

Regular Article

Preliminary indications that the Attachment and Biobehavioral Catch-up Intervention alters DNA methylation in maltreated children

Julie R. Hoye¹ , David Cheishvili², Heather A. Yarger³ , Tania L. Roth⁴, Moshe Szyf² and Mary Dozier⁴

¹Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA; ²Department of Pharmacology and Therapeutics, McGill University, Montreal, Québec, CAN; ³Department of Psychology, University of Maryland, College Park, MD, USA and ⁴Department of Psychological and Brain Sciences, University of Delaware, Newark, DE, USA

Abstract

Maltreatment during development is associated with epigenetic changes to the genome. Enhancing caregiving may mitigate these effects. Attachment and Biobehavioral Catch-Up (ABC) is an intervention that has been shown to improve parent–child relationships and a variety of biological and behavioral outcomes among children that are involved in Child Protective Services. This preliminary study, using a small sample size, explored whether children who received ABC exhibit different methylation patterns than those who received a control intervention. The participants included 23 children aged 6–21 months who were randomized to receive ABC ($n = 12$) or a control intervention ($n = 11$). While the children displayed similar methylation patterns preintervention, DNA methylation varied between the ABC and control groups at 14,828 sites postintervention. Functional pathway analyses indicated that these differences were associated with gene pathways that are involved in cell signaling, metabolism, and neuronal development. This study is one of the first to explore parenting intervention effects on children’s DNA methylation at the whole genome level in infancy. These preliminary findings provide a basis for hypothesis generation in further research with larger-scale studies regarding the malleability of epigenetic states that are associated with maltreatment.

Keywords: DNA methylation, epigenetics, intervention, maltreatment, parenting

(Received 21 January 2019; accepted 1 September 2019)

Children who experience chronic stress are at risk for problematic developmental outcomes including depression, post-traumatic stress disorder (PTSD), and cardiovascular and autoimmune diseases (Cicchetti & Toth, 2005; Melchior, Moffit, Milne, Poulton, & Caspi, 2007; Miller, Chen & Parker, 2011). Stress experienced early in life may affect brain and behavioral development through biological embedding. Early-life experiences may alter biological processes which further influence developmental outcomes. Changes in DNA methylation could serve a mechanistic pathway through which biological embedding occurs. DNA methylation, or the addition of methyl groups to cytosines, is an epigenetic mechanism of gene regulation that is most often associated with gene silencing (Moore, Le, & Fan, 2013; Ziller et al., 2013), though this effect is dependent upon cytosine location in the genome (Cedar & Bergman, 2009; Jones, 2012; Maunakea, Chepelev, Cui, & Zhao, 2013).

Work in various rodent models and humans has associated developmental stress with long-term alterations in DNA methylation (Champagne et al., 2006; Cicchetti, Hetzel, Rogosch, Handley, & Toth, 2016; Doherty, Foster, & Roth, 2016; Khulan et al., 2014; McGowan et al., 2009; Naumova et al., 2012; O’Donnell et al.,

2018; Prados et al., 2015; Roth, Lubin, Funk, & Sweatt, 2009; Unternaehrer et al., 2015; Weaver et al., 2004, Yang et al., 2013). In rodents, these epigenetic changes occur concurrently with behavioral alterations, such as altered stress responsivity and cognitive deficits (Doherty, Blaze, Keller, & Roth, 2017; Weaver et al., 2004). In humans, aberrant epigenetic alterations parallel many disorders and diseases, including depression, PTSD, and cardiovascular and autoimmune diseases (Dalton, Kolshus, & McLaughlin, 2014; Yehuda et al., 2015; Handy, Castro, & Loscalzo, 2011; Farh et al., 2015).

Mounting evidence suggests that enhanced caregiving environments may alter epigenetic states. For example, cross-fostering rat offspring to high quality maternal care can reverse the methylation patterns that are associated with low quality maternal care or maltreatment (Roth et al., 2009; Weaver et al., 2004). Among children, infant attachment, an indication of caregiving environment, is associated with variation in methylation of the genome (Garg et al., 2018). Further, a parenting intervention that is efficacious in reducing harsh parenting has been shown to protect children from the accelerated epigenetic aging that is associated with family risk (Brody, Yu, Chen, Beach, & Miller, 2016). Parenting interventions that are delivered in early childhood appear to have persisting effects on the DNA methylome. Nurse Family Partnership (NFP) is an intervention that is delivered to first-time mothers who are at risk of child abuse. The mothers receive weekly visits from a community nurse beginning in pregnancy and continuing for approximately 30 months. When compared

Author for Correspondence: Julie Hoye or Mary Dozier, 108 Wolf Hall, 105 The Green, Newark, DE 19716. E-mail: jhoye@udel.edu or mdozier@psych.udel.edu

Cite this article: Hoye JR, Cheishvili D, Yarger HA, Roth TL, Szyf M, Dozier M (2019). Preliminary indications that the Attachment and Biobehavioral Catch-up Intervention alters DNA methylation in maltreated children. *Development and Psychopathology* 1–9. <https://doi.org/10.1017/S0954579419001421>

with a control condition, NFP is associated with numerous benefits to the parent–child dyad, including a reduction in child abuse and neglect, fewer behavioral and intellectual problems at age 6, and a reduction in child arrests at age 15 (Olds *et al.*, 1997, 1998, & 2004). Among young adults whose mothers were enrolled in NFP when they were infants, variation in DNA methylation was associated with receipt of the intervention in early childhood, indicating a sustained impact of early intervention on the epigenome (O'Donnell *et al.*, 2018).

