Epigenetic Landscapes of the Adversity-exposed Brain

Tiffany S. Doherty[1] and **Tania L. Roth**[1,*]

[1]Department of Psychological and Brain Sciences, University of Delaware, Newark DE 19716

Abstract

It is understood that adversity during development has the power to alter behavioral trajectories, and the role of the epigenome in that relationship is currently under intense investigation. Several studies in both non-human animals and humans have established a link between early adversity and epigenetic regulation of genes heavily implicated in the stress response, plasticity and cognition, and psychiatric disorders such as depression and anxiety. Thus, the relatively recent surge of studies centering on the epigenetic outcomes of stress has great potential to inform treatments and interventions for psychiatric disorder precipitated by early adversity. Here we review what we know and what we do not know, and suggest approaches to help further elucidate the relationship between early adversity, epigenetics, and behavior.

1. Introduction

In the following sections we discuss ongoing considerations in the field of neuroepigenetics. Discussion will begin with current knowledge of each topic, focusing on DNA methylation. We will then narrow the focus to where we are at with that knowledge at the intersection of early adversity, the epigenome, and behavior. Lastly, discussion will turn to what we do not know within these areas, the direction the literature suggests we go next, and some specific steps necessary to address outstanding issues.

1.1 Early Adversity, Epigenetics, & Psychiatric Disorder

Adversity in early life, particularly in relation to the caregiver, is a major predisposing factor in the development of psychiatric disorder.[1–3] Investigation into the mechanisms of this relationship reveals epigenetic alterations as promising candidates. Exposure to early adversity elicits changes in the epigenome that can vary by gene, brain region, sex, and across time (see Table 1).[4–11] In animal models, these changes are associated with behavioral alterations reflective of psychological dysfunction.[7,9,12] In humans, they are present in individuals with a history of early adversity and in those with psychiatric disorder.

*Address correspondence to: Tania L. Roth, Ph.D., Department of Psychological and Brain Sciences, University of Delaware, 108 Wolf Hall, Newark, DE 19716, Phone: (302) 831-2787, Fax: (302) 831-3645, troth@psych.udel.edu.

[4,13–17] Taken together, these data present a strong argument for the functional role of epigenetics in adverse psychiatric outcomes induced by disruption of the early environment.

1.2 DNA Methylation

Methylation of DNA is an epigenetic modification whereby methyl groups are added to cytosine bases in the DNA sequence. This is generally associated with repression of gene transcription (for review see [18]) though it is known in some cases to give rise to enhanced transcriptional states.[19] While traditionally discussed as occurring at cytosine-guanine (CG) dinucleotides, non-CG methylation is also known to occur and have considerable implications for gene expression.[20–22] Methyl group addition is accomplished by DNA methyltransferases DNMT1, DNMT3a, or DNMT3b depending on whether the mark is new or being maintained during replication.[23] DNA demethylation may occur through oxidation of methyl marks[24] or via enzymes such as Gadd45b.[25] The effects of methylation are partly accomplished via methyl-CpG binding protein 2 (MeCP2), which binds to methylated DNA and facilitates gene silencing or expression via recruitment of corepressors[26] or coactivators (see Figure 1 for schematic of epigenetic enzymes and their roles).[19] DNA methylation is the most heavily studied epigenetic alteration in the realm of early stress and psychiatric disorder, and is thus the focus of the ensuing discussion.

2. Specificity of Experience-induced Methylation Changes

2.1 Tissue Specificity

Though some post-mortem brain studies have been published, human studies of early-stress, psychiatric outcome, and the epigenome generally rely on peripheral measures such as saliva and blood. Use of these peripheral measures has greatly informed the literature on stress-induced methylation statuses of genes critical to mental health such as brain-derived neurotrophic factor (*BDNF*),[27,28] serotonin transporter (*SLC6A4*),[16] and oxytocin receptor (*OXTR*).[29] However, the question remains of whether the epigenetic state of these peripheral tissues accurately reflects what is going in the brain, the target of interest in the study of psychiatric disorder.

