

## **Childhood maltreatment and stress-related psychopathology: the epigenetic memory hypothesis**

Pierre-Eric Lutz, Daniel Almeida, Laura M. Fiori and Gustavo Turecki

Affiliation: McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, 6875 LaSalle Boulevard, Verdun, Quebec, Canada, H4H 1R3

Corresponding author: Gustavo Turecki

Email address: Gustavo.Turecki@mcgill.ca

Tel.: +1 514 761 6131 (ext.3311), Fax: +1 514 762 3023

### **Abstract**

Childhood maltreatment (CM) is all too frequent among western societies, with an estimated prevalence of 10 to 15%. CM associates with increased risk of several psychiatric disorders, and therefore represents a worrying public and socio-economic burden. While associated clinical outcomes are well characterized, determining by which mechanisms early-life adverse experiences affect mental health over the lifespan is a major challenge. Epigenetic mechanisms, in particular DNA methylation, represent a form of molecular memory that may modify brain function over extended periods of time, as well as serve as a bio-marker of behavioral phenotypes associated with CM. Here, we review human studies suggesting that DNA methylation is a crucial substrate mediating neurobiological consequences of CM throughout life, thereby potentiating maladaptive behavioral patterns and psychopathological risk.

### **Keywords**

DNA methylation; epigenetics; early-life adversity; childhood maltreatment; stress

## **Abbreviations**

ASPD, antisocial personality disorder

ALS2, amyotrophic lateral sclerosis 2

BDNF, brain-derived neurotrophic factor

BPD, borderline personality disorder

CM, childhood maltreatment

FAM172A, family with sequence similarity 172, member A (also known as C5orf21)

GR, glucocorticoid receptor

GRIN1, Glutamate Receptor, Ionotropic N-methyl-D-aspartate (NMDA) 1

HPA axis, hypothalamic-pituitary-adrenal axis

HIST2H2AB, histone cluster 2, H2ab

ID3, DNA Binding Protein Inhibitor ID-3

MAOA, monoamine oxidase A

NR1D1, nuclear receptor subfamily 1, group D, member 1

PET, positron emission tomography

PKN1, protein kinase N1

SLC6A4, serotonin transporter

SNP, single nucleotide polymorphisms

TPPP, Tubulin Polymerization Promoting Protein

5-HT, serotonin

## Introduction

Children in our society are all too often subjected to maltreatment, which is a global problem of significant proportion affecting children of all races and socio-economic backgrounds [1]. It is estimated that over 30% of adult psychopathology is directly related to childhood maltreatment (CM), including but not limited to parental neglect, physical, sexual, and psychological abuse [2-4]. In extension to predicting psychiatric illness, CM has been associated with chronic forms of these pathologies, poor clinical course, greater incidence of hospitalization, increased comorbidity and reduced responsiveness to pharmacotherapy [5-8].

An effort to gain insight into the mechanisms through which CM interacts with an individual's biological make-up to trigger psychopathology has pointed towards the emerging field of behavioral epigenetics. Epigenetic modifications refer to the collective chemical and physical alterations of the genome that regulate the activity of genes in a time- and cell-dependent manner [9]. One of the most studied epigenetic marks is DNA methylation, which mostly refers to the covalent addition of a methyl group to cytosines, often followed by a guanine (CpG dinucleotide). Methylation of cytosine has been associated with a variety of molecular events (including the modulation of DNA binding by transcription factors, or the recruitment of methyl-CpG binding proteins), and classically leads to chromatin condensation and transcriptional repression [10]. A majority of studies to date have focused on methylation of CpG sites in gene promoter regions. For additional information on molecular mechanisms of DNA methylation, as well as for methodological considerations, we refer the reader to recent exhaustive reviews [11-13].

A major challenge in the field of CM is to understand how early-life experiences can affect behavior and mental health outcomes over the lifespan. Epigenetic mechanisms, in particular DNA methylation, represent a form of cerebral plasticity that has the potential to modify gene expression

over extended periods of time. Furthermore, while epigenetic processes may potentially occur throughout life, it has been proposed that early development represents a particularly sensitive period to epigenetic modification of the genome [14]. Within this framework, the present mini-review will emphasize the biological memory of CM embedded into the genome and its relation to stress related psychopathology [15-17]; what we refer to as the epigenetic memory hypothesis. We will focus our analysis on DNA methylation, by far the most studied epigenetic substrate linking early-life events with lifelong mental health.

