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

Natalie Weder, MD, Huiping Zhang, PhD, Kevin Jensen, PhD, Bao Zhu Yang, PhD, Arthur Simen, MD, PhD, Andrea Jackowski, PhD, Deborah Lipschitz, MD, Heather Douglas-Palumberi, MA, Margrat Ge, MA, Francheska Perepletchikova, PhD, Kerry O'Loughlin, BA, James J. Hudziak, MD, Joel Gelernter, MD, Joan Kaufman, PhD

Objectives: To determine whether 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 healthy nontraumatized 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 3 genes emerged as genome-wide–significant predictors of depression: DNA-Binding Protein Inhibitor ID–3 (*ID3*); Glutamate Receptor, Ionotropic N-methyl-D-aspartate (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 β values after Bonferroni correction at multiple methylation sites in these candidate genes (e.g., *BDNF, NR3C1, FKBP5*). **Conclusions:** This study suggests that epigenetic changes in *ID3, GRIN1,* and *TPPP* genes, in combination with experiences of maltreatment, may confer risk for depression in children. The study adds to a growing body of literature supporting a role for epigenetic mechanisms in the pathophysiology of stress-related psychiatric disorders. Although 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. *J. Am. Acad. Child Adolesc. Psychiatry,* 2014;53(4):417–424. **Key Words:** child abuse, depression, methylation, epigenetics

C hild 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 a possible mechanism to explain the association between adverse childhood experiences and later health

This article is discussed in an editorial by Dr. Charles B. Nemeroff and Dr. Elisabeth Binder on p. 395.

CG Clinical guidance is available at the end of this article.

Supplemental material cited in this article is available online.

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. Although gene regulation is influenced by DNA methylation in other regions of the genome, the impact of methylation in promoters is currently 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 that 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 2 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 that 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 was 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 that 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 not feasible.

METHOD

Study Sample

Participants included 190 children: 94 maltreated children recruited within 6 months of being removed from their parents' care because of reports of abuse and/or neglect, and 96 healthy control children 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 because of 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 siblings and half-siblings (range, 0–4) in each family. Children ranged in age from 5 to 14 years, with a mean age of 10.2 years. The sample was 42% male, and of mixed racial/ethnic 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, $df = 190$, not significant [NS]), sex ($\chi^2 = 0.1$, $df = 1$, NS), or race/ethnicity ($\chi^2 = 3.3$, $df = 3$, NS). Recruitment and consent procedures are detailed elsewhere.[17,18]

The 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 to 1 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 (J.K.) and a board-certified child psychiatrist (D.L.). 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 the Child Dissociative Checklist (CDC),[22] a 20-item parent-report scale, and the Teachers Report Form (TRF).[20] Maltreated children also completed the Post-traumatic 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 the 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 (major depressive disorder [MDD], 12%; dissociative disorder [DD], 17%; dissociative disorder-not otherwise specified [DD-NOS], 17%), and 25% met criteria for a behavioral disorder (ADHD, 12%; oppositional defiant disorder [ODD], 13%; conduct disorder [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 following: 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 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 3 substantiated reports of abuse or neglect (range, 1–7). In addition, 92% of the children experienced more than 1 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. The MFQ is a 33-item self-report measure that assesses depression in children, with each item rated on a point scale from 0 to 2.[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 $\pm$ standard deviation, 17.4 $\pm$ 11.2; range, 0–46; comparison children: mean $\pm$ standard deviation, 9.9 $\pm$ 7.3; range, 0–29). In all, 26% 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, namely, within 6 months of an incident of maltreatment of sufficient severity to warrant out-of-home placement. Specimens were refrigerated within 2 hours of collection, and DNA was extracted using Puregene (Gentra, Minneapolis, MN) kits. To prepare specimens for methylation study, 500 ng of genomic DNA was treated with bisulfite reagents included in EZ-96 DNA methylation kit (Zymo Research, Orange, CA) according to the manufacturer's protocol. Non-methylated cytosines were converted to uracils, whereas 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 more than 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 β values for each CpG site, with β values ranging from 0.0 to 1.0, quantifying the ratio of methylated allele in fluorescent signals at each CpG site. Raw scanned data were normalized; average β 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 greater than .001 were removed to ensure that only high-confidence probes were included in subsequent analysis (30 of 485,578 CpG sites were removed, 0.006% 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) was used to examine retest methylation levels at 7 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

