21.

Ramsahoye, B.H., Biniszkiewicz, D., Lyko, F., Clark, V., Bird, A.P. & Jaenisch, R. (2000) Non-CpG methylation is prevalent in embryonic stem cells and may be mediated by DNA methyltransferase 3a. *Proc Natl Acad Sci U S A* **97**, 5237–5242.

Razin, A. (1998) CpG methylation, chromatin structure and gene silencing-a three-way connection. *EMBO J* **17**, 4905–4908.

Regland, B., Johansson, B.V. & Gottfries, C.G. (1994) Homocysteinemia and schizophrenia as a case of methylation deficiency. *J Neural Transm Gen Sect* **98**, 143–152.

Reik, W. & Walter, J. (2001) Evolution of imprinting mechanisms: the battle of the sexes begins in the zygote. *Nat Genet* **27**, 255–256.

Renthal, W., Maze, I., Krishnan, V., Covington, H.E. III, Xiao, G., Kumar, A., Russo, S.J., Graham, A., Tsankova, N., Kippin, T.E., Kerstetter, K.A., Neve, R.L., Haggarty, S.J., McKinsey, T.A., Bassel-Duby, R., Olson, E.N. & Nestler, E.J. (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* **56**, 517–529.

Ressler, K.J., Mercer, K.B., Bradley, B., Jovanovic, T., Mahan, A., Kerley, K., Norrholm, S.D., Kilaru, V., Smith, A.K., Myers, A.J., Ramirez, M., Engel, A., Hammack, S.E., Toufexis, D., Braas, K.M., Binder, E.B. & May, V. (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* **470**, 492–497.

Rideout, W.M. III, Coetzee, G.A., Olumi, A.F. & Jones, P.A. (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. *Science* **249**, 1288–1290.