### **HPA Axis**

The most extensively investigated and reviewed gene in epigenetic studies of CM appears to be the glucocorticoid receptor (GR) [18]. Much of excitement surrounding this system arose from i) animal findings pointing towards differential DNA methylation as a result of early-life variations in maternal care [19], as well as ii) dysfunctional hypothalamic-pituitary-adrenal (HPA) axis responses to stress in humans with a history of CM [20, 21]. Building on these findings, our group was the first to provide evidence for an interaction between early-life adversity and the human epigenome [22]. Briefly, DNA methylation of 2 CpG sites in the exon 1<sub>F</sub> promoter region of the GR gene was increased in suicide completers with a history of CM as compared to non-abused suicidees, as well as healthy controls. At the transcriptomic level, this resulted in reduced GR expression. Additional *in vitro* experiments focused on the promoter region where differential DNA methylation occurred as a function of CM. Results showed that low DNA methylation in this region leads to decreased transcriptional activation by the transcription factor NGFI-A, as well as decreased DNA occupancy by NGFI-A, strongly suggesting a molecular mechanism whereby CM may program GR expression.

Following this initial study, increased DNA methylation in the GR gene as a result of CM has been consistently associated with altered GR expression or stress reactivity across a variety of behavioral phenotypes (see [23-26] for recent reviews). Furthermore, consistent results have been obtained in both brain and peripheral tissues, thus establishing GR as a promising candidate gene for use as a biomarker of CM [26].

In addition to studies focusing exclusively on GR, epigenetic associations between GR and the monoamine oxidase A (MAOA) gene were assessed in a Swedish cohort of depressed subjects with a history of early life adversity [27]. MAOA is of particular importance in psychiatric disorders as it plays a key role in the degradation of neurotransmitters such as noradrenaline, dopamine and serotonin (5-HT). One specific type of adversity, namely early parental death, was associated with hypermethylation of the NR3C1 gene proximal to an NGFI-A binding site, as assessed in saliva samples. A regression analysis revealed that this association may be mediated by a well-characterized genetic polymorphism in the MAOA promoter, therefore suggesting the involvement of both gene x gene and gene x environment interactions.

Activity of the HPA axis is tightly controlled by multiple regulatory mechanisms. At the intracellular level, FKBP5 represents a negative feedback loop: the expression of FKBP5 is stimulated by GR, while this protein functionally inhibits GR signaling. Independent groups have identified an interaction between several FKBP5 single nucleotide polymorphisms (SNPs) and a history of CM in PTSD, depression, and suicide attempts [28-30]. Recently, a functional SNP has been identified at a glucocorticoid response element in the FKBP5 intron 2 that moderates the relationship between CM and adult PTSD [31]. Interestingly, individuals carrying the risk allele in intron 2, and with a history of CM, were shown to display decreased methylation in FKBP5 intron 7. Furthermore, intron 7 DNA methylation appeared to be regulated by glucocorticoid levels, and influenced FKBP5 activity.

Altogether, several genes and neuronal pathways are likely epigenetically reprogrammed by CM to alter HPA axis and stress responses over the lifespan.

### **Serotonin**

The serotonergic system has been extensively investigated in its relation to early-life induced epigenetic regulation and psychiatric disorders. The most highly investigated gene being the serotonin transporter (SLC6A4), involved in neurotransmitter reuptake at serotonergic synapses. Early evidence has pointed to an interaction between 5-HTTLPR, a functional polymorphism in the promoter region of the SLC6A4 gene, and psychosocial stressors in conferring a risk for depression [32]. Although some later studies failed to replicate previous findings of genome x environment interactions between the SLC6A4 gene, adversity, and depression [33], there is still considerable support for this relationship [34]. In a recent series of investigations performed in the Iowa Adoptee Sample, methylation of the promoter region of the SLC6A4 gene was linked to a family history of CM, including physical and sexual abuse [35]. While no significant association emerged between specific CpG loci methylation and CM, overall DNA methylation was significantly increased across the promoter region for abused, in comparison to non-abused, males. This was in contrast to females, where two loci were found significantly hyper-methylated for those who had experienced CM. Replicating prior findings in a non-overlapping cohort of females, the authors validated that CM still associated with higher levels of SLC6A4 methylation [36]. Furthermore, in subsequent studies, epigenetic patterns of this gene were suggested to represent a mechanism linking CM to antisocial personality disorder (ASPD) symptoms. The same group of investigators discovered an interaction between parental history of psychopathology and CM as a predictor of the intensity of SLC6A4 DNA methylation and the risk of ASPD symptomology [37]. Finally, the authors aimed at determining how DNA methylation impacts