Examination of human samples of various tissue types (i.e. brain, blood, saliva) has revealed baseline levels of epigenetic variation between tissues, but has also revealed some promising correlations that prompt further investigation. A 2012 study examining methylated regions of the human genome in multiple brain regions and in whole blood reported high variation between tissues, especially at genes critical to developmental processes. However, a correlation between brain and blood was reported for some of the differences found between individual subjects.[30] Importantly, this report shows tissue-specific differences in methylation to occur primarily in intragenic locations, with much less between-tissue variation occurring in promoter regions.[30] Given that promoter and intragenic methylation have distinct effects on transcriptional activity (for review see [31]), each of these findings bear important implications. Another study comparing methylation between brain and blood in humans found blood to be a poor predictor of brain, and in contrast to the previously mentioned study found that the majority of the few sites exhibiting correlation with brain tissue were located in gene bodies.[32] A 2014 study revealed that though overall positively

correlated, methylation within CpG islands is highly dissimilar between saliva and blood in humans, with saliva being a closer match to methylation patterns found in the brain.[33] Blood is also a poor predictor of human brain *BDNF* exon I methylation, though muscle tissue methylation at this locus exhibits a high correlation to brain.[34] Interestingly, methylation in brain and blood have been found similar in an animal model. A study examining correlations between brain and blood in a mouse model of Cushing's disease found the two to be positively correlated for methylation of *Fkbp5*, though methylation changes occurred at different regions in each tissue type.[35] However, the disease variable must be taken into account when interpreting these results in terms of tissue specificity as disease could have multiple epigenetic effects between tissues that may not be seen in a normal state. The results of cross-tissue comparison studies in humans should also be interpreted with caution given that, due to complications typically associated with post-mortem tissue collection, it is not often possible to obtain central and peripheral samples from the same subjects (though some of the aforementioned studies were able to do so). Even so, they provide a valuable foundation for exploring the issue of intra-tissue epigenetic variation in relation to the study of psychiatric disorder.

Despite methodological complications, studies of the epigenome following early stress in humans report findings that are promising in terms of the predictive value of peripheral measures. For example, increased methylation of the glucocorticoid receptor (*GR*) gene following early life stress has been reported in both whole blood[14] and saliva.[28] This is consistent with a 2009 report of increased *GR* methylation in post-mortem brain tissue taken from suicide victims with a history of child abuse.[4] In an interesting cross-species comparison, researchers examined white blood cell progenitor cells in human and nonhuman primate cord blood alongside brain tissue (prefrontal cortex) from rats, all following exposure to early adversity. They reported similar early stress-induced epigenetic patterns between these species and their respective tissue for a handful of genes, one of which is associated with major depressive disorder in humans.[36]

2.2 Cell-type Specificity

Specificity of methylation patterns also occurs between cell types. We know that in the central nervous system neurons and glia display distinct patterns of methylation. Compared to glia, neurons exhibit increased levels of both CG and non-CG methylation as well as enriched methylation at CG island shores, enhancers, and intergenic regions.[37–40] Methylation patterns also differ by neuron type[41,42] in what appears to be a functionally significant manner, especially for developmental processes.[20] These cells also exhibit more variability in their epigenetic state than non-neurons,[43] suggesting that the neuronal epigenome responds dynamically to experience. This has proven true in response to early stress exposure: neurons in the rodent medial prefrontal cortex (mPFC) exhibit increased levels of *Bdnf* methylation in response to adverse caregiving when compared to non-neurons.[44]

Beyond the importance of understanding precisely where in the genome epigenetic modifications are taking place in response to adversity, taking into account cell-type specificity in peripheral samples may be critical for drawing parallels to the brain. For

example, saliva samples containing a higher proportion of leukocytes are more positively correlated with blood methylation patterns whereas saliva samples containing a higher proportion of epithelial cells are more positively correlated with brain methylation patterns. [33]

3. Beyond Methylation

3.1 DNA Hydroxymethylation

Once established, a methyl mark on a cytosine can be oxidized into a hydroxyl group, creating a hydroxymethylated cytosine. This is accomplished by the family of ten-eleven translocation (TET) proteins.[45] This recently discovered mark may play multiple roles in the epigenome. Evidence supports a transient role critical to the process of active demethylation[24] as well as a stable role in neuronal function[46,47] and behavioral outcomes.[48] The relationship of this mark to gene expression appears to be quite dynamic[49,50] and may interact with methylation, changing gene expression outcomes.[51]