Risch, N. & Merikangas, K. (1996) The future of genetic studies of complex human diseases. *Science* **273**, 1516–1517.
Roberts, A.L., Gilman, S.E., Breslau, J., Breslau, N. & Koenen, K.C. (2011) Race/ethnic differences in exposure to traumatic events, development of post-traumatic stress disorder, and treatment-seeking for post-traumatic stress disorder in the United States. *Psychol Med* **41**, 71–83.
Roth, T.L., Zoladz, P.R., Sweatt, J.D. & Diamond, D.M. (2011) Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. *J Psychiatr Res* **45**, 919–926.
Rudek, M.A., Zhao, M., He, P., Hartke, C., Gilbert, J., Gore, S.D., Carducci, M.A. & Baker, S.D. (2005) Pharmacokinetics of 5-azacitidine administered with phenylbutyrate in patients with refractory solid tumors or hematologic malignancies. *J Clin Oncol* **23**, 3906–3911.
Rusiecki, J.A., Chen, L., Srikantan, V., Zhang, L., Yan, L., Polin, M.L. & Baccarelli, A. (2012) DNA methylation in repetitive elements and post-traumatic stress disorder: a case-control study of US military service members. *Epigenomics* **4**, 29–40.
Russo, V.E.A., Martienssen, R.A. & Riggs, A.D. (1996) *Epigenetic mechanisms of gene regulation*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Salomon, R. & Kaye, A.M. (1970) Methylation of mouse DNA in vivo: di- and tripyrimidine sequences containing 5-methylcytosine. *Biochim Biophys Acta* **204**, 340–351.
Sasaki, H. & Matsui, Y. (2008) Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nat Rev Genet* **9**, 129–140.
Segman, R.H., Shefi, N., Goltser-Dubner, T., Friedman, N., Kaminski, N. & Shalev, A.Y. (2005) Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol Psychiatry* **10**, 500–513 425.
Shirane, K., Toh, H., Kobayashi, H., Miura, F., Chiba, H., Ito, T., Kono, T. & Sasaki, H. (2013) Mouse oocyte methylomes at base resolution reveal genome-wide accumulation of non-CpG methylation and role of DNA methyltransferases. *PLoS Genet* **9**, e1003439.
Silverman, L.R., Demakos, E.P., Peterson, B.L., Kornblith, A.B., Holland, J.C., Odchimar-Reissig, R., Stone, R.M., Nelson, D., Powell, B.L., DeCastro, C.M., Ellerton, J., Larson, R.A., Schiffer, C.A. & Holland, J.F. (2002) Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol* **20**, 2429–2440.
Silverman, M.N., Pearce, B.D., Biron, C.A. & Miller, A.H. (2005) Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. *Viral Immunol* **18**, 41–78.
Silverman, L.R., McKenzie, D.R., Peterson, B.L., Holland, J.F., Backstrom, J.T., Beach, C.L. & Larson, R.A. (2006) Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. *J Clin Oncol* **24**, 3895–3903.
Simonini, M.V., Camargo, L.M., Dong, E., Maloku, E., Veldic, M., Costa, E. & Guidotti, A. (2006) The benzamide MS-275 is a potent, long-lasting brain region-selective inhibitor of histone deacetylases. *Proc Natl Acad Sci U S A* **103**, 1587–1592.
Sirianni, N., Naidu, S., Pereira, J., Pillotto, R.F. & Hoffman, E.P. (1998) Rett syndrome: confirmation of X-linked dominant inheritance, and localization of the gene to Xq28. *Am J Hum Genet* **63**, 1552–1558.
Skelton, K., Ressler, K.J., Norrholm, S.D., Jovanovic, T. & Bradley-Davino, B. (2012) PTSD and gene variants: new pathways and new thinking. *Neuropharmacology* **62**, 628–637.
Smith, A.E. & Ford, K.G. (2007) Specific targeting of cytosine methylation to DNA sequences in vivo. *Nucleic Acids Res* **35**, 740–754.
Smith, A.K., Dimulescu, I., Falkenberg, V.R., Narasimhan, S., Heim, C., Vernon, S.D. & Rajeevan, M.S. (2008) Genetic evaluation of the serotonergic system in chronic fatigue syndrome. *Psychoneuroendocrinology* **33**, 188–197.
Smith, A.K., Conneely, K.N., Kilaru, V., Mercer, K.B., Weiss, T.E., Bradley, B., Tang, Y., Gillespie, C.F., Cubells, J.F. & Ressler, K.J. (2011) Differential immune system DNA methylation and cytokine regulation in post-traumatic stress disorder. *Am J Med Genet B Neuropsychiatr Genet* **156B**, 700–708.
Song, C.X., Szulwach, K.E., Fu, Y., Dai, Q., Yi, C., Li, X., Li, Y., Chen, C.H., Zhang, W., Jian, X., Wang, J., Zhang, L., Looney, T.J., Zhang, B., Godley, L.A., Hicks, L.M., Lahn, B.T., Jin, P. & He, C. (2011) Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. *Nat Biotechnol* **29**, 68–72.
Stadler, M.B., Murr, R., Burger, L., Ivanek, R., Lienert, F., Scholer, A., van Nimwegen, E., Wirbelauer, C., Oakeley, E.J., Gaidatzis, D., Tiwari, V.K. & Schubeler, D. (2011) DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* **480**, 490–495.
Stahl, S.M. (2007) Novel therapeutics for schizophrenia: targeting glycine modulation of NMDA glutamate receptors. *CNS Spectr* **12**, 423–427.
Stahl, S.M. (2010) Fooling mother nature: epigenetics and novel treatments for psychiatric disorders. *CNS Spectr* **15**, 358–365.
Sternberg, E.M. (2006) Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol* **6**, 318–328.
Strathdee, G. & Brown, R. (2002) Aberrant DNA methylation in cancer: potential clinical interventions. *Expert Rev Mol Med* **4**, 1–17.
Sultan, F.A., Wang, J., Tront, J., Liebermann, D.A. & Sweatt, J.D. (2012) Genetic deletion of Gadd45b, a regulator of active DNA demethylation, enhances long-term memory and synaptic plasticity. *J Neurosci* **32**, 17059–17066.
Suzuki, M.M. & Bird, A. (2008) DNA methylation landscapes: provocative insights from epigenomics. *Nat Rev Genet* **9**, 465–476.
Szyf, M. (2009) Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu Rev Pharmacol Toxicol* **49**, 243–263.
Szyf, M., Weaver, I.C., Champagne, F.A., Diorio, J. & Meaney, M.J. (2005) Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol* **26**, 139–162.
Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L. & Rao, A. (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930–935.
Taylor, S.M. & Jones, P.A. (1985) Cellular differentiation. *Int J Obes* **9** (Suppl. 1), 15–21.
Tremolizzo, L., Carboni, G., Ruzicka, W.B., Mitchell, C.P., Sugaya, I., Tueting, P., Sharma, R., Grayson, D.R., Costa, E. & Guidotti, A. (2002) An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. *Proc Natl Acad Sci U S A* **99**, 17095–17100.
Tsankova, N.M., Berton, O., Renthal, W., Kumar, A., Neve, R.L. & Nestler, E.J. (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci* **9**, 519–525.
Turek-Plewa, J. & Jagodzinski, P.P. (2005) The role of mammalian DNA methyltransferases in the regulation of gene expression. *Cell Mol Biol Lett* **10**, 631–647.
Turner, B.M. (2002) Cellular memory and the histone code. *Cell* **111**, 285–291.
Uddin, M., Aiello, A.E., Wildman, D.E., Koenen, K.C., Pawelec, G., de Los, S.R., Goldmann, E. & Galea, S. (2010) Epigenetic and immune function profiles associated with posttraumatic stress disorder. *Proc Natl Acad Sci U S A* **107**, 9470–9475.
Valinluck, V. & Sowers, L.C. (2007) Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. *Cancer Res* **67**, 946–950.
Venolia, L. & Gartler, S.M. (1983) Comparison of transformation efficiency of human active and inactive X-chromosomal DNA. *Nature* **302**, 82–83.