gene expression, and whether other non-promoter regions of the SLC6A4 gene served as additional candidates for gene x environment interactions [38]. The analysis identified two methylation sites that associated with variant-specific expression, as well as one site that associated with global SLC6A4 expression. Given the above studies, the relevance of SLC6A4 methylation on pre-treatment characteristics, and treatment outcome of depression are brought into question. Kang *et al.* [39] determined that SLC6A4 promoter DNA methylation status as a function of CM was significantly associated with worse pre-treatment clinical presentation of depression, including severer symptomology, higher perceived stress, and an increased family history of psychopathology. Interestingly, the type of abuse (physical versus sexual) determined methylation site, while higher methylation at specific sites predicted both higher scores on tests of disability and lower scores on quality of life assessments. These data therefore suggest the possibility of interactive relationships between the type of childhood adversity, site of methylation, and clinical outcome.

Additional studies have focused on addressing the role of SLC6A4 DNA methylation and in vivo measures of serotonin synthesis (using positron emission tomography) in childhood physical aggression. Wang and colleagues [40] followed a longitudinal sample of adult males who showed significant levels of high childhood-limited aggression (C-LHPA), a phenotype associated with early-life adversity, and compared methylation patterns in T cells and monocytes of these males to controls. The authors found increased methylation at 4 CpG sites in C-LHPA, which negatively correlated with in vivo levels of brain serotonin synthesis in the orbitofrontal cortex.

## **BDNF**

There is evidence to support an interaction between 5-HT and brain-derived neurotrophic factor (BDNF) in guiding the development and plasticity of neurocircuits that regulate affective behaviors

[41]. In a large population-based cohort study [42], two polymorphisms in the SLC6A4 and BDNF genes (5-HTTLPR and Val66Met, respectively) interacted with an unfavourable early environment to predict depressive symptomology. Depressive symptomology was most common in carriers of either the ll + Met or the ss/sl + Val/Val genotypes in the presence of a history of early-life adversity.

BDNF may also play an important role in energy homeostasis and food intake, thus representing a theoretically supported candidate gene in the pathophysiology of eating-disorders [43]. In a recent study by Thaler *et al.* [44], a significant increase in methylation at specific CpG sites of the BDNF promoter was observed in a bulimia nervosa group as compared to normal eaters. A significant interaction effect was also observed between borderline personality disorder (BPD), CM, and methylation levels of the BDNF gene. Similar interactions were observed between bulimia nervosa, BPD and DNA methylation for the dopamine D2 receptor [45], suggesting that several neuronal networks may be affected. While methylation levels did not differ between bulimia and normal eaters, there was a slight increase in methylation of the D2 receptor in bulimics with BPD, as well as those who reported a history of childhood sexual abuse. Further investigating the BPD phenotype, Perroud *et al.* [46] explored the effects of psychotherapy on BDNF methylation levels. Prior to treatment, BPD subjects had a higher baseline BDNF methylation status as compared to controls. In addition, a positive correlation was observed between the incidence rate of childhood trauma and methylation status in the BPD patients. BDNF methylation status was reassessed following a 4-week course of intensive dialectical behavior therapy, during which responders showed a decrease in methylation status over time. Additional studies will be necessary to further assess the appealing possibility that, in addition to pharmacotherapies and life experiences, psychotherapy might also associate with epigenetic plasticity.



## Genome-wide studies

While hypothesis-driven approaches provide insight into the role that specific genes play in psychopathology, epigenetic reprogramming as a result of early-life adversity may occur on a much larger scale. As such, genome-wide studies seem better equipped to investigate disorders of complex genetic and epigenetic heterogeneity. Such methodological approaches allow for a more comprehensive overview of the molecular pathways potentially involved, and of the relationships between specific methylation sites and nearby genomic regions.