High-quality caregiving following maltreatment may change the epigenetic signatures associated with maltreatment. Following maltreatment, parental behavior can be improved through behavioral interventions that are delivered to the dyad (Bakermans-Kranenburg, Van IJzendoorn, & Juffer, 2003; Toth, Graver-Davis, Guild, & Cicchetti, 2013). Dyadic interventions have been shown to be particularly efficacious in improving relationships and altering parent–child interactions (Toth *et al.*, 2013). The relational focus of dyadic interventions supports the child's development by altering continuous, dynamic interactions between the child and parent (Sameroff & Fiese, 2000). Attachment and Biobehavioral Catch-Up (ABC) is an intervention that is designed to enhance parenting among parents who are identified as being at risk for maltreating their children. The ABC intervention targets parental sensitivity and nurturance, with the objective of improving children's regulatory outcomes. The intervention has been shown to be efficacious at improving parenting quality in parents that are referred to Child Protective Services (Bick & Dozier, 2013), high-risk parents in a community setting (Caron, Weston-Lee, Haggerty, & Dozier, 2016), and parents that are adopting internationally (Yarger, Bernard, Caron, Wallin, & Dozier, 2019). Furthermore, the parents that were randomized to receive ABC in the current sample were observed to be more sensitive in interactions with their children postintervention than those who were randomized to the control condition (Yarger, Hoyer, & Dozier, 2016). The children whose parents received the ABC intervention were more likely to develop secure and organized attachments than those whose parents received a control intervention were (Bernard *et al.*, 2012). Secure attachments in early infancy lead to positive developmental cascades; securely attached children are more likely to have positive peer relationships, higher self-worth, and fewer internalizing and externalizing problems than those who are insecurely attached (Weinfield, Sroufe, Byron, & Carlson, 2008). In addition to improving the quality of parent–child attachment, ABC improves biological functioning that is altered by stress. Children who participated in ABC were more likely to display normalized diurnal cortisol patterns than those whose parents received a control intervention (Bernard, Dozier, Bick, & Gordon, 2015). Preschool-aged children whose parents received ABC in infancy display stronger cognitive flexibility, greater theory of mind abilities, and they display less negative affect during a frustrating task than children whose parents received a control intervention (Lewis-Morriarty, Dozier, Bernard, Terracciano, & Moore, 2012; Lind, Bernard, Ross, & Dozier, 2014). Moreover, changes in the hypothalamic-pituitary-adrenal (HPA) axis have been shown to persist for at least 3 years after having received the intervention (Bernard, Hostinar, & Dozier, 2015). In middle childhood, children whose parents received ABC in infancy exhibited differential neural activity and autonomic nervous system functioning compared with those who received a control intervention (Bick, Palmwood, Zajac, Simons, & Dozier, 2019; Tabachnick, Raby, Goldstein, Zajac, & Dozier, 2019). In summary, ABC is efficacious in altering children's attachment, socioemotional regulation, and biology.

Because epigenetic alterations may underlie the phenotypic outcomes that are associated with ABC, here, within a randomized controlled trial, we explored whether the ABC intervention is associated with changes in DNA methylation in young children who had experienced maltreatment. Based on prior literature demonstrating that in both human and nonhuman animal models early caregiving can change DNA methylation, we hypothesized that we would observe changes in DNA methylation within this sample due to the improvement in caregiving quality observed (Yarger *et al.*, 2016). Furthermore, given the wide array of positive behavioral outcomes associated with ABC (e.g., improved HPA-axis functioning) and evidence documenting the effects of DNA on such outcomes (e.g., Bick *et al.*, 2012; Murgatroyd & Spengler, 2011), we also conducted exploratory analyses to identify the functional gene pathways that harbor differentially methylated CpG sites.

Method

In this study, 23 parent–child dyads with Child Protective Services (CPS) involvement as the result of allegations of maltreatment were randomly assigned to one of two interventions: the experimental intervention, ABC, or the control intervention, Developmental Education for Families (DEF). Upon parental consent, the children provided saliva samples for preintervention methylation analyses. The initial samples were collected in the children's homes following a 7-min period of free-play between child and parent. After the saliva sample was obtained, the children were randomized to ABC ($n = 12$) or DEF ($n = 11$) conditions. One month after completing the intervention, an additional research visit was conducted in the laboratory. Postintervention saliva samples were collected in the laboratory following the same 7-min free-play procedure between parent and child. Individuals who received DEF were not offered ABC after completing the intervention phase in order to allow for longitudinal assessment. All of the study procedures were approved by the Institutional Review Board at the University of Delaware.

Participants

The primary participants included 23 children who were involved with CPS. The parent–child dyads were referred to a local child welfare agency due to concerns of possible maltreatment. At the time of enrollment, the children ranged in age from 6 to 21 months ($M = 14.07$, $SD = 4.18$). The mothers were between the ages of 19.6 and 42.4 years old ($M = 27.09$, $SD = 5.66$). Demographic information did not differ between groups (see Table 1).

Interventions

ABC is a 10-session manualized intervention that is designed to enhance parenting behaviors that are associated with effective self-regulation in children. The intervention was delivered in the participants' homes by a certified parent coach. During the intervention, the parents are encouraged to provide nurturance, follow their children's lead with delight, and decrease frightening behaviors. Psychoeducation regarding the importance of these behaviors is provided through didactic content at each session. The parent coaches further reinforce desired parent–child interactions by making “in-the-moment” (ITM) comments that describe and label the parent behaviors and link these behaviors to associated outcomes. The frequency of in-the-moment comments predicts

Table 1. Demographic characteristics by intervention group

	ABC (<i>n</i> = 12) Mean (<i>SD</i>) or <i>n</i> (%)	DEF (<i>n</i> = 11) Mean (<i>SD</i>) or <i>n</i> (%)
Child Age in Months	13.11 (4.56)	15.14 (3.64)
Child Sex		
Male	7 (58.3)	5 (45.5)
Female	5 (41.7)	6 (54.5)
Child Race		
European American	5 (41.7)	4 (36.3)
African American	5 (41.7)	7 (63.6)
Biracial	1 (8.3)	0
Other	1 (8.3)	0
Maternal Age in Years	25.42 (3.43)	28.90 (7.12)
Household Income	16,079.33 (12,896.09)	17,800.00 (13,505.93)
Maternal Education		
Did Not Complete High School	3 (25.0)	2 (18.2)
High School or GED	6 (50.0)	5 (45.5)
Some College	3 (25.0)	4 (36.3)
Relationship Status		
Partnered	4 (33.3)	5 (45.5)
Single	8 (66.7)	6 (54.5)

behavioral change among parents (Caron, Dozier, & Bernard, 2016). The DEF intervention is structurally similar to ABC, with families participating in 10 weekly hour-long sessions within their homes. However, the interventionists provide psychoeducation and developmentally appropriate activities for parents to engage in with their child that are related to language development (e.g., labeling colors and shapes), motor skills (e.g., improving balance), and cognitive development (e.g., engaging in symbolic play) rather than parental sensitivity. The content of DEF was modeled after the daycare curriculum from the Carolina Abecedarian Project, but content regarding sensitive parenting was omitted in order to keep the interventions distinct (Ramey, McGinness, Cross, Collier, & Barrie-Blackley, 1982).

