HHS Public Access

Author manuscript

J Am Acad Child Adolesc Psychiatry. Author manuscript; available in PMC 2015 April 01.

Published in final edited form as:

J Am Acad Child Adolesc Psychiatry. 2014 April ; 53(4): 417–24.e5. doi:10.1016/j.jaac.2013.12.025.

Child Abuse, Depression, and Methylation in Genes Involved with Stress, Neural Plasticity, and Brain Circuitry

Natalie Weder, M.D.,
Child Mind Institute

Huiping Zhang, Ph.D.,
Yale University School of Medicine

Kevin Jensen, Ph.D.,
Yale University School of Medicine

Bao Zhu Yang, Ph.D.,
Yale University School of Medicine

Arthur Simen, M.D., Ph.D.,
Yale University School of Medicine

Merck Research Laboratories

Andrea Jackowski, Ph.D.,
LiNC, Universidade Federal de São Paulo, São Paulo, Brazil

Deborah Lipschitz, M.D.,
Yale University School of Medicine

Heather Douglas-Palumberi, M.A.,
Yale University School of Medicine

Margrat Ge, M.A.,
Yale University School of Medicine

Francheska Perepletchikova, Ph.D.,
Yale University School of Medicine

Kerry O'Laughlin, B.A.,
University of Vermont, Vermont Center for Children, Youth, and Families

James J. Hudziak, M.D.,

Correspondence to: Joan Kaufman, Ph.D., Yale University, Department of Psychiatry, Child and Adolescent Research and Education (CARE) Program, Congress Place, 301 Cedar Street, P.O. Box 208098, New Haven, CT. 06520. joan.kaufman@yale.edu.

Dr. Kaufman has served as a consultant for Merck Pharmaceutical. Drs. Zhang, Jensen, Yang, Simen, Jackowski, Lipschitz, Perepletchikova, and Gelernter, and Ms. Douglas-Palumberi, Ge, and O'Loughlin report no biomedical financial interests or potential conflicts of interest.

University of Vermont, Vermont Center for Children, Youth, and Families

Joel Gelernter, M.D., and
Yale University School of Medicine

Joan Kaufman, Ph.D.
Child Mind Institute

Yale University School of Medicine

Abstract

Objectives—Determine if epigenetic markers predict dimensional ratings of depression in maltreated children.

Method—A Genome-wide methylation study was completed using the Illumina 450K BeadChip array in 94 maltreated and 96 non-traumatized children with saliva-derived DNA. The 450K BeadChip does not include any methylation sites in the exact location as sites in candidate genes previously examined in the literature, so a test for replication of prior research findings was not feasible.

Results—Methylation in three genes emerged as genomewide-significant predictors of depression: DNA-Binding Protein Inhibitor ID-3 (*ID3*); Glutamate Receptor, Ionotropic NMDA 1 (*GRIN1*); and Tubulin Polymerization Promoting Protein (*TPPP*) ($p<5.0 \times 10^{-7}$, all analyses). These genes are all biologically relevant–with *ID3* involved in the stress response, *GRIN1* involved in neural plasticity, and *TPPP* involved in neural circuitry development. Methylation in CpG sites in candidate genes were not predictors of depression at significance levels corrected for whole genome testing, but maltreated and control children did have significantly different beta values after Bonferroni correction at multiple methylation sites in these candidate genes (e.g., *BDNF, NR3C1, FKBP5)*.

Conclusion—This study suggests epigenetic changes in *ID3*, *GRIN1*, and *TPPP* genes, in combination with experiences of maltreatment, may confer risk for depression in children. It adds to a growing body of literature supporting a role for epigenetic mechanisms in the pathophysiology of stress-related psychiatric disorders. While epigenetic changes are frequently long lasting, they are not necessarily permanent. Consequently, interventions to reverse the negative biological and behavioral sequelae associated with child maltreatment are briefly discussed.

Keywords

Child Abuse; Depression; Methylation; Epigenetics

INTRODUCTION

Child abuse is highly prevalent and is associated with increased risk for a range of health problems including: cancer,[1,2] cardiovascular disease,[2,3] diabetes,[2,3] and multiple psychiatric disorders, including depression.[4,5] Epigenetics has been hypothesized as one possible mechanism to explain the association between adverse childhood experiences and later health problems.[6,7] Epigenetics refers to chemical modifications to the genome that

regulate gene activity, but do not involve a change in DNA nucleotide sequence.[8] DNA methylation, which occurs mainly at CpG sites, regions where cytosine nucleotides occur next to guanine nucleotides,[9] is one of the most studied epigenetic mechanisms.

As a preliminary test of the hypothesis that child abuse may confer risk for a range of health problems through epigenetic mechanisms, we examined genomewide methylation differences in a sample of 96 maltreated and 96 healthy non-traumatized comparison children using the Illumina 450K BeadChip.[10] After controlling for multiple comparisons, maltreated and comparison children had significantly different saliva derived DNA methylation values at 2,868 CpG sites ($p < 5.0 \times 10^{-7}$, all sites), with the set of genes showing significant methylation differences including numerous known markers for cancer, cardiovascular disease, diabetes, and psychiatric disorders.