To take familial correlations into consideration while examining methylation predictors of depression, data were analyzed using a 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/ethnicity were not related to children's depression scores but were included in the

LME model to normalize residuals. Given the heteroscedasticity of β values, as recommended by Du et al.,[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 the level recommended by Rakyan et al.[32]

After identifying methylation sites that individually predicted children's depression scores, a generalized estimating equation (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 3 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

TABLE 1 Genes Associated With CpG Site Methylation Values That Predict Depression (N = 190)

Gene	Illumina ID	Uncorrected Significance	Corrected Significance
ID3	cg03535461	**5.43 x 10^{-8}**	**.005**
TPPP	cg04230438	**1.79 × 10^{-7}**	**.02**
GRIN1	cg14055193	**2.68 × 10^{-7}**	**.03**
MYT1L	cg03235479	6.16 × 10^{-7}	.06

Note: A linear mixed effects model was used to examine association between children's depression scores and methylation values derived using Illumina 450K BeadChip. Boldface type reflects significant findings after correcting for whole-genome testing. GRIN1 = glutamate N-methyl-D-aspartate (NMDA) receptor, NR1 subunit; ID3 = DNA binding protein inhibitor ID-3; MYT1L = myelin transcription factor 1—like; TPPP = tubulin polymerization promoting protein.

methylation values of each of the 3 significant CpG sites in 1 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/ethnicity, and sex were not related to methylation values in these genes; 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, comprising 44 maltreated and 23 control children. A GEE analysis found a significant main effect for maltreatment (Wald statistic 15.99, $p < .0001$) and a maltreatment $\times$ ID3 methylation interaction (Wald statistic 14.12, $p < .0001$) in predicting diurnal cortisol secretion, a measure that was previously shown to predict depression in maltreated children.[34] ID3 methylation was negatively related to diurnal cortisol secretion in control children but was 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 3 significant CpG sites identified in ID3, GRIN1, and TPPP were located on the 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

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 N-methyl-D-aspartate (NMDA) 1 (*GRIN1*), and Tubulin Polymerization Promoting Protein (*TPPP*) (Wald Type 3 Statistic)

Source	Wald χ^2	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). df = degree of freedom.