DNA Methylation Measurement

Cotton swabs were used to collect saliva from the children (DNA Genotek, Ottawa, ON, Canada). Using saliva provides a minimally invasive collection procedure, and saliva methylation patterns often correlate positively with those in the blood and brain (Smith et al., 2015; Thompson et al., 2013). The saliva swabs were placed in Oragene discs (OGR-250) and stored at room temperature until extraction (DNA Genotek). The DNA was extracted with prepIT L2P following the manufacturer's instructions (DNA Genotek), with minor modifications. The modifications included centrifuging the samples at 14,000 rpm and rehydrating the dehydrated DNA pellet in 500 μ L of TE buffer. Quality and quantity were verified by spectrophotometry (NanoDrop2000). The DNA samples were stored at -80°C until further analysis.

Bisulfite treatment, DNA amplification, sample hybridization to the Infinium HumanMethylation450 BeadChip, and measurement of the methylation values were performed by GenomeQuebec (Montréal, Canada). Methylation was measured using the Infinium HumanMethylation450 BeadChip array, which measures 485,000 CpG sites related to 99% of the genes that are included in the National Center for Biotechnology Information Reference Sequence Database (Illumina, San Diego, CA). The Infinium Methylation Assay provided a β -value for each CpG site, which indicates the proportion of methylated to unmethylated DNA that is present in the sample. The values ranged from 0, indicating a sample with no methylation present, to 1, indicating a fully methylated CpG site.

Analytic Plan

The methylation analyses were conducted in R (R Core Team, 2014). The raw values were normalized by using the ChAMP package (Morris et al., 2013). Sites were removed from further analyses if the probes had a detection rate of less than 0.01 ($n = 2,023$), aligned with known single nucleotide polymorphisms ($n = 28,585$), aligned with multiple locations ($n = 8,489$), or were present on the X or Y chromosome ($n = 11,167$). The data were further normalized via beta mixture quantile dilation, and the batch and chip effects were removed (Teschendorff et al., 2012). The differentially methylated CpG sites between groups were calculated in R, using empirical Bayes statistics for differential expression (Ritchie et al., 2015). To account for multiple comparisons, the p -values were adjusted with false discovery rate (FDR) Benjamini-Hochberg procedures. The primary analyses addressed whether the methylation sites differed as a function of the intervention. The methylation values for the ABC and DEF groups were compared before and after the interventions. The differentially methylated sites with an adjusted p -value $< .05$ were included in subsequent analyses.

The effects of potential covariates were accounted for by removing the sites that were associated with demographic variables (sex, minority status, and age) from the list of CpG sites that were used for further analyses. Due to our small sample size, racial categories were collapsed into "nonminority" and "minority" groups. The sites that were associated with sex (male and female) and minority status (minority and nonminority) groups were identified by using empirical Bayes statistics for differential expression (Ritchie et al., 2015). The sites that were associated with age were identified by regressing normalized β -values for each CpG site on child age. The CpG sites that were significantly associated (adjusted p -value $< .05$) with any demographic covariate (sex, minority status, or age) were removed ($n = 755$).

Secondary analyses were conducted to determine the likelihood of potential functional impact of changes in DNA methylation as a result of the intervention. Differentially methylated sites were first mapped to genic region, as the location of a CpG site within the gene has important implications for whether DNA methylation affects transcription. Next, we identified genes that contained several differentially methylated CpG sites. Next, we identified a subset of differentially methylated sites that were robustly associated with intervention status. A change in methylation score ($\Delta\beta$) was calculated by subtracting the preintervention β from the postintervention β for each CpG site that differed between groups postintervention. Sites that changed in a consistent direction among all individuals in either group were selected and included in functional analyses that were performed in

Table 2. Gene locations of CpG sites showing differential DNA methylation in association with the ABC intervention

Location	Number of CpG sites
Gene Body	4,302
1st Exon	1,724
5' UTR	2,654
3' UTR	428
TSS200	3,344
TSS1500	3,296

Note: CpG sites may be aligned with more than one gene region.

PANTHER version 11 (Mi, Muruganujan, Casagrande, & Thomas, 2013; Mi et al., 2017).

Results

Primary Analysis

As previously reported, the parents who received ABC exhibited a greater increase in parental sensitivity and decrease in intrusiveness than those who received DEF (Yarger, Hoye, & Dozier, 2016). Prior to beginning the intervention, 31,593 sites were differentially methylated between the ABC and DEF groups, but after FDR correction only one CpG site maintained statistical significance, $t = -9.29$, adjusted $p < 0.01$ (0.0017), cg09931793, Olfactory Receptor Family 2 Subfamily K Member 2 Gene (OR2K2). After the intervention, 82,937 sites were differentially methylated between the ABC and DEF groups, and after FDR

correction 14,828 sites maintained their statistical significance (adjusted $p < .05$). Compared with the DEF group, 5,050 sites in the ABC group were hypermethylated and 9,778 were hypomethylated.

Secondary Analyses

The differentially methylated CpG sites that survived FDR correction were mapped onto gene regions (Table 2). Of the CpG sites that were associated with intervention groups, 42.16% fell within 1,500 base pairs of a transcription start site (TSS), or the location where transcription starts in a gene sequence, indicating that methylation changes at these sites are more likely to have functional relevance. Several genes contained multiple CpG sites that were differentially methylated between the groups, indicating that downstream processes are likely to be altered (Table 3). The gene functions that were associated with the list of genes were identified through Gene Ontology annotations (Ashburner et al., 2000). While a large number of CpG sites were associated with intervention group differences, the functional relevance of these differences remains unknown. To identify the sites that are most likely to be associated with biological consequences, additional analyses were conducted. Selecting CpG sites with the largest between-group differences improves confidence in their functional relevance because larger between-group differences at a particular CpG site indicate more discrepant levels of methylation and, in theory, gene products, between the two intervention conditions. The average difference in β -values between groups was calculated for each CpG site. The top 10% of differentially methylated CpG sites (average difference in $\beta > .025$; $n = 1,552$) were used to identify the functional relevance of the intervention group differences

Table 3. Genes containing 10 or more CpG sites associated with intervention group

Gene Symbol	Number of CpG Sites	Gene	GO annotation
7SK	23	RNA, 7SK Small Nuclear	n/a
DIP2C	16	Disco Interacting Protein 2 Homolog C	Transcription factor binding
Metazoa_SRP	16	Metazoan signal recognition particle RNA	ncRNA
COL11a2	15	Collagen Type XI Alpha 2 Chain	Extracellular matrix structural constituent and extracellular matrix structural constituent conferring tensile strength
TRIM26	15	Tripartite Motif Containing 26	Innate immune response, interferon-gamma-mediated signaling pathway
EBF3	12	Early B-Cell Factor 3	Protein dimerization activity
LOC401242	12	Uncharacterized	
TPO	12	Thyroid Peroxidase	Calcium ion binding and peroxidase activity
HDAC4	11	Histone Deacetylase 4	Sequence-specific DNA binding and transcription factor binding
LOC100506585	11	Uncharacterized	
SMOC2	11	SPARC Related Modular Calcium Binding 2	Calcium ion binding and glycosaminoglycan binding
MAD1L1	10	Mitotic Arrest Deficient Like 1	Kinetochore binding, mitotic cell cycle check point, negative regulation of T cell proliferation
OCA2	10	Melanosomal Transmembrane Protein	Transporter activity and L-tyrosine transmembrane transporter activity
VPS52	10	VPS52, GARP Complex Subunit	Syntaxin binding

Note: GO refers to gene ontology.