3.2 Early Adversity and the Hydroxymethylome

Significantly different patterns of hydroxymethylation are seen in non-human primates exposed to early-life adversity (i.e. peer-reared) when compared to their maternally-reared counterparts.[51] Our lab has also found significant changes in this mark using a rodent model of adverse caregiving. Specifically, male rats exposed to our model of early adversity (i.e. the scarcity-adversity model of low nesting resources) exhibit decreased levels of hydroxymethylation within amygdala tissue in adolescence.[52]

The recent discovery of this mark means there has been much less time to address it in studies of early adversity. In fact, methods most commonly used in the field up to this point are unable to distinguish between methylation and hydroxymethylation, though methods do now exist for this purpose[53–55] and are certain to become more commonly used. For example, oxidative bisulfite sequencing (oxBS-seq) entails the oxidation of hydroxymethylcytosines followed by conversion to uracil in a bisulfite treatment, allowing for sole detection of methylated cytosines.[54] Another option is Tet-assisted bisulfite sequencing (TAB-seq) wherein hydroxymethylcytosines are preserved via glucosylation and methylated cytosines are oxidized to carboxylcytosines via Tet1. The carboxylcytosines are then converted to thymine in a bisulfite treatment, allowing for sole detection of hydroxymethylated cytosines.[55]

To our knowledge, no studies yet exist that examine hydroxymethylation at any time point following developmental stress exposure in humans. However, this modification has been studied in both the developing and adult human brain[56–58] as well as in human peripheral samples (i.e. blood and saliva),[59] and we know it is responsive to environmental conditions. [60] These factors comprise the necessary foundation to move forward with examination of this mark within the realm of early adversity in humans.

4. Sex Differences

4.1 Animal Models

The epigenome and its many mechanisms are unarguably sexually dimorphic in development and throughout the lifespan. Sex differences have been reported in MeCP2, Gadd45b, and DNMT3a expression in the developing rat amygdala,[61–63] in MeCP2 expression in the developing rat ventromedial hypothalamus and preoptic area,[62] and in methylation patterns of steroid receptors (e.g. estrogen receptor α) in sexually dimorphic regions of the brain (e.g. mediobasal hypothalamus, preoptic area).[64,65] Post development, sex-specific histone modifications have been reported for the bed nucleus of the stria terminalis and preoptic area in adult mice[66] and again, in methylation patterns of steroid receptors in sexually dimorphic brain regions.[64,65] In humans, baseline (i.e. in non-stress/disease samples) sex differences in methylation have been reported.[67,68] Bear in mind that as the axis of this review spins on early adversity, these lists of baseline epigenetic differences are for illustration and thus are not exhaustive.

Though foundational studies of the epigenome in the early stress literature were conducted only in males, a large amount of data have since been produced to support the sex-specific nature of epigenetic modifications following early adversity. One of these foundational studies found that natural variations in maternal care (i.e. high versus low licking and grooming by a dam) resulted in altered methylation patterns and anxiety-like behavior in adult male offspring.[7] A study published approximately a decade later examined outcomes in female offspring and found interesting differences (i.e. different in many respects from the male data) in both epigenetic and behavioral responses.[69] Though methodological differences between these studies restrict a direct comparison, the collective results nevertheless point to the need for sex-specific examination.

Using a version of the scarcity-adversity model of low nesting resources, wherein pups experience daily exposure to adverse conditions outside the home cage during the first week of life, our lab has observed sex-specific alterations in epigenetic regulators in the mPFC as late as postnatal day 90.[70] We have also reported numerous sex differences in adversity-induced *Bdnf* methylation patterns. We find that females exhibit changes in *Bdnf* methylation that very rarely match male patterns.[5,6,52] We also observe significant, brain-region specific differences in genome-wide DNA methylation and hydroxymethylation in adversity-exposed adolescent males, an effect not observed in females.[52] These alterations are accompanied by sex-specific deficits in select behavioral tasks,[71] data which support the hypothesis that altered epigenetic patterns are behaviorally functional. Exposure to maternal separation, another commonly used model of early adversity, results in similar sex-specific outcomes. For example, in a mouse model of maternal separation, altered *Nr3c1* (*GR*) methylation is seen in the adolescent male but not female hippocampus of C57BL/6J mice, a change accompanied by various male-specific behavioral alterations.[72]

4.2 Humans

Interestingly, methylation of *NR3C1* in humans exposed to early adversity is not reported to differ between males and females in adulthood.[15] The same appears to be true for early

stress-induced methylation changes in genes whose methylation status significantly predicts depression.[13] However, sex differences in methylation have been reported in children[8] and adolescents[73] exposed to early stress. Many variables could account for these opposing results. Tissue types less representative of the brain (e.g. blood) may not pick up sex differences that would normally be seen in the brain. Differing types, intensities, and timing of adversity may have more or less of a sex effect. Lastly, gene-specific studies not reporting a sex difference may simply be examining a gene that does not exhibit sex differences in methylation in response to adversity.