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 1 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 Previously 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 introductory section of this article, 450K BeadChip does not include any CpG sites in promoter regions of *SLC6A4* or *BDNF,* and sites that it does include in promoter regions of *NR3C1* and *FKBP5* are different from sites previously examined in the literature, so a test for replication of prior research findings is not feasible. Figures 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.* 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 3 CpG sites in *SLC6A4,* 6 sites in *BDNF,* 2 sites in *NR3C1,* and 3 sites in *FKBP5* significantly predicted children's depression scores, at uncorrected significance levels, the level of significance used 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,* 1 of which was significant after correcting for whole-genome testing ($p = 2.0 \times 10^{-7}$). All of the sites in *BDNF* are located on the gene body, all sites in *NR3C1* are located in promoter regions, and 1 site in *FKBP5* is located on the gene body: 1 at the 3'UTR site and 2 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 of 10 gene body sites, and at 6 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 Candidate Genes
Correlations were examined between morning cortisol values and methylation values in the 6 *NR3C1* and 4 *FKBP5* CpG sites identified in the analyses above. Methylation in 2 sites in *NR3C1* (cg04111177 r = 0.49, $p < .001$; cg11152298 r = 0.33, $p < .01$) and 1 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 that methylation in 3 genes, namely, *ID3, TPPP,* and *GRIN1,* significantly predicted depression scores in children. 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 co-morbid 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 is believed to have a role in development and maintenance of white matter tracts in the brain.[41,42] *GRIN1* transcription is downregulated in the 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 the differences in the CpG sites included on the 450K Illumina BeadChip and the CpG sites examined in prior studies. Methylation values of 3 CpG sites in *SLC6A4*, 6 sites in *BDNF*, 2 sites in *NR3C1*, and 3 sites in *FKBP5* significantly predicted children's depression scores, at uncorrected significance levels. Differences in methylation values of maltreated and comparison children reached traditional levels of significance at 74 sites within these previously studied candidate genes, with significance thresholds withstanding Bonferroni correction for 9 sites in *BDNF*, 4 sites in *FKBP5,* and 6 sites in *NR3C1,* 1 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, 1 at the 3'UTR site, and 2 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. Although 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 introductory section of this article, 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 in 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, that although 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 although 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 the following: developing secure attachment relations;[17,57-59] facilitating enrichment opportunities;[56,60] and providing clinical interventions to address child and parent psychopathology.[61-66] Although 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 will help to identify novel, more effective treatments. &

CG Clinical Guidance

- There is a growing body of literature that suggests that 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.

- Although the influence of early experience can be profound, emerging data suggests that negative biological and behavioral sequelae associated with early adversity can be reversed.

- Attachment-focused interventions, enrichment opportunities, and treatment to address both child and parent psychopathology are key in tipping the scale in favor of positive outcomes for maltreated children.

Drs. Weder and Kaufman are with the Child Mind Institute. Drs. Zhang, Jensen, Yang, Simen, Lipschitz, Douglas-Palumberi, Ge, Perepletchikova, Gelernter, and Kaufman are with Yale University School of Medicine. Dr. Simen is also with Merck Research Laboratories.

Dr. Jackowski is with LiNC, Universidade Federal de São Paulo, São Paulo, Brazil. Drs. O'Laughlin and Hudziak are with the University of Vermont, Vermont Center for Children, Youth, and Families.

Correspondence to Joan Kaufman, PhD, 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; e-mail: 06520.joan.kaufman@yale.edu

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 Jan 19;10:20.
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 Med. 1998;14:245-258.
3. Romans S, Belaise C, Martin J, Morris E, Raffi A. Childhood abuse and later medical disorders in women. An epidemiological study. Psychother Psychosom. 2002;71:141-150.
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. 2000;57:953-959.
5. Molnar BE, Buka SL, Kessler RC. Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. Am J Public Health. 2001;91:753-760.
6. McEwen BS, Eiland L, Hunter RG, Miller MM. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology. 2012;62:3-12.
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;301:2252-2259.
8. Zhang TY, Meaney MJ. Epigenetics and the environmental regulation of the genome and its function. Annu Rev Psychol. 2010;61:439-466, C431-C433.
9. Szyf M. The early life environment and the epigenome. Biochim Biophys Acta. 2009;1790:878-885.
10. Yang B-Z, Zhang H, Ge W, et al. Child abuse and epigenetic mechanisms of disease risk. Am J Prev Med. 2013;44:101-107.
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. 2010;83:159-165.
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:e23881.
13. Klengel T, Mehta D, Anacker C, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci. 2013;16:33-41.
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. 2012;72:41-48.
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. 2009;12:342-348.
16. Bibikova M, Barnes B, Tsan C, et al. High density DNA methylation array with single CpG site resolution. Genomics. 2011;98:288-295.
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. 2004;101:17316-17321.
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. Biol Psychiatry. 2006;59:673-680.
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;36:980-988.

20. Achenbach TM, Rescorla LA. Manual for ASEBA School-Age Forms and Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, and 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;39:879-883.