Moving away from a GR centered locus, our group recently broadened our explorations in the human brain hippocampus to a 6.5 million base pair region surrounding the GR [47], and to the genome-wide level [48] (using immuno-precipitation of methylated DNA and hybridization to custom-designed promoter arrays, MeDIP-chip). The latter study identified 362 sites that were differentially methylated in suicide completers with a history of CM compared to psychiatrically normal controls, 248 of which were hypermethylated and 114 hypomethylated [48]. Differentially methylated sites followed a non-random distribution and were clustered in specific genomic regions, suggesting wide reprogramming of the epigenome. Furthermore, fluorescence-assisted cell sorting using an antibody raised against the neuron specific protein Neu-N, allowed us to separate neuronal versus non-neuronal nuclear fractions, and revealed that most CM-associated DNA methylation changes occurred in neurons [48]. Highlighted sites included genes involved in neuronal plasticity: histone cluster 2, H2ab (*HIST2H2AB*); nuclear receptor subfamily 1, group D, member 1 (*NR1D1*); and amyotrophic lateral sclerosis 2 (*ALS2*), with DNA methylation level in the *ALS2* promoter region displaying functional effects on gene expression *in vitro*. Thus, these studies provide additional evidence supporting the hypothesis that CM leads to functionally relevant, genome-wide and cell-type specific reprogramming of the epigenome.

In addition to post-mortem brain analyses, researchers have also assessed epigenetic consequences of CM in peripheral samples. One such study characterized methylation patterns in peripheral blood samples of 14 institutionalized and 14 children raised by their biological parents [49]. Of the 26,214 sites tested (using the Infinium 27 array), 914 were differentially methylated in the two groups. These differences were mostly due to increased DNA methylation in the genomes of institutionalized children. In another genome-wide study [50], salivary specimens were analyzed in 96 maltreated children who were removed from their parents due to neglect or CM, in comparison to 96 matched controls. The analysis found 2868 CpG sites that showed significantly different methylation values between maltreated children and controls. The next level of inquiry then, is to question whether whole-genome epigenetic reprogramming in response to CM could predict dimensional ratings of childhood psychiatric disorders. Weder *et al.* [51] aimed to address this difficult question using saliva-derived DNA in 94 maltreated and 96 healthy non-traumatized controls, while also assessing child depressive symptomology via the Mood and Feelings Questionnaire (MFQ). Methylation values at CpG sites in 3 genes significantly predicted depression scores, beyond the effects of CM: DNA Binding Protein Inhibitor ID-3 (*ID3*); Glutamate Receptor, Ionotropic N-methyl-D-aspartate (NMDA) 1 (*GRIN1*); and Tubulin Polymerization Promoting Protein (*TPPP*). In a follow-up analysis to investigate the impact of CM on DNA methylation of these 3 genes, all main effects reached significance while no significant interaction was observed.

While these studies have focused primarily on effects of CM on epigenetic programming early-on in life, other genome-wide studies have performed similar investigations at latter points in development. In the study by Essex *et al.*, DNA methylation was investigated in buccal epithelial cells obtained from adolescents whose parents themselves experienced high levels of stress earlier in their child's life [52]. Differential methylation was observed as a function of parental stress, child's sex, and

maternal versus paternal stress. Specifically, maternal and paternal stress resulted in 139 and 31 differentially methylated CpG sites, respectively. Parental stress resulted in differential methylation of the protein kinase N1 (PKN1) gene promoter in boys, and the FAM172A (family with sequence similarity 172, member A; also known as C5orf21) gene promoter in girls. In another study by Suderman *et al.*, methylation profiles of 40 males in a 1958 British Birth cohort were compared between those with and without a history of CM [53]. 20,000 genes and 489 microRNAs were analyzed by MeDIP-chip. The result of their investigation revealed 997 differentially methylated gene promoters, 311 of which were hyper-methylated and 686 hypo-methylated. Functionally, these genes have implications in cell signaling cascades related to development and transcriptional regulation. Abuse-associated differential methylation was found in 39 microRNA genes; among these, hyper-methylation in 6 microRNA genes associated with hypo-methylation in downstream gene targets. Lastly, the metalloproteinase gene (PM20D1) displayed abuse-associated differential methylation that withstood validation and replication in an additional 27 males.