5. Methylation across the lifespan

We now know that methylation across the genome is tied to age in both non-human animals[9,74] and humans.[68,75,76] In addition, data suggest that the effect of early environmental alterations on health at a later age is of an epigenetic nature. For example, prenatal exposure to famine is related to altered methylation of growth- and metabolic-related genes more than half a century after exposure.[77,78] Adversity in the context of caregiving in humans and non-humans is known to leave its epigenetic mark early on[14,79,80] and/or in adulthood.[4,9,79] That is, in some cases these marks are seen persistently across development and into adulthood while in other cases they are dynamic across the lifespan, appearing in some life stages and not in others. These stress-induced changes in methylation have even been reported to override changes typically seen in the aging methylome.[9]

In non-human primates, serotonin transporter methylation appears to be elevated in both infancy[80] and adulthood[81] following early stress, though it is important to note that different types of early stress were employed in these two studies. In a mouse model of early stress, *Avp* hypomethylation was detected at 6 weeks of age and persisted up to the last measurement at 1 year of age.[9] In a similar model, *Pomc* hypomethylation is maintained within the same time frame.[79] In contrast to these sustained marks, our lab has found developmental changes in methylation of the *Bdnf* gene in our adversity-exposed rats. In infancy, we find that adversity-exposed females exhibit a brain region-specific decrease in methylation of *Bdnf* exon I alongside increased methylation of exon IV, the reverse of which is then found in adulthood.[6] In males, the only developmental change detected in *Bdnf* methylation is at exon I in adolescence.[5] Not only is that mark found to be reversed (i.e. opposite direction in methylation) in adulthood, six additional alterations in *Bdnf* methylation emerge at this time point.[5,6] Additionally we find several significant, maltreatment-specific alterations in epigenetic regulators in the mPFC of our adult animals that are not detected in infancy.[70] Taken together, these data underline the importance of longitudinal studies of environmental effects upon the epigenome, a type of study that is somewhat lacking in the field at present.

6. What we do not know and how we get there

6.1 Tissue- and Cell-type Specificity

Currently, one of the largest issues in studying epigenetic signatures following adversity in humans is the lack of understanding of the relationship between central and peripheral epigenetic marks. Though we may not necessarily expect similar marks between central and

peripheral measures, thorough investigation and comparison of the epigenome between tissue types will aid in understanding if there is a relationship between them, and if there is, how we can use that relationship to understand what is happening in the brain when only peripheral measures are available. An example of the utility of this approach can be seen in work by Walton and colleagues.[32] Following initial examination of tissue-specific methylation in samples obtained from epileptic patients during neurosurgery, these researchers isolated a small number of sites in which significant correlations existed between methylation in blood and brain. Using these sites they examined methylation in blood samples of schizophrenia subjects, an approach that led them to discover an association between schizophrenia and methylation of a gene (*AVP*) and its receptor (*AVPR1A*) previously associated with this disorder.[32]

Collection of more biosamples in rodent studies will be incredibly useful in this endeavor. Previous reports of correlation of tissue-specific methylation between-species (i.e. mouse and human)[41,82] support the examination of peripheral tissues in animal models to draw parallels in humans, though more studies are needed to know if correlations also exist for adversity-induced methylation patterns. Peripheral and central tissue collection in rodent studies can be easily accomplished within subjects and will greatly contribute to the quest of understanding the peripheral-central relationship of the psychiatric epigenome. However, there are important caveats to consider in making cross-species comparisons. For example, the previously discussed correlation between methylation patterns in mouse and human brain is more highly conserved in inhibitory than excitatory neurons.[41]

When possible, collecting multiple types of peripheral samples within human subjects will help us better understand intra-tissue epigenetic variation in humans. Given that methylation patterns exhibit brain region-specific changes in response to adversity, it will also be necessary to examine multiple regions individually to understand which of them exhibit a relationship to peripheral measures and what each relationship looks like. Though access to human brain tissue is difficult to obtain (especially when specific conditions, like adversity, are required), the collection of more brain regions from the same subject is warranted.