22. Putnam FW, Helmers K, Trickett PK. Development, reliability, and validity of a child dissociation scale. Child Abuse Negl. 1993;17:731-741.

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 24-29, 2000; New York, NY.

24. Kaufman J, Jones B, Steiglitz E, Vitulano L, Mannarino A. The use of multiple informants to assess children's maltreatment experiences. J Fam 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;36:340-348.

26. Bernstein D. A new screening measure for detecting 'hidden' domestic violence. Psychiatric Times. 1998;15:448-453.

27. Costello EJ, Angold A. Scales to assess child and adolescent depression: checklists, screens, and nets. J Am Acad Child Adolesc Psychiatry. 1988;27:726-737.

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. 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:e6058.

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. 1995;36:327-334.

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 Bioinform. 2010;11:587.

32. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. Nat Rev Genet. 2011;12:529-541.

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. 2006;137:89-94.

34. Kaufman J. Depressive disorders in maltreated children. J Am Acad Child Adolesc Psychiatry. 1991;30:257-265.

35. Ball MP, Li J, Gao Y, et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat Biotechnol. 2009;27:361-368.

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. 2010;169:1527-1534.

37. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. Nature. 2011;470:492-497.

38. Chang SC, Xie P, Anton RF, et al. No association between ADCYAP1R1 and post-traumatic stress disorder in two independent samples. Mol Psychiatry. 2012;17:239-241.

39. Farioli-Vecchioli 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. 2009;4:e8339.

40. Lehotzky A, Lau P, Tökési N, Muja N, Hudson LD, Ovádi J. Tubulin polymerization-promoting protein (TPPP/p25) is critical for oligodendrocyte differentiation. Glia. 2010;58:157-168.

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. 2008;56:1736-1746.

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. 2011;409:137-141.

43. Tordera RM, Garcia-García AL, Elizalde N, et al. Chronic stress and impaired glutamate function elicit a depressive-like phenotype

44. and common changes in gene expression in the mouse frontal cortex. Eur Neuropsychopharmacol. 2011;21:23-32.

44. Krystal JH, Mathew SJ, D'Souza DC, Garakani A, H. G-B, Charney DS. Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. CNS Drugs. 2010;24:669-693.

45. Sanacora G, Treccani G, Popoli M. Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology. 2012;62:63-77.

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. 2001;8:229-242.

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. 2012;72:2304-2313.

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. 2011;6:20-28.

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. 2012;17:728-740.

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. 2013;84:58-75.

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. 2011;29:1-13.

52. Orr CA, Kaufman J. Neuroscience and child maltreatment: the role of epigenetics in risk and resilience in maltreated children. Soc Res Child Dev (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.

54. Maya Vetencourt JF, Sale A, Viegi A, et al. The antidepressant fluoxetine restores plasticity in the adult visual cortex. Science. 2008;320:385-388.

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. 2007;10:679-681.

56. Curley JP, Jensen CL, Mashoodh R, Champagne FA. Social influences on neurobiology and behavior: epigenetic effects during development. Psychoneuroendocrinology. 2011;36:352-371.

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.

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. 2008;20:845-859.

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. 2004;29:279-289.

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. 2008;65:625-633.

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. 2013;73:93-101.

62. Oliveros A, Kaufman J. Addressing substance abuse treatment needs of parents involved with the child welfare system. Child Welfare. 2011;90:25-41.

63. Weissman MM, Pilowsky DJ, Wickramaratne PJ, et al. Remissions in maternal depression and child psychopathology: a STAR*D-child report. JAMA. 2006;29:1389-1398.

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:e207.