Because epigenetic modifications as a result of CM could disrupt complex physiological systems involved in stress regulation and behavioral regulation, researchers also explored epigenetic patterns in PTSD populations. In the study by Mehta *et al.* [54], expression microarray profiles revealed a total of 303 transcripts differentially expressed between PTSD subjects with a history of CM and controls. Meanwhile, 244 transcripts showed differential expression between PTSD subjects lacking a history of CM and control samples. Interestingly, an overlap of only 14 transcripts (2%) was found between these 2 gene lists, suggesting dissociable biomarkers for the PTSD phenotype in the presence and absence of a history of CM. Illumina 450K arrays were then used to assess whether gene expression changes associated with DNA methylation differences. At least one CpG site was differentially methylated in 69.3% of the transcripts specific to PTSD with a history of CM, while only

33.6% of the transcripts specific to PTSD without CM satisfied this requirement. When the criteria selectivity was increased to methylation of 5 or more CpG sites, the difference was accentuated to 11.7% compared with 0.8%. These results suggest that distinct biological pathways may be perturbed in clinical PTSD populations with and without a history of CM, with epigenetic adaptations being more prominent in the group of CM victims.

Finally, CM-induced epigenetic modifications have also started being addressed in the context of addictive disorders, and as a function of ethnicity. Zhang *et al.* performed a methylome analysis in African and European Americans with or without a history of CM, and with or without a diagnosis of alcohol dependence [55]. In European Americans, CM resulted in several sites of increased DNA methylation in both alcohol dependent and non-dependent subjects that had a history of CM. In African Americans, CM-associated DNA methylation changes in dependent patients were not observed in non-dependent controls, suggesting interactive effects between ethnicity, clinical status, and epigenetic plasticity.

Altogether, recent data suggest that CM-induced DNA methylation changes at specific genes, in both peripheral and brain tissues, have major physiological implications for stress regulation, neural plasticity, and neurodevelopment.

### **Future directions**

While the present review has attempted to highlight available human data indicating potential mechanisms through which the epigenome may represent a long lasting imprint of childhood adversity, the complexities of biological processes mediating this relationship have only begun to be unraveled. Several lines of converging evidence direct us towards DNA methylation as a potential epigenetic mechanism through which CM may exert long lasting effects on gene expression and

human behavior. Nevertheless, this rapidly evolving field of investigation faces numerous key methodological limitations and challenges. Among these, we need to better understand at the molecular level how exposure to adversity results in differential methylation. Within this line, animal models should be instrumental in determining which epigenetic reprogramming events truly contribute to the emergence of behavioral dysregulation, or merely represent epiphenomenons (i.e. the difficult question of causality).

Researchers will also have to determine whether or not different forms of maltreatment trigger specific methylation patterns and neurobiological alterations [56], an issue that remains debated. Developmental timing [57] and cell-specificity [58] of CM-induced DNA methylation adaptations remain also largely unknown. In fact, there is some evidence that the timing of CM might be important in determining the severity of epigenetic alterations [14]. It is possible that abuse occurring earlier in development might lead to severer consequences, suggesting that there may be a critical period where CM exerts its greatest effects on the human epigenome [59]. As such, researchers have begun to incorporate epigenetics assessments into longitudinal studies, so as to tap into the developmental course of CM on the epigenome. Another major consideration is whether genome-wide epigenetic consequences of CM in peripheral tissue are reflective of plastic changes in the CNS [60-63]. Advances in cell sorting methods, as well as DNA collection from multiple tissue sources will allow us to gain a more comprehensive overview of CM-dependent epigenetic memory [58], at the “body-wide” level. Finally, studies should standardize methodologies to improve comparison of findings and facilitate multicenter initiatives.

In conclusion, we summarized in the present review our current knowledge on epigenetic mechanisms mediating lifelong consequences of early-life adversity in human. As the understanding of molecular epigenetic processes is rapidly progressing, future studies will likely provide deeper

insight on the crucial role of DNA methylation, in combination with other epigenetic marks and mechanisms, at the interface of genes, life experiences, behavioral regulation and mental health.