To date, most studies examining epigenetic changes associated with depression have used candidate gene approaches, and all studies have examined methylation in gene promoter regions. While gene regulation is influenced by DNA methylation in other regions of the genome, the impact of methylation in promoters is presently best understood; it usually leads to reduced expression. Methylation in the promoter region of the serotonin transporter *(SLC6A4)* gene determined from peripheral DNA has been reported to interact with *SLC6A4* genotype to predict depressive symptoms in adolescents;[11] brain derived neurotrophic factor (*BDNF*) methylation profiles derived from peripheral blood cells have been found to correctly classify patients with major depressive disorder,[12] and preliminary data suggest promoter associated methylation of the FK506 binding protein 5 (*FKBP5*) gene mediates the combined effect of genetic (e.g., *FKBP5* high-risk polymorphisms) and environmental (e.g., child abuse) risk for stress related psychiatric disorders.[13] Increased promoter associated glucocorticoid receptor (*NR3C1)* gene methylation in the hippocampus has also been associated with suicide completion in individuals with a history of early child abuse in two independent studies.[14,15] Suicide completers without a history of childhood abuse did not have increased methylation of the *NR3C1* gene when compared to controls, suggesting depression-associated methylation profiles may be different in depressed individuals with and without a history of early adversity.[14,15]

The goal of this study is to identify novel methylation markers associated with depression in maltreated children using the Illumina 450K BeadChip. The 450K BeadChip, in addition to examining methylation in promoter associated CpG sites, also assays CpG sites involved in gene regulation located on the gene body, 3′ untranslated regions (3′UTR), 5′UTRs, and intergenic regions.[16] Unfortunately the Illumina 450K Beadchip does not include any methylation sites in the promoter regions of *SLC6A4* or *BDNF*, and the sites it does include in *FKBP5* and *NR3C1* are not identical to the sites previously examined in the literature, making tests of replicability of prior research findings infeasible.

METHOD AND MATERIALS

Sample

Participants included 190 children: 94 maltreated children recruited within six months of being removed from their parents' care due to reports of abuse and/or neglect, and 96

healthy controls with no history of maltreatment or exposure to intrafamilial violence, and no lifetime history of psychiatric illness. Two maltreated children who were included in our prior report comparing genome-wide methylation values between maltreated and control children were excluded here due to missing depression scale data.[10] All maltreated children in this investigation were also included in our published reports of genetic and environmental factors associated with depression;[17,18] the cohort of controls was expanded for this current investigation. The 190 children were from 136 families with various numbers of sibs and half-sibs (range, 0–4) in each family. Children ranged in age from 5–14 years, with a mean age of 10.2. The sample was 42% male, and of mixed racial origin (17% European-American, 38% Hispanic, 30% African-American, and 15% biracial). Maltreated and control cohorts did not differ in terms of age ($t = 0.2$, df=190, ns), sex ($\chi^2 = 0.1$, df=1, ns), or race ($\chi^2 = 3.3$, df=3, ns). Recruitment and consent procedures are detailed elsewhere.[17,18]

Yale University Human Investigations Committee and Connecticut Department of Children and Families Institutional Review Board approved this research.

Psychiatric Diagnoses

The semi-structured child psychiatric diagnostic interview the Schedule for Affective Disorders and Schizophrenia (K-SADS-PL),[19] was administered to each child and one biological parent or a relative caregiver. A foster parent or residential staff member completed the Child Behavior Checklist (CBCL)[20] when no biological relative was available to complete the psychiatric interview (n=32). In deriving 'best estimate' psychiatric diagnoses,[21] all clinical material was reviewed during a multi-disciplinary team meeting led by a licensed child psychologist (JK) and a board certified child psychiatrist (DL). Final diagnoses were assigned by consensus agreement between the chairs of this meeting and the researcher responsible for collecting the interview data with the child. In addition to K-SADS-PL and CBCL data, clinical data obtained and reviewed to derive best estimate diagnoses included Child Dissociative Checklist (CDC),[22] a 20-item parent-report scale, and Teachers Report Form (TRF).[20] Maltreated children additionally completed the Posttraumatic Stress Disorder Checklist (PTSD-CL),[23] a 17-item measure that assesses PTSD re-experiencing, avoidance, and hyperarousal symptoms. Healthy controls were selected for this pilot study, so by inclusion criteria definition, no controls met diagnostic criteria for any psychiatric diagnosis. Among maltreated children, PTSD was most common diagnosis, with 50% of maltreated children meeting full diagnostic criteria for the disorder. In addition, 35% of maltreated children met criteria for a depressive disorder (MDD-12%; DD-17%; DD-NOS-17%); and 25% met criteria for a behavioral disorder (ADHD-12% ODD-13%; CD-5%). There was considerable comorbidity, with 88%% of the children meeting criteria for a depressive disorder also meeting full diagnostic criteria for PTSD.

Maltreatment

Multiple informants and data sources (e.g., parents, children, and protective services case records) were used to obtain a best estimate of each child's maltreatment history using procedures detailed previously.[24] Specific data sources examined included: the child protective services child abuse and neglect investigation reports; parent and child responses

to the trauma screen items included on the KSADS child psychiatric interview,[19] child responses on the Child Trauma Questionnaire,[25] and mother's reports of domestic violence on the Partner Violence Inventory.[26] Before the maltreated children's removal from their parents' care, the children in this study had a mean of three substantiated reports of abuse or neglect (range: 1–7). In addition, 92% of the children experienced more than one type of maltreatment: 65% had a history of physical abuse, 24% sexual abuse, 83% neglect, 65% emotional abuse, and 70% witnessed domestic violence.