Currently there are few studies on cell-type specific methylation following early stress. However, existing literature clearly demonstrates stress-induced epigenetic responses and the field would greatly benefit from expanding these studies to understand more precisely where these changes are occurring. Given the known mental health outcomes of developmental stress exposure, this understanding is critical to the intersection of epigenetics and psychopathology.

6.2 Hydroxymethylation and non-CG methylation

Another necessary step in the field will be increased utilization of the techniques referenced in section 3.2 in order to distinguish between methylated and hydroxymethylated cytosines. Considering what is known at this point about the distinct effects of these two modifications on gene expression, it will be critical to parse them apart if we wish to understand epigenetic regulation of behavior following early adversity. Additionally, investigation of hydroxymethylation in human early adversity studies will be essential in moving the field forward.

Another component of the epigenome requiring more attention is non-CG methylation. Examining methylation at CG sites is the dominant practice at present. However, non-CG methylation is not only known to occur (as referenced in section 1.2) but is reportedly the preeminent form of methylation in neurons.[38] Furthermore, when occurring in the gene body, it has been reported to be more predictive of neuronal gene expression than both CG methylation and measures of chromatin accessibility.[20] Given this powerful relationship to functional outcome, examination of non-CG methylation is paramount to understanding how epigenetic alterations subserve the relationship between early adversity and psychiatric dysfunction.

6.3 Sex Differences

In terms of understanding sex differences in the epigenome following early life stress, female offspring need to be included more consistently in animal research, something that is already occurring based on recently introduced NIH policies on balancing sex in cell and animal studies. Something else to consider is that studies in post-mortem brains suffer from a lack of female tissue, making it difficult to verify sex differences in human studies using peripheral samples. Sex differences are prevalent in psychiatric disorders,[83,84] particularly in disorders for which stress is a precipitating factor.[85] Given these facts alongside our knowledge of a sexually dimorphic epigenome in individuals exposed to early adversity and in those with psychiatric disorder, it is highly plausible that epigenetic mechanisms are a causal factor in these sex-specific outcomes. Failing to consistently study sex differences will greatly undermine our understanding of and ability to treat psychiatric dysfunction.

6.4 Lifespan Methylation

Concerning aging, we know the human epigenome bears the marks of early stress at individual time points (e.g. adolescence, adulthood) but we have very little information on how methylation changes across the lifespan in these individuals. The literature would greatly benefit from longitudinal studies in early-stress cohorts (in both animal models and humans) that report epigenetic states at various age points following the initial exposure. Understanding the state of the epigenome at a given point in time has great potential to inform the medical community based on the age a given treatment or intervention might be administered.

7. Concluding Remarks

For a young field, great strides have certainly been made. The foundation that exists in the current literature gives us every opportunity to address these challenges and others that will surely arise. Given the nature of these studies, some obstacles will be more difficult to address than others. For example, obtaining post-mortem brain tissue under specific conditions (i.e. from subjects with known histories of early stress) is difficult, as is obtaining peripheral samples from the same subjects due to timing (of death and of the research) as well as the conditions of death. Longitudinal studies also present unique challenges due to issues such as subject attrition. However, the potential of this work to improve the human condition definitively outweighs the cost. The implications are far-reaching, with the

potential to shape both policy and medicine in a manner that will meaningfully affect the lives of many in terms of development and/or in the prevention and treatment of disease.