## References

- [1] Gilbert R, Widom CS, Browne K, Fergusson D, Webb E, Janson S. Burden and consequences of child maltreatment in high-income countries. *Lancet*, 2009; 373: 68-81.
- [2] Afifi TO, Enns MW, Cox BJ, Asmundson GJ, Stein MB, Sareen J. Population attributable fractions of psychiatric disorders and suicide ideation and attempts associated with adverse childhood experiences. *Am J Public Health*, 2008; 98: 946-52.
- [3] Green JG, McLaughlin KA, Berglund PA, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Arch Gen Psychiatry*, 2010; 67: 113-23.
- [4] McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM, Kessler RC. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication II: associations with persistence of DSM-IV disorders. *Arch Gen Psychiatry*, 2010; 67: 124-32.
- [5] Tyrka AR, Burgers DE, Philip NS, Price LH, Carpenter LL. The neurobiological correlates of childhood adversity and implications for treatment. *Acta Psychiatr Scand*, 2013; 128: 434-47.
- [6] Tunnard C, Rane LJ, Wooderson SC, Markopoulou K, Poon L, Fekadu A, Juruena M, Cleare AJ. The impact of childhood adversity on suicidality and clinical course in treatment-resistant depression. *J Affect Disord*, 2014; 152-154: 122-30.
- [7] Klein DN, Arnow BA, Barkin JL, Dowling F, Kocsis JH, Leon AC, Manber R, Rothbaum BO, Trivedi MH, Wisniewski SR. Early adversity in chronic depression: clinical correlates and response to pharmacotherapy. *Depress Anxiety*, 2009; 26: 701-10.
- [8] Du Rocher Schudlich T, Youngstrom EA, Martinez M, KogosYoungstrom J, Scovil K, Ross J, Feeny NC, Findling RL. Physical and Sexual Abuse and Early-Onset Bipolar Disorder in Youths Receiving Outpatient Services: Frequent, but Not Specific. *J Abnorm Child Psychol*, 2014.
- [9] Tsankova N, Renthal W, Kumar A, Nestler EJ. Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci*, 2007; 8: 355-67.
- [10] Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci*, 2006; 31: 89-97.
- [11] Bock C. Analysing and interpreting DNA methylation data. *Nat Rev Genet*, 2012; 13: 705-19.
- [12] Plongthongkum N, Diep DH, Zhang K. Advances in the profiling of DNA modifications: cytosine methylation and beyond. *Nat Rev Genet*, 2014; 15: 647-61.
- [13] Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet*, 2012; 13: 484-92.
- [14] Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol*, 2012; 233: 102-11.
- [15] Hertzman C. Putting the concept of biological embedding in historical perspective. *Proc Natl Acad Sci U S A*, 2012; 109 Suppl 2: 17160-7.
- [16] Lutz PE, Turecki G. DNA methylation and childhood maltreatment: from animal models to human studies. *Neuroscience*, 2013; *In press*.
- [17] Zhou Y, Lutz PE, Turecki G. Epigenetics of Suicidal Behavior. In *Advancing the Science of Suicidal Behavior: Understanding and Intervention*, Nova Science Publishers, In press.
- [18] Zhang TY, Labonte B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology*, 2013; 38: 111-23.
- [19] Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci*, 2004; 7: 847-54.
- [20] Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH, Nemeroff CB. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA*, 2000; 284: 592-7.

- [21] Frodl T, O'Keane V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiol Dis*, 2013; 52: 24-37.
- [22] McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci*, 2009; 12: 342-8.
- [23] Labonte B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, Turecki G. Differential glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide completers with a history of childhood abuse. *Biol Psychiatry*, 2012; 72: 41-8.
- [24] Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS One*, 2012; 7: e30148.
- [25] Perroud N, Paoloni-Giacobino A, Prada P, Olie E, Salzmann A, Nicastro R, Guillaume S, Mouthon D, Stouder C, Dieben K, Huguelet P, Courtet P, Malafosse A. Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl Psychiatry*, 2011; 1: e59.
- [26] Turecki G. The epigenetic basis of behavioral phenotypes: is there reason for continued optimism? *Depress Anxiety*, 2013; 30: 1147-50.
- [27] Melas PA, Wei Y, Wong CC, Sjöholm LK, Aberg E, Mill J, Schalling M, Forsell Y, Lavebratt C. Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *Int J Neuropsychopharmacol*, 2013; 16: 1513-28.
- [28] Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, Tang Y, Gillespie CF, Heim CM, Nemeroff CB, Schwartz AC, Cubells JF, Ressler KJ. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA*, 2008; 299: 1291-305.
- [29] Roy A, Gorodetsky E, Yuan Q, Goldman D, Enoch MA. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology*, 2010; 35: 1674-83.
- [30] Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K, Stender J, Barnow S, John U, Teumer A, Biffar R, Nauck M, Volzke H, Freyberger HJ, Grabe HJ. Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. *Neuropsychopharmacology*, 2011; 36: 1982-91.
- [31] Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, Pace TW, Mercer KB, Mayberg HS, Bradley B, Nemeroff CB, Holsboer F, Heim CM, Ressler KJ, Rein T, Binder EB. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci*, 2013; 16: 33-41.
- [32] Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*, 2003; 301: 386-9.
- [33] Surtees PG, Wainwright NW, Willis-Owen SA, Luben R, Day NE, Flint J. Social adversity, the serotonin transporter (5-HTTLPR) polymorphism and major depressive disorder. *Biol Psychiatry*, 2006; 59: 224-9.
- [34] Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry*, 2010; 167: 509-27.
- [35] Beach SR, Brody GH, Todorov AA, Gunter TD, Philibert RA. 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*, 2010; 153B: 710-3.
- [36] Beach SR, Brody GH, Todorov AA, Gunter TD, Philibert RA. Methylation at 5HTT mediates the impact of child sex abuse on women's antisocial behavior: an examination of the Iowa adoptee sample. *Psychosom Med*, 2011; 73: 83-7.