Depression

The Mood and Feelings Questionnaire (MFQ) was used to assess children's depression symptomatology. MFQ is a 33-item self-report measure that assesses depression in children, with each item rated on a 0–2 point scale.[27] It has excellent psychometric properties and has been used extensively in clinical and epidemiological research.[17,18,28–30] The measure was individually administered. Research assistants read the MFQ items to children and used pictorial scoring aids to facilitate administration with younger children. Maltreated and comparison children reported a significant degree and wide range of depression symptoms, with depression scores of maltreated children, as expected, significantly greater than the scores of comparison children (Wald Statistic = 30.1, $p < .001$; Maltreated Children: Mean 17.4 ± 11.2, Range 0–46; Comparison Children: Mean 9.9 ± 7.3, Range 0–29). Twenty-six percent of maltreated children, and 4% of controls scored 27 or above, the clinical threshold on the MFQ depression scale.

DNA Specimens

Saliva for DNA extraction was collected from maltreated children at a time of acute stress: within six months of an incident of maltreatment of sufficient severity to warrant out-of-home placement. Specimens were refrigerated within two hours of collection and DNA extracted using Puregene (Gentra, Minneapolis, MN, USA) kits. To prepare specimens for methylation study, 500 ng of genomic DNA were treated with bisulfite reagents included in EZ-96 DNA methylation kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. Unmethylated cytosines were converted to uracils while methylated cytosines remained unchanged. Bisulfite-converted DNA samples were then used in the array-based DNA methylation assay.

Array-based genome-wide DNA methylation assays

The Illumina 450K Methylation BeadChip was used in the current investigation. This BeadChip interrogates>485,000 CpG sites per sample at single-nucleotide resolution covering most (96%) designable RefSeq genes. Array-based epigenome-wide methylation analyses were completed at Keck Biotechnology Laboratory at Yale University using standard procedures. GenomeStudio software (Illumina, San Diego, CA) was used to generate beta values for each CpG site, with beta values ranging from 0.0–1.0, quantifying the ratio of methylated allele in fluorescent signals at each CpG site. Raw scanned data were normalized; average beta values were recalculated using background intensity measured by negative background probes present on array. Standard quality control tests were run. CpG sites with detection p values>.001 were removed to ensure only high-confidence probes were included in subsequent analysis (30/485,578 CpG sites were removed, .006% of sites).

Validation of Array Methylation Values

To validate DNA methylation values observed with the Illumina 450K methylation BeadChip assay, the Sequenom MassARRAY EpiTYPER approach (Sequenom, San Diego, CA, USA) was used to examine retest methylation levels at seven CpG sites. The methods and forward and backward primers (plus tags) used for these analyses are available from the corresponding author upon request.

Data Analyses

In order to take familial correlations into consideration while examining methylation predictors of depression, data were analyzed using linear mixed effects model (LME), which addresses familial correlations in the sample by assigning a random effect to each family. Demographic variables age, sex, or race were not related to children's depression scores, but were included in the LME model to normalize residuals. Given heteroscedasticity of beta values, as recommended by Du and colleagues,[31] M-values (logit transformation in log2 scale) were used in all analyses. To correct for multiple comparison testing, significance threshold for analyses was set to 5.0×10^{-7}, consistent with level recommended by Rakyan, Beck and colleagues.[32]

After identifying methylation sites that individually predicted children's depression scores, a generalized estimating equations (GEE) analysis was conducted to examine in a single model the combined effect of children's maltreatment status and methylation values at each significant CpG site. GEE analysis was used to control for familial correlations between subjects resulting from the inclusion of siblings in the sample, and square root transformed depression scores were used in this analysis. Pearson correlations were conducted to determine the similarity in methylation values derived using the Illumina BeadChip array and follow-up Sequenom methods.

RESULTS

Epigenetic Predictors of Depression in Children

After correction for multiple comparisons, methylation values at CpG sites in three genes emerged as significant predictors of depression scores ($p<5.0 \times 10^{-7}$, all analyses), and methylation of a CpG site in a fourth gene fell just short of significance. The genes associated with these CpG sites and results of analysis are depicted in Table 1. Lower depression scores were associated with greater methylation at the CpG sites within *ID3* ($r = -0.34$, $p<.001$), *GRIN1* ($r = -0.37$, $p<.001$), and *TPPP* ($r = -0.39$, $p<.001$).

Methylation changes in these genes appear to be independent predictors of depression, above and beyond the effects of maltreatment history. When a follow-up GEE analysis was conducted examining the impact of maltreatment history and methylation values of each of the three significant CpG sites in one analysis, as depicted in Table 2, all main effect terms were significant ($p<.01$, all terms). No significant interactions were observed ($p>.05$, all interactions). Age, race, and sex were not related to methylation values in these genes, and as noted previously, these covariates were not predictors of depression scores.

Validation of Array Methylation Values—Methylation values derived using the Illumina array were highly correlated with values derived using the Sequenom MassARRAY EpiTYPER approach (r=0.96, p<.0001).