Table 4. Relevant biological pathways associated with intervention group differences in DNA methylation

Gene ontology biological process	Fold enrichment	<i>p</i> -value
Regulation of signaling (GO:0023051)	1.49	1.74E-04
Regulation of cell communication (GO:0010646)	1.47	6.29E-04
Neuron differentiation (GO:0030182)	1.96	8.68E-04
negative regulation of cellular metabolic process (GO:0031324)	1.54	9.50E-04
Neurogenesis (GO:0022008)	1.72	9.66E-04
Negative regulation of nitrogen compound metabolic process (GO:0051172)	1.56	1.31E-03
Negative regulation of macromolecule metabolic process (GO:0010605)	1.5	3.32E-03
Regulation of signal transduction (GO:0009966)	1.47	4.26E-03
Negative regulation of nucleobase-containing compound metabolic process (GO:0045934)	1.68	1.09E-02
Regulation of cell-substrate adhesion (GO:0010810)	3.24	1.27E-02
Neuron development (GO:0048666)	1.94	2.81E-02

Note: For the full table see Supplemental Table 1.

through pathway analysis. Group differences were associated with 74 biological processes (Supplemental Table 1). Notably, the pathways that emerged as different between the two groups included cellular adhesion, cell signaling, neuronal development, signal transduction, and the regulation of metabolic processes (Table 4). The fold enrichment of each pathway indicates the degree to which pathway-associated genes appear among the 1,552 CpG sites relative to the expected frequency of sites associated with that pathway as estimated from the genome.

To identify robust changes in methylation associated with intervention, methylation patterns were investigated within each individual. The change in methylation scores ($\Delta\beta$) was calculated for each individual at each of the 14,828 CpG sites that were associated with intervention group differences. The directionality of the changes in DNA methylation over time (preintervention to postintervention) was identified. Sites that changed in the same direction among all individuals within a group were identified. Within children who received DEF, methylation of 599 CpG sites changed in the same direction among all of the individuals

within the group. Five CpG sites were associated with consistent changes in both groups, though the intervention groups changed in opposite directions (Table 5). The methylation values for the children who received ABC consistently changed in 181 CpG sites. To further investigate changes over time within the ABC group, the preintervention and postintervention methylation values were compared. The methylation values for 115 sites were significantly different before and after the intervention within the ABC group (Figure 1). The *p*-values were Bonferroni adjusted to account for multiple comparisons. The hierarchical cluster analysis of these sites is represented by dendrograms on the heatmap. Interestingly, all of the ABC postintervention cases clustered apart from both ABC pre- and DEF pre- and postcases, highlighting the potential influence of the ABC intervention on the DNA methylation profile. Whereas the majority of CpG sites postABC were hypomethylated in comparison to preABC, preDEF, and postDEF measurements, 13 CpG sites were hypermethylated. The 115 CpG sites aligned to 115 distinct genes. Of these sites, 55 were associated with promoter regions. Thirty-six CpG sites were located within 200 bp of the TSS, and 19 additional sites fell within 1500 bp of the TSS. Twenty CpG sites were located within the gene body, 12 sites were located within the 5' UTR, and 10 sites fell within the 1st exon.

Discussion

In the current exploratory study, ABC was implemented with parent-child dyads that had been referred to child welfare because of concerns regarding maltreatment. Over the 10 sessions, parents who received ABC displayed increases in sensitive, attuned behaviors and decreases in intrusive interactions (Yarger et al., 2016). The ABC intervention was also associated with widespread changes in DNA methylation. Prior to receiving the intervention, children within the ABC and DEF groups on average had similar methylation levels after FDR correction. After the intervention, 14,828 CpG sites were differentially methylated in the two groups. The ABC intervention resulted in consistent changes in DNA methylation of 115 CpG sites among all of the individuals who received the intervention. Clustering of the children's DNA methylation by state of methylation of these sites separated the ABC intervention from all of the other groups, illustrating the specific influence of the ABC intervention on the DNA methylation profile. Children who received the control intervention displayed consistent changes in DNA methylation sites at 599 sites. These data suggest that children who continue to experience low parental sensitivity exhibit a methylation profile that worsens over time. While longitudinal studies of whole-genome methylation profiles in relation to early-life stress do not yet exist, these findings are

Table 5. CpG sites that changed consistently in all of the children in both ABC and DEF groups

Site	Gene	Distance from TSS	GO annotation	Direction of change in ABC	Direction of change in DEF
cg11344614	JAM2	-1,433	Protein heterodimerization activity	Hyper	Hypo
cg20944548	GSTM2	3,674	Protein homodimerization activity and enzyme binding	Hyper	Hypo
cg24160082	YIPF1	-85	RNA binding and ribonuclease activity	Hypo	Hyper
cg25917609	GOLGA5	264	Protein homodimerization activity and Rab GTPase binding	Hypo	Hyper
cg26593262	GRID2IP	17,808	Long-term synaptic depression	Hyper	Hypo

Note: TSS refers to transcription start site. GO refers to gene ontology.