SUPPLEMENT 1

FIGURE S1 Location of Illumina 450K CpG Sites on FKBP5 relative to sites previously examined in the literature. http://genome.ucsc.edu (GRCh37/hg19).
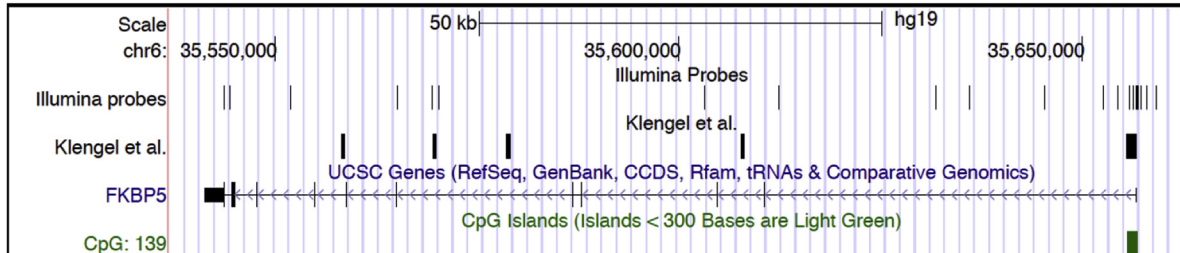
FIGURE S2 Location of Illumina 450K CpG sites on NR3C1 relative to sites previously examined in the literature. http://genome.ucsc.edu (GRCh37/hg19).
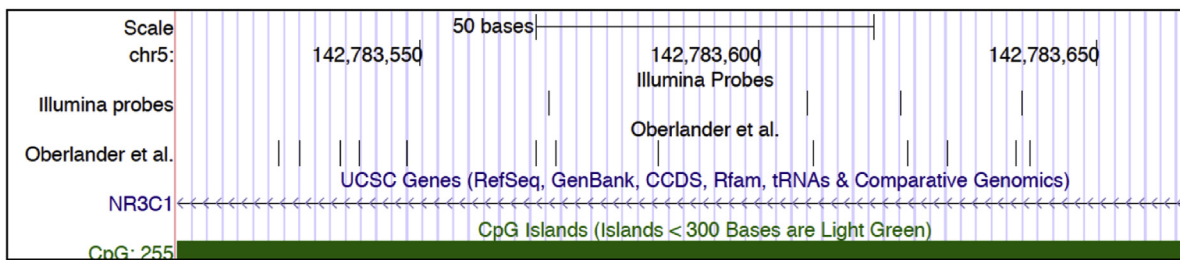
TABLE S1 Group Differences Between Maltreated and Control Children and Associations Between Children's Depression Scores and Methylation in CpG Sites in *SLC6A4, BDNF, NR3C1,* and *FKBP5*

| SLC6A4 Sites[a] | Maltreated vs. Control Group Differences | | Predictor of Depression Severity Score | | |
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	CpG Location
cg25725890	0.389547077	NS	0.009375991	NS	TSS200
cg22584138	0.890677671	NS	0.017763675	NS	5′UTR
cg18584905	0.362947352	NS	0.044410081	NS	TSS1500
cg03363743	0.606875611	NS	0.126198768	NS	5′UTR
cg24984698	0.166207482	NS	0.286084111	NS	Body
cg01330016	0.563631422	NS	0.422569194	NS	5′UTR
cg20592995	0.007292171	NS	0.424334536	NS	3′UTR
cg14692377	0.042962662	NS	0.498005157	NS	1stExon;5′UTR
cg26126367	0.119189965	NS	0.530719827	NS	5′UTR
cg05016953	0.363220673	NS	0.569368012	NS	1stExon;5′UTR
cg05951817	0.588369197	NS	0.601353988	NS	5′UTR
cg10901968	0.981190937	NS	0.619723094	NS	TSS200
cg06841846	0.214420909	NS	0.840393939	NS	TSS1500
cg27569822	0.461393774	NS	0.864032558	NS	TSS200
cg12074493	0.103272514	NS	0.968488712	NS	TSS1500
cg26741280	0.058636884	NS	0.97783844	NS	TSS200