Exploratory Analyses – Cortisol Data—It was hypothesized that variation in salivary cortisol would be predicted by *ID3* CpG site methylation given upregulation of *ID3* in the pituitary in response to stress.[33] Exploratory analyses were performed on a preexisting dataset of basal salivary cortisol data available for a subset of 67 children: 44 Maltreated; 23 Control Children. A GEE analysis found a significant main effect for maltreatment (Wald statistic 15.99, p<.0001) and a maltreatment x *ID3* methylation interaction (Wald statistic 14.12, p<.0001) in predicting diurnal cortisol secretion, a measure which was previously shown to predict depression in maltreated children.[34] *ID3* methylation was negatively related to diurnal cortisol secretion in controls, but positively associated with diurnal cortisol secretion in maltreated children (Maltreated r = 0.35, p<.02; Control r = −0.53, p<.01). Within the maltreated cohort, *ID3* methylation also correlated significantly with morning cortisol measures (r = 0.49, p<.001). Methylation levels in *TPPP* and *GRIN1* did not predict cortisol secretion (p>.05, all comparisons), providing convergent and discriminant validity data.

Examination of Methylation Values at Additional CpG Sites in ID3, GRIN1, and TPPP—All three significant CpG sites identified in *ID3*, *GRIN1*, and *TPPP* were located on gene body, where methylation is believed to enhance gene transcription.[35] The 450K Illumina chip includes a total of 19 CpG sites in *ID3*, 40 CpG sites in *GRIN1*, and 56 CpG sites in *TPPP*. Methylation values in one 3′UTR and one promoter CpG site in *ID3*, seven gene body CpG sites in *GRIN1*, and two 3′UTR, one 5′UTR, and one gene body CpG site in *TPPP* significantly predicted children's depression scores, at uncorrected significance levels, more than twice the number expected by chance. None of these CpG sites, however, withstood controlling for genomewide testing, and only one CpG site on the gene body in *GRIN1* was still significant after Bonferroni correction for the number of CpG sites examined within *GRIN1* (e.g., 40 sites, p<.00125). Among the CpG sites contained in *ID3*, *TPPP*, and *GRIN1*, differences in the methylation values of maltreated and comparison children reached uncorrected significance levels at 33 sites, 11 of which withstood Bonferroni correction. Results of these analyses are available from the corresponding author upon request.

Secondary Analyses-Examination of CpG Sites in Prior Investigated Candidate Genes—Methylation values in CpG sites in candidate genes examined in prior studies (e.g., *SLC6A4, BDNF, NR3C1, FKBP5)* were not predictors of depression in children at significance levels corrected for whole genome testing ($p < 5.0 \times 10^{-7}$). As noted in the introduction, 450K BeadChip does not include any CpG sites in promoter regions of *SLC6A4* or *BDNF*, and sites it does include in promoter regions of *NR3C1* and *FKBP5* are different than sites previously examined in the literature, so a test for replication of prior research findings is not feasible. Figure S1 and S2, available online depict the proximity of Illumina 450K CpG sites examined in *NR3C1* and *FKBP5* to sites in the genes previously examined in the literature (See Supplement 1, available online). The Illumina 450K

Beadchip includes 16 CpG sites in *SLC6A4*, 77 sites in *BDNF*, 41 sites in *NR3C1* and 34 sites in *FKBP5*, and Table S1, available online contains the results of analyses examining methylation in candidate genes *SLC6A4*, *BDNF*, *NR3C1*, and *FKBP5* as predictors of children's depression scores. Methylation values of three CpG sites in *SLC6A4*, six sites in *BDNF*, two sites in *NR3C1*, and three sites in *FKBP5* significantly predicted children's depression scores, at uncorrected significance levels, the level of significance utilized in most a priori hypothesized candidate gene studies. Also presented in Table S1 are maltreated versus control group differences in candidate gene CpG sites. Differences in methylation values of maltreated and comparison children reached traditional levels of significance at 74 sites, with significance thresholds withstanding Bonferroni correction for 9 sites in *BDNF*, 4 sites in *FKBP5, and* 6 sites in *NR3C1*, one which was significant after correcting for whole genome testing ($p = 2.0 \times 10^{-7}$). All the sites in *BDNF* are located on gene body, all sites in *NR3C1* are located in promoter regions, and 1 site in *FKBP5* is located on gene body, one at 3′UTR site, and two within promoter regions. The means and standard deviations of these 19 sites for maltreated and control children are available from the corresponding author upon request. Maltreated children had significantly reduced methylation at one 3′UTR site, at 8 out of 10 gene body sites, and at 6 out of 8 promoter associated sites. Maltreated children who met criteria for PTSD (n=47) and who did not meet criteria for PTSD (n=47) had comparable methylation values at each of these candidate gene sites ($p > .05$, all comparisons).

Cortisol and Methylation in Canddiate Genes—Correlations were examined between morning cortisol values and methylation values in the six *NR3C1* and four *FKBP5* CpG sites identified in the analyses above. Methylation in two sites in *NR3C1* (cg04111177 r=0.49, $p < .001$; cg11152298 r=0.33 $p < .01$) and one site in *FKBP5* (cg00610228 r=0.34, $p < .03$) significantly predicted morning cortisol values, and methylation in a second *FKBP5* site showed a trend toward significance (cg07633853 r=−0.25, p<.10).