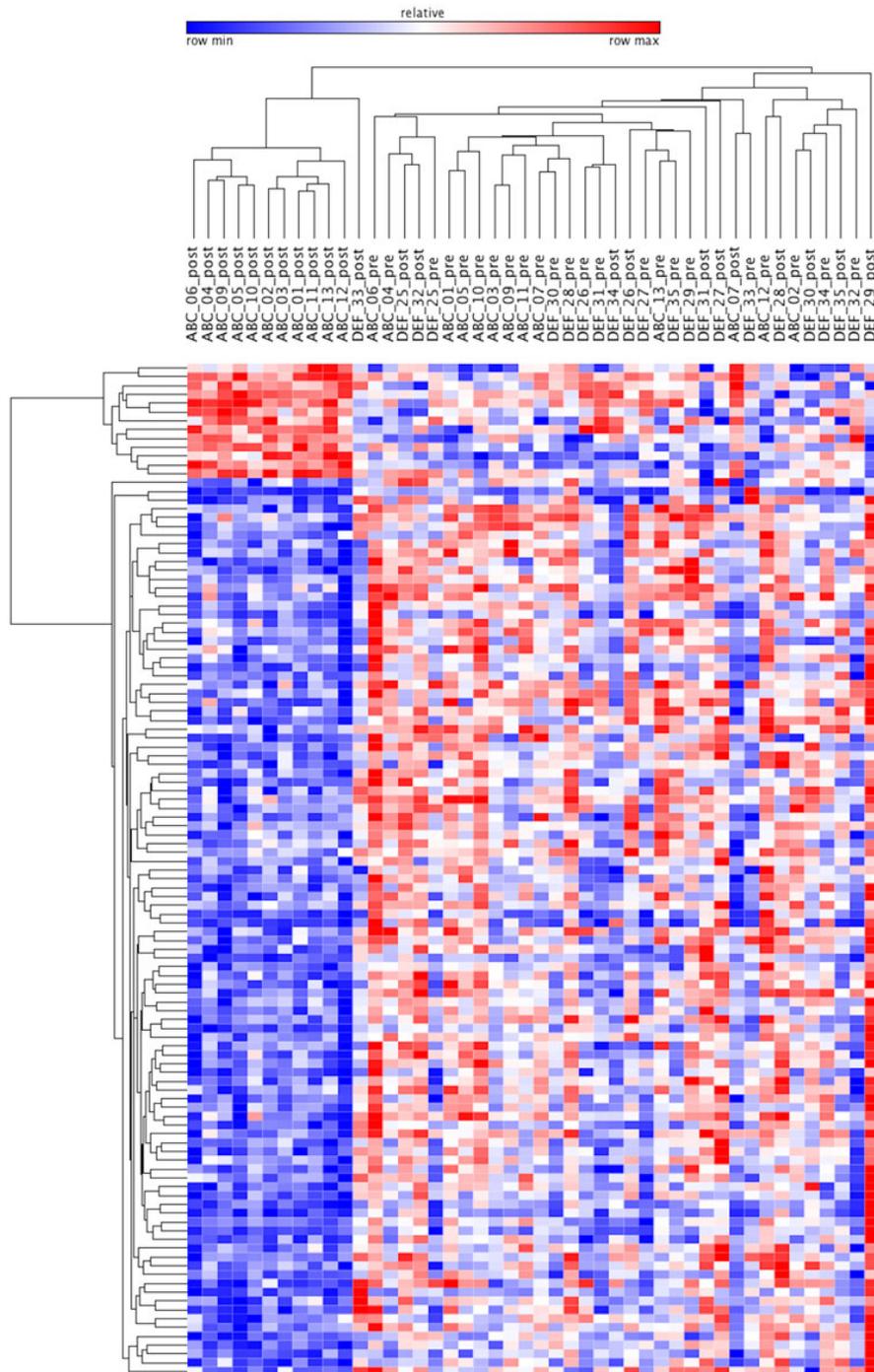


Figure 1. Heatmap representing DNA methylation levels of 115 robustly differentially methylated CpG sites. Each column represents an individual and each row represents one CpG site. The color gradient scale represents relative methylation values. Hierarchical clustering was performed with city block distance.

consistent with literature regarding the effects of institutional care, which indicate that prolonged exposure to adverse environments are associated with increasingly aberrant brain functioning (Stamoulis, Vanderwert, Zeanah, Fox, & Nelson, 2015; Tottenham *et al.*, 2010; Vanderwert, Marshall, Nelson, Zeanah, & Fox, 2010). Overall, our data are consistent with findings

among older children, suggesting that interventions that enhance parenting can alter children's DNA methylation patterns (Brody *et al.*, 2016; O'Donnell *et al.*, 2018).

Sites showing methylation changes mapped to various gene regions, all of which could have functional consequences on gene expression (Table 2; Brenet *et al.*, 2011; Cedar & Bergman,

2009; Moore, Le, & Fan, 2013; Toperoff et al., 2011). The CpG sites that were differentially methylated between the intervention groups aligned to 9,381 individual genes. Thirteen genes contained 10 or more differentially methylated CpG sites (Table 3). In determining the functional relevance of DNA methylation differences, genes that contain multiple differentially methylated sites are more likely to be affected, although a change in methylation at only a few sites within a gene is sufficient to significantly reduce the activity of that gene (Baker-Andersen, Ratnu, & Bredy, 2013; Lubin, Roth, & Sweatt, 2008). The affected genes are involved in a wide range of cellular processes, including calcium ion binding, transcription factor binding, and DNA binding.

Pathway analyses were conducted using the CpG sites that had the greatest degree of differential methylation between children who had received ABC and those who had received DEF (Table 4). These sites are associated with numerous biological pathways, including the regulation of cell signaling, regulation of signal transduction, neuronal differentiation, regulation of a wide range of metabolic processes, and neuron development.

Though speculative, the functional analyses that were conducted within the present study are consistent with previous research on the outcomes that are associated with ABC. Attachment and Biobehavioral Catch-Up is associated with a wide variety of behavioral and biological outcomes. When compared with a control intervention, the children whose parents received ABC exhibited more normative patterns of diurnal cortisol production, neural activity, and autonomic nervous system functioning (Bernard et al., 2015; Bick et al., 2019; Tabachnick et al., 2019). This broad array of outcomes suggests that fundamental biological functions, such as cell-signaling, cell adhesion, and neuronal development may be affected by the intervention. The present analyses of the biological pathways that are associated with differential DNA methylation suggest that ABC alters calcium ion signaling, neurogenesis, cell-substrate adhesion and metabolism. These biological functions may have broad effects on various types of biological regulation and behavior, such as those that were observed as outcomes of ABC.