Acknowledgments

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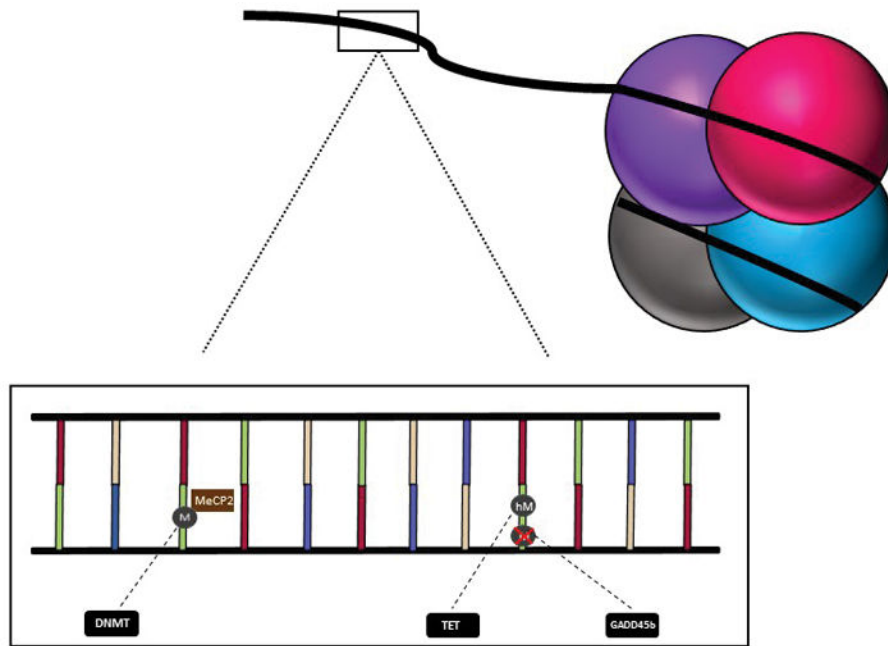
Figure 1.
Schematic of DNA wrapped around four core histones. Zoom-in of DNA strand demonstrates the addition of methyl groups (M) to cytosines by DNMT enzymes, association of MeCP2 with methyl groups, oxidation of methyl groups by TET enzymes to create hydroxymethylcytosines (hM), and demethylation activity of GADD45b.

Table 1

Models of early adversity and their associated epigenetic outcomes, including the brain region and time point at which they were found and any sex differences reported.

Type of Early Adversity	Epigenetic Outcome	Brain Region/Peripheral Measure	Age	Sex Differences	Reference
Low Levels of Licking/Grooming	Increased methylation *GR*	Hippocampus	Adult	Only male brains analyzed	7
	Decreased methylation *GR*	Hippocampus	Adult	Only female brains analyzed	69
	Increased methylation *ERa*	Medial Preoptic Area	Adult	Only female brains analyzed	11
Maternal Separation	Decreased methylation *Avp*	Paraventricular nucleus	Adult	Only male brains analyzed	9
	Increased methylation *GR*	Hippocampus	Adolescent	Present only in males	72
	Increased methylation *Nrsr1*	Amygdala	Adult	Only male brains analyzed	12
	Decreased methylation *Pomc*	Pituitary	Adult	Only male brains analyzed	79
	Increased methylation *Bdnf* IX	Whole Prefrontal Cortex	Infant, Adolescent, Adult	Males & Females	10
	Increased methylation *Bdnf* I	Medial Prefrontal Cortex	Adolescent	Present only in males	5
	Decreased methylation *Bdnf* IV	Medial Prefrontal Cortex	Adolescent	Present only in females	5
	Decreased methylation *Bdnf* I	Medial Prefrontal Cortex	Adult	Males & Females	5
	Increased methylation *Bdnf* IV	Medial Prefrontal Cortex	Adult	Present only in females	5
Scarcity-Adversity Model Of Low Nesting Resources	Decreased methylation *Bdnf* I	Amygdala	Infant	Present only in females	6
	Increased methylation *Bdnf* IV	Amygdala	Adolescent	Present only in females	6
	Decreased methylation *Bdnf* I	Amygdala	Adult	Present only in males	6
	Decreased methylation *Bdnf* IV	Amygdala	Adult	Males & Females	6
	Decreased methylation *Bdnf* I, IV	Dorsal Hippocampus	Adult	Present only in males	6
	Increased methylation *Bdnf* I	Ventral Hippocampus	Adult	Present only in females	6
	Increased methylation *Bdnf* IV	Ventral Hippocampus	Infant, Adolescent	Present only in females	6
	Increased methylation *NR3Cl*	Hippocampus	Adult	Only male brains analyzed	4
Childhood Abuse/Neglect (Humans)	Site-specific differential methylation, *BDNF, NR3C1, FKBP5*	Saliva	Child/Adolescent, 5–14 years	Males & Females	13
	Increased methylation, *BDNF, OXTR*	Blood	Adult	Males & Females	29
	Differential methylation Genome-wide	Saliva	Child, Average age 9	Gene-dependent sex differences	8