DISCUSSION

After controlling for whole genome testing, this study found methylation in three genes significantly predicted depression scores in children: *ID3*, *TPPP*, and *GRIN1*. These genes are all biologically relevant – involved in the stress response, neural plasticity, and neural circuitry. Specifically, *ID3* is upregulated in the pituitary in response to chronic stress,[36] and in the current study, predicted basal cortisol levels in the children. *ID3* is also upregulated with stimulation by pituitary adenylate cyclase-activating polypeptide (PACAP).[33] This is interesting as variation in the gene that encodes for PACAP has recently been associated with risk for PTSD, a stress-related neuropsychiatric disorder that is frequently comorbid with depression,[37] although this result was not replicated in a second study.[38] *ID3* is also involved in neurogenesis and has been implicated in neural plasticity.[39] *TPPP* is critical for oligodendrocyte differentiation,[40] and *TPPP* is present in myelinating oligodendrocytes, and believed to have a role in development and maintenance of white matter tracts in brain.[41,42] *GRIN1* transcription is downregulated in frontal cortex in response to stress in animal models of depression,[43] glutamate is implicated in pathophysiology of depression and anxiety disorders,[44,45] and NMDA receptors play a critical role in synaptic plasticity,

memory, and fear conditioning.[46] Methylation changes in these genes appear to be independent predictors of depression, above and beyond the effects of maltreatment history.

In this investigation it was not feasible to examine replicability of prior candidate gene findings, given differences in the CpG sites included on the 450K Illumina BeadChip and the CpG sites examined in prior studies. Methylation values of three CpG sites in *SLC6A4*, six sites in *BDNF*, two sites in *NR3C1*, and three sites in *FKBP5* significantly predicted children's depression scores, at uncorrected significance levels; and differences in methylation values of maltreated and comparison children reached traditional levels of significance at 74 sites within these prior studied candidate genes, with significance thresholds withstanding Bonferroni correction for 9 sites in *BDNF*, 4 sites in *FKBP5, and* 6 sites in *NR3C1*, one that was significant after correcting for whole genome testing. All the sites in *BDNF* were located on the gene body, all sites in *NR3C1* were located in promoter regions, and 1 site in *FKBP5* was located on the gene body, one at 3′UTR site, and two within promoter regions. Knowledge about epigenetic mechanisms of gene regulation is advancing rapidly, however, the full implication of methylation changes in various areas of the genome are not fully understood.

The current investigation is limited by its modest sample size, the absence of gene expression data, and failure to examine polymorphisms that may have moderated the impact of child maltreatment on methylation values and depression outcomes. This study lays the groundwork, however, for future work in this area. While there is controversy in the field about use of peripheral DNA methylation markers to study tissue-specific disease processes, there are emerging research findings across multiple areas of medicine documenting the utility of peripheral DNA methylation measures in understanding disease pathology and deriving biomarker sets to predict risk, diagnosis, and prognosis.[10,47–49]

This study adds to a growing body of literature highlighting the importance of epigenetic modifications in the pathophysiology of early adversity-related psychiatric illnesses, and expands the focus of research beyond genes selected based on a priori hypotheses. As discussed in the introduction, child abuse is associated with a whole host of adverse health outcomes. Recent studies have found early adversity to be linked to epigenetic changes in genes involved in metabolic processes,[50] immune functioning,[51], and genes implicated diabetes, cardiovascular disease, and cancer, in addition to genes implicated in psychiatric disease.[10] Epigenetic mechanisms appear to hold significant promise in understanding how adverse early childhood experiences confer risk for a range of health problems later in life.

It is important to note, however, while epigenetic changes are frequently long lasting, they are not necessarily permanent.[52,53] Some brain and behavior changes previously perceived as permanent secondary to epigenetic modifications resulting from adverse early experiences have now been shown to be reversible and amenable to treatment.[54,55] In addition, emerging data suggest that the window of opportunity for intervention is wider than initially perceived. It is now appreciated that while there are "sensitive periods" when children are more susceptible to environmental influences, the opportunity to promote positive brain and behavioral changes persists into adulthood.[53,56] Positive adaptation in cohorts of maltreated children can be promoted with interventions that focus on: 1)

developing secure attachment relations;[17,57–59] 2) facilitating enrichment opportunities;[56,60] and 3) providing clinical interventions to address child and parent psychopathology.[61–66] While a history of abuse is frequently associated with deleterious outcomes, not all abused individuals develop problems. Ongoing multidisciplinary and translational work in this area will increase our understanding of the mechanisms by which early abuse confers risk for depression, and help to identify novel, more effective treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Leon Levy Foundation (NW), a Brain and Behavior Research NARSAD Young Investigator award (B-ZY), a pilot grant from the Yale Center (AS); a grant from the Conway family (AS), funding from the National Institutes of Health – T32 MH067763 (NW), K01 DA24758 (B-ZY), DA022251 (AS), K99/R00DA022891 (HZ), K23 MH01789 (DL), DA12849 (JG), DA12690 (JG), AA017535 (JG), AA11330R01 (JG), MH077087 (JK), MH65519, MH098073 (JK, JH), the National Center for Posttraumatic Stress Disorder-Veterans Affairs Connecticut (HD-P, JG, JK), and the VA Depression Research Enhancement Award Program (Veterans Affairs Connecticut; JG, JK).

Dr. Bao-Zhu Yang, PhD, served as the statistical expert for this research.

The authors thank the children and families who participated in this research, and the administration of the Connecticut Department of Children and Families for their collaboration on this effort.