Limitations

This study has several limitations that should be considered. First, these results should be considered preliminary because this was an exploratory study of methylation within a randomized controlled trial with no replication sample. Second, the present study was limited by a small sample size. However, using a small sample size for whole genome analyses may provide information regarding candidate genes to be analyzed in a large sample using more cost-effective methods. Though it is plausible that the ABC intervention caused beneficial changes in DNA methylation and/or avoided detrimental changes in DNA methylation that are associated with continued exposure to maltreatment, the data presented here should be interpreted with caution and viewed as groundwork for the generation of several hypotheses to be tested in future studies that explore the malleability of epigenetic states that are associated with maltreatment. Specifically, future research may consider whether a similar, consistent epigenetic signature of maltreatment is observed among a cohort of young children. Moreover, future studies may investigate whether such a signature persists over time, is affected by the quality of parenting, or is associated with various outcomes that are typically associated with early experiences of stress, such as biological dysregulation

and deficits in impulse control. Lastly, DNA methylation was examined in saliva samples, which contain various proportions of epithelial cells, leukocytes, and bacteria. As methylation values vary by tissue type, differences in cell-type proportions may affect the measurement of methylation (Smith et al., 2015). Further, we were unable to directly assess methylation within the brain, the organ that is most relevant to the behavioral intervention. It is obviously impossible to measure DNA methylation in the brain in living individuals, and several studies have shown strong correlations between peripheral and central measures of DNA methylation (Smith et al., 2015; Thompson et al., 2013). Consistent with previous studies, we discovered informative DNA methylation associations with a behavioral intervention in a peripheral tissue (Brody et al., 2016; O'Donnell et al., 2018; Perroud et al., 2013; Roberts et al., 2014). Interestingly, many of the pathways that were identified in the present study involve neuronal pathways. These data further support the relevance of peripheral DNA methylation measures to behavioral phenotypes.

Conclusions

In summary, this study is one of the first to explore parenting intervention effects on children's DNA methylation in this young age group, with results suggesting that enhancing parental behaviors during a child's early life changes DNA methylation. Importantly, these changes occurred in a short time span, indicating that an early intervention caused rapid alterations to the methylome. These findings warrant further large-scale research, including identifying whether changes are long-lived and are associated with positive behavioral change in children. Our data suggest several specific CpG sites and genes that may be influenced by early adversity and early intervention, providing a framework for candidate-gene approaches in larger cohorts. Nonetheless, these data suggest the remarkable malleability of a child's epigenome and the potential reversibility of epigenetic states that are associated with maltreatment, contributing to a growing body of research that seeks to understand whether DNA methylation could play a meaningful role in linking early-life experiences to physical and mental health.

Supplementary Material. To view supplementary material for this article, please visit <https://doi.org/10.1017/S0954579419001421>

Acknowledgments. This work was supported by donor funds from Edna Bennett Pierce to M. Dozier.

References

- Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M. ... Sherlock, G. (2000). Gene Ontology: Tool for the unification of biology. *Nature Genetics*, 25, 25–29. doi: 10.1038/75556
- Baker-Andersen, D., Ratnu, V. S., & Bredy, T. W. (2013). Dynamic DNA methylation: A prime candidate for genomic metaplasticity and behavioral adaptation. *Trends in Neuroscience*, 36, 3–13. doi: 10.1016/j.tins.2012.09.003
- Bakermans-Kranenburg, M. J., Van Ijzendoorn, M. H., & Juffer, F. (2003). Less is more: Meta-analyses of sensitivity and attachment interventions in early childhood. *Psychological Bulletin*, 129, 195–215. doi:10.1037/0033-2909.129.2.195
- Bernard, K., Dozier, M., Bick, J., & Gordon, M. K. (2015). Intervening to enhance cortisol regulation among children at risk for neglect: Results of a randomized clinical trial. *Development Psychopathology*, 27, 829–841. doi:10.1017/S095457941400073X
- Bernard, K., Dozier, M., Bick, J., Lewis-Morrarty, E., Lindhiem, O., & Carlson, E. (2012). Enhancing attachment organization among maltreated