- [37] Beach SR, Brody GH, Lei MK, Gibbons FX, Gerrard M, Simons RL, Cutrona CE, Philibert RA. Impact of child sex abuse on adult psychopathology: A genetically and epigenetically informed investigation. *J Fam Psychol*, 2013; 27: 3-11.
- [38] Vijayendran M, Beach SR, Plume JM, Brody GH, Philibert RA. Effects of genotype and child abuse on DNA methylation and gene expression at the serotonin transporter. *Front Psychiatry*, 2012; 3: 55.
- [39] Kang HJ, Kim JM, Stewart R, Kim SY, Bae KY, Kim SW, Shin IS, Shin MG, Yoon JS. Association of SLC6A4 methylation with early adversity, characteristics and outcomes in depression. *Prog Neuropsychopharmacol Biol Psychiatry*, 2013; 44: 23-8.
- [40] Wang D, Szyf M, Benkelfat C, Provencal N, Turecki G, Caramaschi D, Cote SM, Vitaro F, Tremblay RE, Boon L. Peripheral SLC6A4 DNA methylation is associated with in vivo measures of human brain serotonin synthesis and childhood physical aggression. *PLoS One*, 2012; 7: e39501.
- [41] Martinowich K, Lu B. Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology*, 2008; 33: 73-83.
- [42] Comasco E, Aslund C, Orelund L, Nilsson KW. Three-way interaction effect of 5-HTTLPR, BDNF Val66Met, and childhood adversity on depression: a replication study. *Eur Neuropsychopharmacol*, 2013; 23: 1300-6.
- [43] Tortorella A, Brambilla F, Fabrazzo M, Volpe U, Monteleone AM, Mastromo D, Monteleone P. Central and peripheral peptides regulating eating behaviour and energy homeostasis in anorexia nervosa and bulimia nervosa: a literature review. *Eur Eat Disord Rev*, 2014; 22: 307-20.
- [44] Thaler L, Gauvin L, Joobor R, Groleau P, de Guzman R, Ambalavanan A, Israel M, Wilson S, Steiger H. Methylation of BDNF in women with bulimic eating syndromes: Associations with childhood abuse and borderline personality disorder. *Prog Neuropsychopharmacol Biol Psychiatry*, 2014; 54C: 43-49.
- [45] Groleau P, Joobor R, Israel M, Zeramardini N, DeGuzman R, Steiger H. Methylation of the dopamine D2 receptor (DRD2) gene promoter in women with a bulimia-spectrum disorder: associations with borderline personality disorder and exposure to childhood abuse. *J Psychiatr Res*, 2014; 48: 121-7.
- [46] Perroud N, Salzmann A, Prada P, Nicastro R, Hoeppli ME, Furrer S, Ardu S, Krejci I, Karege F, Malafosse A. Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Transl Psychiatry*, 2013; 3: e207.
- [47] Suderman M, McGowan PO, Sasaki A, Huang TC, Hallett MT, Meaney MJ, Turecki G, Szyf M. Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, 2012; 109 Suppl 2: 17266-72.
- [48] Labonte B, Suderman M, Maussion G, Navaro L, Yerko V, Mahar I, Bureau A, Mechawar N, Szyf M, Meaney MJ, Turecki G. Genome-wide epigenetic regulation by early-life trauma. *Arch Gen Psychiatry*, 2012; 69: 722-31.
- [49] Naumova OY, Lee M, Kuposov R, Szyf M, Dozier M, Grigorenko EL. Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. *Dev Psychopathol*, 2012; 24: 143-55.
- [50] Yang BZ, Zhang H, Ge W, Weder N, Douglas-Palumberi H, Perepletchikova F, Gelernter J, Kaufman J. Child abuse and epigenetic mechanisms of disease risk. *Am J Prev Med*, 2013; 44: 101-7.
- [51] Weder N, Zhang H, Jensen K, Yang BZ, Simen A, Jackowski A, Lipschitz D, Douglas-Palumberi H, Ge M, Perepletchikova F, O'Loughlin K, Hudziak JJ, Gelernter J, Kaufman J. Child abuse, depression, and methylation in genes involved with stress, neural plasticity, and brain circuitry. *J Am Acad Child Adolesc Psychiatry*, 2014; 53: 417-24 e5.
- [52] Essex MJ, Boyce WT, Hertzman C, Lam LL, Armstrong JM, Neumann SM, Kobor MS. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Dev*, 2013; 84: 58-75.
- [53] Suderman M, Borghol N, Pappas JJ, Pinto Pereira SM, Pembrey M, Hertzman C, Power C, Szyf M. Childhood abuse is associated with methylation of multiple loci in adult DNA. *BMC Med Genomics*, 2014; 7: 13.