Disclosures. Dr. Weder receives royalties from Lippincott, Williams, and Wilkins. Dr. Hudziak has received grant or research funding from the National Institute of Mental Health and the National Institute of Diabetes and Digestive and Kidney Disease. His primary appointment is with the University of Vermont. He has additional appointments with Erasmus University in Rotterdam, Netherlands, Washington University School of Medicine in St. Louis, Missouri, Dartmouth School of Medicine in Hanover, New Hampshire, and Avera Institute of Human Behavioral Genetics in Sioux Falls, South Dakota. He is the Associate Editor of *JAACAP*.

References

1. Brown DW, Anda RF, Felitti VJ, et al. Adverse childhood experiences are associated with the risk of lung cancer: a prospective cohort study. BMC Public Health. 2010; 10(20):20. [PubMed: 20085623]
2. Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. Am J Prev Medicine. 1998; 14(4):245–258. 19980831 DCOM- 19980831.
3. Romans S, Belaise C, Martin J, Morris E, Raffi A. Childhood abuse and later medical disorders in women. An epidemiological study. Psychother Psychosom. May-Jun;2002 71(3):141–150. [PubMed: 12021556]
4. Kendler KS, Bulik CM, Silberg J, Hettema JM, Myers J, Prescott CA. Childhood sexual abuse and adult psychiatric and substance use disorders in women: an epidemiological and cotwin control analysis. Arch Gen Psychiatry. Oct; 2000 57(10):953–959. [PubMed: 11015813]
5. Molnar BE, Buka SL, Kessler RC. Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. Am J Public Health. May; 2001 91(5):753–760. [PubMed: 11344883]
6. McEwen BS, Eiland L, Hunter RG, Miller MM. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology. Jan; 2012 62(1):3–12. [PubMed: 21807003]
7. Shonkoff JP, Boyce WT, McEwen BS. Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. JAMA. 2009; 301(21):2252–2259. 20090603 DCOM- 20090608. [PubMed: 19491187]

8. Zhang TY, Meaney MJ. Epigenetics and the environmental regulation of the genome and its function. Annu Rev Psychol. 2010; 61:439–466. C431–433. [PubMed: 19958180]
9. Szyf M. The early life environment and the epigenome. Biochim Biophys Acta. Sep; 2009 1790(9): 878–885. [PubMed: 19364482]
10. Yang B-Z, Zhang H, Ge W, et al. Child Abuse and Epigenetic Mechanisms of Disease Risk. American Journal of Preventive Medicine. 2013; 44(2):101–107. [PubMed: 23332324]
11. Olsson CA, Foley DL, Parkinson-Bates M, et al. Prospects for epigenetic research within cohort studies of psychological disorder: a pilot investigation of a peripheral cell marker of epigenetic risk for depression. Biol Psychol. Feb; 2010 83(2):159–165. [PubMed: 20018225]
12. Fuchikami M, Morinobu S, Segawa M, et al. DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. PLoS One. 2011; 6(8):e23881. [PubMed: 21912609]
13. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci Jan. 2013; 16(1):33–41.
14. Labonte B, Yerko V, Gross J, et al. 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. Jul 1;2012 72(1):41–48. [PubMed: 22444201]
15. McGowan PO, Sasaki A, D'Alessio AC, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci. Mar; 2009 12(3):342–348. [PubMed: 19234457]
16. Bibikova M, Barnes B, Tsan C, et al. High density DNA methylation array with single CpG site resolution. Genomics. Oct; 2011 98(4):288–295. Epub 2011 Aug 2012. [PubMed: 21839163]
17. Kaufman J, Yang BZ, Douglas-Palumberi H, et al. Social supports and serotonin transporter gene moderate depression in maltreated children. Proc Natl Acad Sci U S A. Dec 7; 2004 101(49): 17316–17321. [PubMed: 15563601]
18. Kaufman J, Yang BZ, Douglas-Palumberi H, et al. Brain-Derived Neurotrophic Factor-5-HTTLPR Gene Interactions and Environmental Modifiers of Depression in Children. Biological Psychiatry. 2006; 59:673–680. [PubMed: 16458264]
19. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997; 36(7):980–988. [PubMed: 9204677]
20. Achenbach, TM.; Rescorla, LA. Manual for ASEBA School-Age Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families; 2001.
21. Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM. Best estimate of lifetime psychiatric diagnosis: a methodological study. Arch Gen Psychiatry. 1982; 39(8):879–883. [PubMed: 7103676]
22. Putnam FW, Helmers K, Trickett PK. Development, reliability, and validity of a child dissociation scale. Child Abuse Negl. 1993; 17(6):731–741. [PubMed: 8287286]
23. Amaya-Jackson, L.; Newman, E.; Lipschitz, DS. The Child PTSD Checklist; Paper presented at: Annual Meeting of the American Academy of Child and Adolescent Psychiatry; October 2000; 2000.
24. Kaufman J, Jones B, Steiglitz E, Vitulano L, Mannarino A. The Use of Multiple Informants to Assess Children's Maltreatment Experiences. Journal of Family Violence. 1994; 9:227–248.
25. Bernstein D, Ahluvalia T, Pogge D, Handelsman L. Validity of the Childhood Trauma Questionnaire in an adolescent psychiatric population. J Am Acad Child Adolesc Psychiatry. 1997; 36(3):340–348. [PubMed: 9055514]
26. Bernstein D. A New Screening Measure for Detecting 'Hidden' Domestic Violence. Psychiatric Times. 1998; 15(11):448–453.
27. Costello EJ, Angold A. Scales to assess child and adolescent depression: checklists, screens, and nets. J Am Acad Child Adolesc Psychiatry. 1988; 27(6):726–737. [PubMed: 3058677]
28. Culpin I, Heron J, Araya R, Melotti R, Joinson C. Father absence and depressive symptoms in adolescence: findings from a UK cohort. Psychol Med. May 14;2013 14:1–12.