- children: Results of a randomized clinical trial. *Child Development*, 83, 623–636. doi:10.1111/j.1467-8624.2011.01712.x
- Bernard, K., Hostinar, C. E., & Dozier, M. (2015). Intervention effects on diurnal cortisol rhythms of Child Protective Services-referred infants in early childhood: Preschool follow-up results of a randomized clinical trial. *JAMA Pediatrics*, 169, 112–119. doi:10.1001/jamapediatrics.2014.2369
- Bick, J., & Dozier, M. (2013). The effectiveness of an attachment-based intervention in promoting foster mothers' sensitivity toward foster infants. *Infant Mental Health Journal*, 34, 95–103. doi:10.1002/imhj.21373
- Bick, J., Naumova, O., Hunter, S., Barbot, B., Lee, M., Luthar, S. S., ... & Grigorenko, E. L. (2012). Childhood adversity and DNA methylation of genes involved in the hypothalamus-pituitary-adrenal axis and immune system: Whole-genome and candidate-gene associations. *Development and Psychopathology*, 24, 1417–1425. doi: 10.1017/S0954579412000806
- Bick, J., Palmwood, E., Zajac, L., Simons, R., & Dozier, M. (2019). Early parenting intervention and adverse family environments affect neural function in middle childhood. *Biological Psychiatry*, 85, 326–335. doi: 10.1016/j.biopsych.2018.09.020.
- Brenet, F., Moh, M., Funk, P., Feierstein, E., Viale, A. J., Socci, N. D., & Scandura, J. M. (2011). DNA methylation of the first exon is tightly linked to transcriptional silencing. *PLoS One*, 6, e14524. doi:10.1371/journal.pone.0014524
- Brody, G. H., Yu, T., Chen, E., Beach, S. R.H., & Miller, G. E. (2016). Family-centered prevention ameliorates the longitudinal association between risky family processes and epigenetic aging. *Journal of Child Psychology and Psychiatry*, 57, 566–574. doi:10.1111/jcpp.12495
- Caron, E. B., Bernard, K., & Dozier, M. K. (2016). In Vivo feedback predicts parent behavior change in the Attachment and Biobehavioral Catch-up Intervention. *Journal of Clinical Child and Adolescent Psychology*, 47, S35–S46. doi:10.1080/15374416.2016.1141359
- Caron, E., Weston-Lee, P., Haggerty, D., & Dozier, M. (2016). Community implementation outcomes of attachment and biobehavioral catch-up. *Child Abuse & Neglect*, 53, 128–137. doi:10.1016/j.chiabu.2015.11.010
- Cedar, H., & Bergman, Y. (2009). Linking DNA methylation and histone modification: Patterns and paradigms. *Nature Review Genetics*, 10, 295–304. doi:10.1038/nrg2540
- Champagne, F. A., Weaver, I. C., Diorio, J., Dymov, S., Szyf, M., & Meaney, M. J. (2006). Maternal care associated with methylation of the estrogen receptor- α 1b promoter and estrogen receptor- α expression in the medial preoptic area of female offspring. *Endocrinology*, 147, 2909–2915. doi:10.1210/en.2005-1119
- Cicchetti, D., Hetzel, S., Rogosch, F. A., Handley, E. D., & Toth, S. L. (2016). An investigation of child maltreatment and epigenetic mechanisms of mental and physical health risk. *Developmental Psychopathology*, 28, 1305–1317. doi:10.1017/S0954579416000869
- Cicchetti, D., & Toth, S. L. (2005). Child maltreatment. *Annual Review of Clinical Psychology*, 1, 409–438. doi: 10.1146/annurev.clinpsy.1.102803.144029
- Dalton, V. S., Kolshus, E., & McLoughlin, D. M. (2014). Epigenetics and depression: Return of the repressed. *Journal of Affective Disorders*, 155, 1–12. doi:10.1016/j.jad.2013.10.028
- Doherty, T. S., Blaze, J., Keller, S. M., & Roth, T. L. (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental Psychobiology*, 59, 703–714. doi:10.1002/dev.21547
- Doherty, T. S., Forster, A., & Roth, T. L. (2016). Global and gene-specific DNA methylation alterations in the adolescent amygdala and hippocampus in an animal model of caregiver maltreatment. *Behavioural Brain Research*, 298, 55–61. doi:10.1016/j.bbr.2015.05.028
- Farh, K. K., Marson, A., Zhu, J., Kleinewietfeld, M., Housley, W. J., Beik, S., ... Bernstein, B. E. (2015). Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*, 518, 337–343. doi:10.1038/nature13835
- Garg, E., Chen, L., Nguyen, T. T.T., Pokhivneva, I., Chen, L. M., Unternaehrer, E., ... Mavan Study Team (2018). The early care environment and DNA methylome variation in childhood. *Developmental Psychopathology*, 30, 891–903. doi:10.1017/S0954579418000627.
- Handy, D. E., Castro, R., & Loscalzo, J. (2011). Epigenetic modifications: Basic mechanisms and role in cardiovascular disease. *Circulation*, 123, 2145–2156. doi:10.1161/CIRCULATIONAHA.110.956839
- Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond. *Nature Review Genetics*, 13, 484–492. doi:10.1038/nrg3230
- Khulan, B., Manning, J. R., Dunbar, D. R., Seckl, J. R., Raikonen, K., Eriksson, J. G., & Drake, A. J. (2014). Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current mental state. *Translational Psychiatry*, 4, e448. doi:10.1038/tp.2014.94
- Lewis-Morrarty, E., Dozier, M., Bernard, K., Terracciano, S. M., & Moore, S. V. (2012). Cognitive flexibility and theory of mind outcomes among foster children: Preschool follow-up results of a randomized clinical trial. *Journal of Adolescent Health*, 51, S17–22. doi:10.1016/j.jadohealth.2012.05.005
- Lind, T., Bernard, K., Ross, E., & Dozier, M. (2014). Intervention effects on negative affect of CPS-referred children: Results of a randomized clinical trial. *Child Abuse & Neglect*, 38, 1459–1467. doi:10.1016/j.chiabu.2014.04.004
- Lubin, F. D., Roth, T. L., & Sweatt, J. D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *Journal of Neuroscience*, 28, 10576–10586. doi:10.1523/jneurosci.1786-08.2008
- Maunakea, A. K., Chepelev, I., Cui, K., & Zhao, K. (2013). Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Research*, 23, 1256–1269. doi:10.1038/cr.2013.110
- McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonté, B., Szyf, M., ... Meaney, M. J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neuroscience*, 12, 342–348. doi:10.1038/nn.2270
- Melchior, M., Moffitt, T. E., Milne, B. J., Poulton, R., & Caspi, A. (2007). Why do children from socioeconomically disadvantaged families suffer from poor health when they reach adulthood? A life-course study. *American Journal of Epidemiology*, 166, 966–974. doi:10.1093/aje/kwm155
- Mi, H., Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D., & Thomas, P. D. (2017). PANTHER version 11: Expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Research*, 45, D183–189. doi:10.1093/nar/gkw1138
- Mi, H., Muruganujan, A., Casagrande, J. T., & Thomas, P. D. Large-scale gene function analysis with the PANTHER classification system. *Nature Protocols*, 8, 1551–1566. doi:10.1038/nprot.2013.092
- Miller, G. E., Chen, E., & Parker, K. J. (2011). Psychological stress in childhood and susceptibility to the chronic diseases of aging: Moving toward a model of behavioral and biological mechanisms. *Psychological Bulletin*, 137, 959–997. doi:10.1037/a0024768
- Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology*, 38, 23–38. doi:10.1038/npp.2012.112
- Morris, T. J., Butcher, L. M., Feber, A., Teschendorff, A. E., Chakravathy, A. R., Wojdacz, T. K., & Beck, S. (2013). ChAMP: 450k chip analysis methylation pipeline. *Bioinformatics*, 30, 428–430. doi:10.1093/bioinformatics/btt684
- Murgatroyd, C., & Spengler, D. (2011). Epigenetic programming of the HPA axis: early life decides. *Stress*, 14, 581–589. doi: 10.3109/10253890.2011.602146
- Naumova, O. Y., Lee, M., Kuposov, R., Szyf, M., Dozier, M., & Grigorenko, E. L. (2012). Differential patterns of whole-genome DNA methylation in institutionalized children and children raised by their biological parents. *Developmental Psychopathology*, 24, 143–155. doi:10.1017/S0954579411000605
- O'Donnell, K. J., Chen, L., MacIsaac, J. L., McEwen, L. M., Nguyen, T., Beckmann, K., ... Meaney, M. J. (2018). DNA methylome variation in a perinatal nurse-visitation program that reduces child maltreatment: A 27 year follow-up. *Translational Psychiatry*, 8(15), 1–9. doi:10.1038/s41398-017-0063-9
- Olds, D. L., Eckenrode, J., Henderson, C. R., Kitzman, H., Powers, J., Cole, R., ... & Luckey, D. (1997). Long-term effects of home visitation on maternal life course and child abuse and neglect: Fifteen-year follow-up of a randomized trial. *JAMA*, 278, 637–643. doi:10.1001/jama.1997.03550080047038
- Olds, D., Henderson Jr, C. R., Cole, R., Eckenrode, J., Kitzman, H., Luckey, D., ... & Powers, J. (1998). Long-term effects of nurse home