- [54] Mehta D, Klengel T, Conneely KN, Smith AK, Altmann A, Pace TW, Rex-Haffner M, Loeschner A, Gonik M, Mercer KB, Bradley B, Muller-Myhsok B, Ressler KJ, Binder EB. Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proc Natl Acad Sci U S A*, 2013; 110: 8302-7.
- [55] Zhang H, Wang F, Kranzler HR, Zhao H, Gelernter J. Profiling of childhood adversity-associated DNA methylation changes in alcoholic patients and healthy controls. *PLoS One*, 2013; 8: e65648.
- [56] Heim CM, Mayberg HS, Mletzko T, Nemeroff CB, Pruessner JC. Decreased cortical representation of genital somatosensory field after childhood sexual abuse. *Am J Psychiatry*, 2013; 170: 616-23.
- [57] Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmuhl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OF, Spengler D. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci*, 2009; 12: 1559-66.
- [58] Mellen M, Ayata P, Dewell S, Kriaucionis S, Heintz N. MeCP2 Binds to 5hmC Enriched within Active Genes and Accessible Chromatin in the Nervous System. *Cell*, 2012; 151: 1417-30.
- [59] Lister R, Mukamel EA, Nery JR, Urich M, Puddifoot CA, Johnson ND, Lucero J, Huang Y, Dwork AJ, Schultz MD, Yu M, Tonti-Filippini J, Heyn H, Hu S, Wu JC, Rao A, Esteller M, He C, Haghighi FG, Sejnowski TJ, Behrens MM, Ecker JR. Global epigenomic reconfiguration during mammalian brain development. *Science*, 2013; 341: 1237905.
- [60] Tylee DS, Kawaguchi DM, Glatt SJ. On the outside, looking in: a review and evaluation of the comparability of blood and brain "-omes". *Am J Med Genet B Neuropsychiatr Genet*, 2013; 162B: 595-603.
- [61] Aberg KA, McClay JL, Nerella S, Clark S, Kumar G, Chen W, Khachane AN, Xie L, Hudson A, Gao G, Harada A, Hultman CM, Sullivan PF, Magnusson PK, van den Oord EJ. Methylome-wide association study of schizophrenia: identifying blood biomarker signatures of environmental insults. *JAMA Psychiatry*, 2014; 71: 255-64.
- [62] Provencal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, Wang D, Bennett AJ, Pierre PJ, Friedman DP, Cote SM, Hallett M, Tremblay RE, Suomi SJ, Szyf M. The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. *J Neurosci*, 2012; 32: 15626-42.
- [63] Davies MN, Volta M, Pidsley R, Lunnon K, Dixit A, Lovestone S, Coarfa C, Harris RA, Milosavljevic A, Troakes C, Al-Sarraj S, Dobson R, Schalkwyk LC, Mill J. Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biol*, 2012; 13: R43.