29. Stallard P, Sayal K, Phillips R, et al. Classroom based cognitive behavioural therapy in reducing symptoms of depression in high risk adolescents: pragmatic cluster randomised controlled trial. Bmj. 2012; 345(345):e6058. [PubMed: 23043090]
30. Wood A, Kroll L, Moore A, Harrington R. Properties of the mood and feelings questionnaire in adolescent psychiatric outpatients: a research note. J Child Psychol Psychiatry. Feb; 1995 36(2): 327–334. [PubMed: 7759594]
31. Du P, Zhang X, Huang C, et al. Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. BMC Bioinformatics. Nov 30.2010 11:587. [PubMed: 21118553]
32. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nat Rev Genet. Aug; 2011 12(8):529–541. [PubMed: 21747404]
33. Ghzili H, Grumolato L, Thouennon E, Vaudry H, Anouar Y. Possible implication of the transcriptional regulator Id3 in PACAP-induced pro-survival signaling during PC12 cell differentiation. Regul Pept. Nov 15; 2006 137(1–2):89–94. Epub 2006 Aug 2022. [PubMed: 16928405]
34. Kaufman J. Depressive disorders in maltreated children. J Am Acad Child Adolesc Psychiatry. 1991; 30(2):257–265. [PubMed: 2016230]
35. Ball MP, Li J, Gao Y, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat Biotechnol. Apr; 2009 27(4):361–368. Epub 2009 Mar 2029. [PubMed: 19329998]
36. Konishi H, Ogawa T, Nakagomi S, Inoue K, Tohyama M, Kiyama H. Id1, Id2 and Id3 are induced in rat melanotrophs of the pituitary gland by dopamine suppression under continuous stress. Neuroscience. Sep 15; 2010 169(4):1527–1534. Epub 2010 Jun 1520. [PubMed: 20600660]
37. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature. Feb 24; 2011 470(7335):492–497. [PubMed: 21350482]
38. Chang SC, Xie P, Anton RF, et al. No association between ADCYAP1R1 and post-traumatic stress disorder in two independent samples. Mol Psychiatry. Mar; 2012 17(3):239–241. [PubMed: 21912390]
39. Farioli-Veccochioli S, Saraulli D, Costanzi M, et al. Impaired terminal differentiation of hippocampal granule neurons and defective contextual memory in PC3/Tis21 knockout mice. PLoS One. Dec 17.2009 4(12):e8339. [PubMed: 20020054]
40. Lehotzky A, Lau P, Tokési N, Muja N, Hudson LD, Ovádi J. Tubulin polymerization-promoting protein (TPPP/p25) is critical for oligodendrocyte differentiation. Glia. Jan 15; 2010 58(2):157–168. [PubMed: 19606501]
41. Goldbaum O, Jensen PH, Ritcher-Landsberg C. The expression of tubulin polymerization promoting protein TPPP/p25alpha is developmentally regulated in cultured rat brain oligodendrocytes and affected by proteolytic stress. Glia. Dec; 2008 56(16):1736–1746. [PubMed: 18563798]
42. Vincze O, Oláh J, Zádori D, Klivényi P, Vécsei L, Ovádi J. A new myelin protein, TPPP/p25, reduced in demyelinated lesions is enriched in cerebrospinal fluid of multiple sclerosis. Biochem Biophys Res Commun. May 27; 2011 409(1):137–141. Epub 2011 May 2011. [PubMed: 21565174]
43. Tordera RM, Garcia-García AL, Elizalde N, et al. Chronic stress and impaired glutamate function elicit a depressive-like phenotype and common changes in gene expression in the mouse frontal cortex. Eur Neuropsychopharmacol. Jan; 2011 21(1):23–32. [PubMed: 20937555]
44. Krystal JH, Mathew SJ, D'Souza DC, Garakani A, G-B H, Charney DS. Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. CNS Drugs. Aug 1; 2010 24(8):669–693. [PubMed: 20658799]
45. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression An emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology. 2011; 3:3.
46. Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem. Sep-Oct;2001 8(5):229–242. [PubMed: 11584069]