TABLE S1 Continued

BDNF Sites[b]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		CpG Location
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	
cg25962210	0.148960564	NS	0.020507296	NS	Body;5'UTR;TSS200
cg00298481	0.000557109	Significant	0.028709789	NS	5'UTR;TSS200;Body;TSS1500
cg10635145	0.14659086	NS	0.02908057	NS	5'UTR;TSS200;Body;TSS1500
cg23619332	0.023205762	NS	0.039309183	NS	5'UTR;TSS200;Body;TSS1500
cg04106006	0.142973911	NS	0.041643427	NS	5'UTR;TSS200;Body;TSS1500
cg20954537	0.150810252	NS	0.054886979	NS	Body;5'UTR;TSS1500
cg11718030	0.058877206	NS	0.064524889	NS	TSS1500
cg07704699	0.223299722	NS	0.06755146	NS	Body;TSS1500
cg15914769	0.013072878	NS	0.069687082	NS	TSS200;TSS1500
cg08362738	0.035693931	NS	0.096556257	NS	5'UTR;TSS200;Body;TSS1500
cg21010859	0.000343308	Significant	0.1030634	NS	Body;5'UTR
cg05189570	0.126257248	NS	0.111817901	NS	Body;5'UTR;1stExon
cg24377657	0.002917329	NS	0.122568359	NS	TSS1500;Body;5'UTR;TSS200
cg24065044	0.005555087	NS	0.13900972	NS	TSS1500;Body;5'UTR
cg12448003	0.028474844	NS	0.15344048	NS	Body;TSS1500;TSS200
cg25412831	0.039189333	NS	0.178482374	NS	Body;5'UTR;TSS1500;1stExon
cg15462887	0.657220934	NS	0.183028344	NS	TSS1500
cg25328597	0.552470319	NS	0.186466565	NS	TSS200;Body;5'UTR;TSS1500
cg05818894	0.247413162	NS	0.187061209	NS	Body;5'UTR
cg27193031	0.10934742	NS	0.207814321	NS	Body;1stExon;5'UTR
cg02613510	0.211746516	NS	0.238614422	NS	Body;5'UTR;TSS1500
cg15688670	0.107325261	NS	0.251216822	NS	TSS1500;Body;5'UTR;TSS200
cg01418645	0.031908348	NS	0.262345561	NS	Body
cg06991510	0.772728099	NS	0.274757776	NS	Body;TSS1500;5'UTR;TSS200
cg01225698	0.0000129	Significant	0.27917886	NS	Body;TSS1500;TSS200
cg06816235	0.000473413	Significant	0.28144659	NS	Body;5'UTR;TSS1500; 1stExon
cg15014679	0.023341201	NS	0.290533862	NS	Body;5'UTR
cg22043168	0.00011159	Significant	0.30060822	NS	Body;5'UTR;5'UTR;1stExon
cg07238832	0.250154077	NS	0.323235813	NS	Body;5'UTR;Body;TSS1500
cg16257091	0.065911525	NS	0.328988544	NS	TSS1500;1stExon;5'UTR
cg18867480	0.041518355	NS	0.330180292	NS	TSS1500
cg01642653	0.889787755	NS	0.347888785	NS	TSS1500;1stExon;5'UTR
cg24249411	0.029591783	NS	0.359233193	NS	TSS1500
cg09606766	0.63313769	NS	0.38585578	NS	TSS1500;Body;5'UTR;TSS1500
cg27351358	0.00467074	NS	0.392241455	NS	TSS1500;1stExon;5'UTR
cg18595174	0.000771768	NS	0.400139989	NS	Body;5'UTR
cg14291693	0.002609618	NS	0.414334195	NS	Body;5'UTR
cg05733135	0.005146437	NS	0.416264112	NS	Body;5'UTR
cg25457956	0.030943413	NS	0.419027337	NS	TSS200;TSS1500
cg11241206	0.128532073	NS	0.427020778	NS	TSS1500;Body;5'UTR;1stExon
cg20108357	0.106184987	NS	0.427040518	NS	Body;5'UTR
cg14589148	0.00079039	NS	0.438135311	NS	TSS200;TSS1500
cg06684850	0.005465605	NS	0.467552665	NS	Body;TSS1500;TSS200
cg04672351	0.450442775	NS	0.470239527	NS	TSS1500;Body;5'UTR;1stExon
cg07159484	0.001236769	NS	0.49293865	NS	Body;5'UTR;1stExon;TSS200
cg26949694	0.377345425	NS	0.51315985	NS	Body;TSS1500;1stExon;5'UTR
cg03747251	0.00000742	Significant	0.533941568	NS	TSS200;TSS1500;Body;5'UTR
cg06260077	0.307915617	NS	0.549126505	NS	Body;5'UTR;TSS200
cg23426002	0.007019715	NS	0.552944591	NS	Body
cg09492354	0.817219078	NS	0.564147055	NS	Body;5'UTR
cg15710245	0.00000935	Significant	0.57872989	NS	TSS200;Body;5'UTR;TSS1500
cg25381667	0.031851159	NS	0.582690755	NS	TSS200;TSS1500