- visitation on children's criminal and antisocial behavior: 15-year follow-up of a randomized controlled trial. *JAMA*, 280, 1238–1244. doi:10.1001/jama.280.14.1238
- Olds, D. L., Kitzman, H., Cole, R., Robinson, J., Sidora, K., Luckey, D. W., ... & Holmberg, J. (2004). Effects of nurse home-visiting on maternal life course and child development: Age 6 follow-up results of a randomized trial. *Pediatrics*, 114, 1550–1559. doi:10.1542/peds.2004-0962
- Perroud, N., Salzmann, A., Prada, P., Nicastrò, R., Hoeppli, M. E., Furrer, S. ... Malafosse, A. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Translational Psychiatry*, 3, e207. doi:10.1038/tp.2012.140
- Prados, J., Stenz, L., Courtet, P., Prada, P., Nicastrò, R., Adouan, W., ... Perroud, N. (2015). Borderline personality disorder and childhood maltreatment: A genome-wide methylation analysis. *Genes Brain Behavior*, 14, 177–188. doi:10.1111/gbb.12197
- Ramey, C. T., McGinness, G. D., Cross, L., Collier, A. M., & Barrie-Blackley, S. (1982). The Abecedarian approach to social competence: Cognitive and linguistic intervention for disadvantaged preschoolers. In K. Borman (Ed.), *The social life of children in a changing society* (145–174). Hillsdale, NJ: Erlbaum.
- R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, 43, e47. doi:10.1093/nar/gkv007
- Roberts, S., Lester, K. J., Hudson, J. L., Rapee, R. M., Creswell, C., Cooper, P. J., ... Eley, T. C. (2014). Serotonin transporter methylation and response to cognitive behaviour therapy in children with anxiety disorders. *Translational Psychiatry*, 4, e444. doi:10.1038/tp.2014.83
- Roth, T. L., Lubin, F. D., Funk, A. J., & Sweatt, J. D. (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry*, 65, 760–769. doi:10.1016/j.biopsych.2008.11.028
- Sameroff, A. J., & Fiese, B. H. (2000). Transactional regulation: The developmental ecology of early intervention. *Handbook of early childhood intervention*, 2, 135–159.
- Smith, A. K., Kilaru, V., Klengel, T., Mercer, K. B., Bradley, B., Conneely, K. N., ... Binder, E. B. (2015). DNA extracted from saliva for methylation studies of psychiatric traits: evidence tissue specificity and relatedness to brain. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, 168, 36–44. doi:10.1002/ajmg.b.32278
- Stamoulis, C., Vanderwert, R. E., Zeanah, C. H., Fox, N. A., & Nelson, C. A. (2015). Early psychosocial neglect adversely impacts developmental trajectories of brain oscillations and their interactions. *Journal of Cognitive Neuroscience*, 27(12), 2512–2528. doi:10.1162/jocn_a_00877
- Tabachnick, A. R., Raby, K. L., Goldstein, A., Zajac, L., & Dozier, M. (2019). Effects of an attachment-based intervention in infancy on children's autonomic regulation during middle childhood. *Biological Psychology*, 143, 22–31. doi:10.1016/j.biopsycho.2019.01
- Teschendorff, A. E., Marabita, F., Lechner, M., Bartlett, T., Tegner, J., Gomez-Cabrero, D., & Beck, S. (2012). A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics*, 29, 189–196. doi:10.1093/bioinformatics/bts680
- Thompson, T. M., Sharfi, D., Lee, M., Yrigollen, C. M., Naumova, O. Y., & Grigorenko, E. L. (2013). Comparison of whole-genome DNA methylation patterns in whole blood, saliva, and lymphoblastoid cell lines. *Behavior Genetics*, 43, 168–176. doi:10.1007/s10519-012-9579-1
- Toperoff, G., Aran, D., Kark, J. D., Rosenberg, M., Dubnikov, T., Nissan, B. ... Hellman, A. (2011). Genome-wide survey reveals predisposing diabetes type 2-related DNA methylation variations in human peripheral blood. *Human Molecular Genetics*, 21, 371–383. doi:10.1093/hmg/ddr472
- Toth, S. L., Gravener-Davis, J. A., Guild, D. J., & Cicchetti, D. (2013). Relational interventions for child maltreatment: Past, present, and future perspectives. *Development Psychopathology*, 25, 1601–1617. doi:10.1017/S0954579413000795
- Tottenham, N., Hare, T. A., Quinn, B. T., McCarry, T. W., Nurse, M., Gilhooly, T., ... & Thomas, K. M. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Developmental Science*, 13, 46–61. doi:10.1111/j.1467-7867.2009.00852.x
- Unternaehrer, E., Meyer, A. H., Burkhardt, S. C., Dempster, E., Staehli, S., Theill, N., ... Meinschmidt, G. (2015). Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women. *Stress*, 18, 451–461. doi:10.3109/10253890.2015.1038992
- Vanderwert, R. E., Marshall, P. J., Nelson III, C. A., Zeanah, C. H., & Fox, N. A. (2010). Timing of intervention affects brain electrical activity in children exposed to severe psychosocial neglect. *PLoS One*, 5, e11415. doi:10.1371/journal.pone.0011415
- Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., ... Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, 7, 847–854. doi:10.1038/nn1276
- Weinfeld, N. S., Sroufe, L. A., Egeland, B., & Carlson, E. (2008). Individual differences in infant-caregiver attachment: Conceptual and empirical aspects of security. In Cassidy, J., & Shaver, P. R. (Eds.) *Handbook of attachment: Theory, research, and clinical applications* (2nd ed., pp. 78–101). NY: Guilford Press.
- Yang, B. Z., Zhang, H., Ge, W., Weder, N., Douglas-Palumberi, H., Perepletchikova, F. ... Kaufman, J. (2013). Child abuse and epigenetic mechanisms of disease risk. *American Journal of Preventive Medicine*, 44, 101–107. doi:10.1016/j.amepre.2012.10.012
- Yarger, H. A., Bernard, K., Caron, E. B., Wallin, A., & Dozier, M. (2019). Enhancing parenting quality for young children adopted internationally: Results of a randomized controlled trial. *Journal of Clinical Child & Adolescent Psychology*, 16, 1–13. doi:10.1080/15374416.2018.1547972
- Yarger, H. A., Hoye, J. R., & Dozier, M. (2016). Trajectories of change in Attachment and Biobehavioral Catch-Up among high-risk mothers: A randomized clinical trial. *Infant Mental Health Journal*, 37, 525–536. doi:10.1002/imhj.21585
- Yehuda, R., Flory, J. D., Bierer, L. M., Henn-Haase, C., Lehrner, A., Desarnaud, F., ... Meaney, M. J. (2015). Lower methylation of glucocorticoid receptor gene promoter 1 F in peripheral blood of veterans with post-traumatic stress disorder. *Biological Psychiatry*, 77, 356–364. doi:10.1016/j.biopsych.2014.02.006
- Ziller, M. J., Gu, H., Müller, F., Donaghey, J., Tsai, L. T., Kohlbacher, O., ... Meissner, A. (2013). Charting a dynamic DNA methylation landscape of the human genome. *Nature*, 500, 477–481. doi:10.1038/nature12433