47. Brennan K, Garcias-Closas M, Orr N, et al. Intragenic ATM methylation in peripheral blood DNA as a biomarker of breast cancer risk. Cancer Res. Feb 28.2012 28:28.
48. Sapienza C, Lee J, Powell J, et al. DNA methylation profiling identifies epigenetic differences between diabetes patients with ESRD and diabetes patients without nephropathy. Epigenetics. Jan; 2011 6(1):20–28. [PubMed: 21150313]
49. Kaminsky Z, Tochigi M, Jia P, et al. A multi-tissue analysis identifies HLA complex group 9 gene methylation differences in bipolar disorder. Mol Psychiatry. Jun 7.2011 7:7.
50. Essex MJ, Thomas Boyce W, Hertzman C, et al. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. Child Dev. Jan-Feb; 2013 84(1):58–75. [PubMed: 21883162]
51. Naumova OY, Lee M, Koposov 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. Nov 29.2011 29:1–13.
52. Orr CA, Kaufman J. Neuroscience and Child Maltreatment: The Role of Epigenetics in Risk and Resilience in Maltreated Children. Society for Research in Child Development: Social Policy Report. in press.
53. Weder N, Kaufman J. Critical periods revisited: implications for intervention with traumatized children. J Am Acad Child Adolesc Psychiatry. Nov; 2011 50(11):1087–1089. [PubMed: 22023994]
54. Maya Vetencourt JF, Sale A, Viegi A, et al. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science. Apr 18; 2008 320(5874):385–388. [PubMed: 18420937]
55. Sale A, Maya Vetencourt JF, Medini P, et al. Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. Nat Neurosci. Jun; 2007 10(6): 679–681. [PubMed: 17468749]
56. Curley JP, Jensen CL, Mashoodh R, Champagne FA. Social influences on neurobiology and behavior: epigenetic effects during development. Psychoneuroendocrinology. Apr; 2011 36(3): 352–371. [PubMed: 20650569]
57. Dozier, M.; Kaufman, J.; Kobak, R., et al. Consensus Statement on Group Care; Paper presented at: Applying Research in Child and Adolescent Development to Child Welfare Placement Practices Meeting Participants; August 9–10, 2012; New York, NY: 2012.
58. Dozier M, Peloso E, Lewis E, Laurenceau JP, Levine S. Effects of an attachment-based intervention on the cortisol production of infants and toddlers in foster care. Dev Psychopathol. Summer;2008 20(3):845–859. [PubMed: 18606034]
59. Huot RL, Gonzalez ME, Ladd CO, Thrivikraman KV, Plotsky PM. Foster litters prevent hypothalamic-pituitary-adrenal axis sensitization mediated by neonatal maternal separation. Psychoneuroendocrinology. Feb; 2004 29(2):279–289. [PubMed: 14604606]
60. Kessler RC, Pecora PJ, Williams J, et al. Effects of enhanced foster care on the long-term physical and mental health of foster care alumni. Arch Gen Psychiatry. Jun; 2008 65(6):625–633. [PubMed: 18519820]
61. Kircher T, Arolt V, Jansen A, et al. Effect of cognitive-behavioral therapy on neural correlates of fear conditioning in panic disorder. Biol Psychiatry. Jan 1; 2013 73(1):93–101. [PubMed: 22921454]
62. Oliveros A, Kaufman J. Addressing substance abuse treatment needs of parents involved with the child welfare system. Child Welfare. 2011; 90(1):25–41. [PubMed: 21950173]
63. Weissman MM, Pilowsky DJ, Wickramaratne PJ, et al. Remissions in maternal depression and child psychopathology: a STAR*D-child report. Jama. Mar 22; 2006 295(12):1389–1398. [PubMed: 16551710]
64. Cohen, J.; Mannarino, A.; Deblinger, E. Treating Trauma and Traumatic Grief in Children and Adolescents. New York, New York: Guilford Press; 2006.
65. Dorsey, S. Evaluation of the Implementation of Three Evidence-Based Practices to Address Trauma for Children and Youth Who Are Wards of the State of Illinois. Northwestern University; Mental Health and Policy Program; 2010.

66. Perroud N, Salzmann A, Prada P, et al. Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. Transl Psychiatry. 2013; 3(3):e207. [PubMed: 23422958]

Clinical Guidance

- There is a growing body of literature that suggests early experience can promote long-term changes in gene expression that confers risk for depression and a range of other mental health and medical health problems.
- While the influence of early experience can be profound, emerging data suggests negative biological and behavioral sequelae associated with early adversity can be reversed.
- Attachment focused interventions, enrichment opportunities, and treatment to address child *and* parent psychopathology are key in tipping the scale in favor of positive outcomes for maltreated children.

Table 1

Genes Associated with CpG Site Methylation Values that Predict Depression (N=190)

Gene	Illumina ID	Uncorrected Significance	Corrected Significance
***ID3* – DNA binding protein inhibitor ID-3**	**cg03535461**	**5.43×10^{-8}**	**0.005**
***TPPP* – tubulin polymerization promoting protein**	**cg04230438**	**1.79×10^{-7}**	**0.02**
***GRIN1* – glutamate NMDA receptor, NR1 subunit**	**cg14055193**	**2.68×10^{-7}**	**0.03**
MYT1L – myelin transcription factor 1-like	cg03235479	6.16×10^{-7}	0.06

Note: Linear Mixed Effects model used to examine association between children's depression scores and methylation values derived using Illumina 450K BeadChip. Bolded text reflects significant findings after correcting for whole-genome testing.

Table 2

Predictors of Depression in Children: Effect of Maltreatment Status and Methylation Values in DNA-Binding Protein Inhibitor ID-3 (*ID3*); Glutamate Receptor, Ionotropic NMDA 1 (*GRIN1*); and Tubulin Polymerization Promoting Protein (*TPPP*) (Wald Type 3 Statistic)

Source	Wald Chi-Square	Df	Significance
Maltreatment Status	15.12	1	.0001
ID3	11.41	1	.001
TPPP	4.98	1	.03
GRIN1	7.32	1	.007

Note: Square root transformed depression scores were used in this analysis. Maltreatment status and methylation values in each of the three genes uniquely predicted variation in children's depression scores (N=190).