Venter, J.C., Adams, M.D., Myers, E.W. *et al.* (2001) The sequence of the human genome. *Science* **291**, 1304–1351.
Vermetten, E. & Bremner, J.D. (2002) Circuits and systems in stress. II. Applications to neurobiology and treatment in posttraumatic stress disorder. *Depress Anxiety* **16**, 14–38.
Weaver, I.C., Szyf, M. & Meaney, M.J. (2002) From maternal care to gene expression: DNA methylation and the maternal programming of stress responses. *Endocr Res* **28**, 699.
Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M. & Meaney, M.J. (2004) Epigenetic programming by maternal behavior. *Nat Neurosci* **7**, 847–854.
Weaver, I.C., Champagne, F.A., Brown, S.E., Dymov, S., Sharma, S., Meaney, M.J. & Szyf, M. (2005) Reversal of maternal programming of stress responses in adult offspring through methyl supplementation: altering epigenetic marking later in life. *J Neurosci* **25**, 11045–11054.
Weaver, I.C., Meaney, M.J. & Szyf, M. (2006) Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A* **103**, 3480–3485.
Weiss, E.L., Longhurst, J.G. & Mazure, C.M. (1999) Childhood sexual abuse as a risk factor for depression in women: psychosocial and neurobiological correlates. *Am J Psychiatry* **156**, 816–828.
Wolf, S.F., Jolly, D.J., Lunnen, K.D., Friedmann, T. & Migeon, B.R. (1984) Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: implications for X-chromosome inactivation. *Proc Natl Acad Sci U S A* **81**, 2806–2810.
Wong, C.M. (2002) Post-traumatic stress disorder: advances in psychoneuroimmunology. *Psychiatr Clin North Am* **25**, 369–383 vii.
Xie, S., Wang, Z., Okano, M., Nogami, M., Li, Y., He, W.W., Okumura, K. & Li, E. (1999) Cloning, expression and chromosome locations of the human DNMT3 gene family. *Gene* **236**, 87–95.
Xie, W., Barr, C.L., Kim, A., Yue, F., Lee, A.Y., Eubanks, J., Dempster, E.L. & Ren, B. (2012) Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. *Cell* **148**, 816–831.
Xie, P., Kranzler, H.R., Yang, C., Zhao, H., Farrer, L.A. & Gelernter, J. (2013) Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. *Biol Psychiatry* **74**, 656–663.
Yang, X., Ewald, E.R., Huo, Y., Tamashiro, K.L., Salvatori, R., Sawa, A., Wand, G.S. & Lee, R.S. (2012) Glucocorticoid-induced loss of DNA methylation in non-neuronal cells and potential involvement of DNMT1 in epigenetic regulation of Fkbp5. *Biochem Biophys Res Commun* **420**, 570–575.
Yehuda, R. & Bierer, L.M. (2009) The relevance of epigenetics to PTSD: implications for the DSM-V. *J Trauma Stress* **22**, 427–434.
Yehuda, R. & LeDoux, J. (2007) Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron* **56**, 19–32.
Yehuda, R., Flory, J.D., Pratchett, L.C., Buxbaum, J., Ising, M. & Holsboer, F. (2010) Putative biological mechanisms for the association between early life adversity and the subsequent development of PTSD. *Psychopharmacology (Berl)* **212**, 405–417.
Yoder, J.A., Walsh, C.P. & Bestor, T.H. (1997) Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* **13**, 335–340.
Zhang, Y., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Bird, A. & Reinberg, D. (1999) Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev* **13**, 1924–1935.
Zieker, J., Zieker, D., Jatzko, A., Dietzsch, J., Nieselt, K., Schmitt, A., Bertsch, T., Fassbender, K., Spanagel, R., Northoff, H. & Gebicke-Haerter, P.J. (2007) Differential gene expression in peripheral blood of patients suffering from post-traumatic stress disorder. *Mol Psychiatry* **12**, 116–118.

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