TABLE S1 Continued

BDNF Sites[b]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	CpG Location
cg08388004	0.116936987	NS	0.590928634	NS	Body
cg23497217	0.002196795	NS	0.599162536	NS	TSS1500;Body;5'UTR;TSS200
cg03984780	0.009619105	NS	0.599429881	NS	TSS200;Body;5'UTR;TSS1500
cg06025631	0.008003534	NS	0.6250328	NS	Body;TSS200;5'UTR; TSS1500
cg18117895	0.037366543	NS	0.626348621	NS	Body;5'UTR;TSS200;TSS1500
cg24650785	0.0000197	Significant	0.654835065	NS	Body;TSS1500;5'UTR;1stExon
cg06979684	0.017061052	NS	0.671790686	NS	3'UTR;1stExon;Body
cg26057780	0.099601076	NS	0.709230963	NS	Body;5'UTR;TSS200
cg11806762	0.006052462	NS	0.740428343	NS	Body;5'UTR
cg02527472	0.22502039	NS	0.752372802	NS	TSS1500;1stExon;5'UTR
cg10022526	0.453195175	NS	0.788322515	NS	TSS1500
cg15313332	0.538617606	NS	0.81797577	NS	Body;5'UTR;TSS200R
cg13974632	0.036178462	NS	0.852902569	NS	Body;5'UTR
cg01583131	0.034589838	NS	0.856446599	NS	TSS1500
cg01636003	0.018513732	NS	0.864585633	NS	TSS1500;Body;5'UTR
cg23947039	0.000164476	Significant	0.867265182	NS	Body;5'UTR;TSS200;TSS1500
cg10558494	0.589713883	NS	0.869104723	NS	Body;5'UTR;TSS200
cg17413943	0.179279859	NS	0.921298129	NS	Body;5'UTR
cg03167496	0.143323283	NS	0.933131655	NS	TSS200;TSS1500
cg20340655	0.869652263	NS	0.934905121	NS	TSS1500;Body;5'UTR;1stExon
cg04481212	0.210920383	NS	0.938996435	NS	Body;5'UTR
cg06046431	0.201799568	NS	0.939515731	NS	TSS1500
cg18354203	0.043632706	NS	0.952418477	NS	Body;5'UTR
cg05218375	0.007188174	NS	0.972883503	NS	TSS1500;Body;5'UTR;TSS200
cg26840770	0.010643066	NS	0.976212265	NS	TSS1500;Body;5'UTR;TSS200
NR3C1 Sites[c]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	CpG Location
cg11152298	0.000514005	Significant	0.007245998	NS	Promoter_Associated
cg07733851	0.096474453	NS	0.029673053	NS	Promoter_Associated
cg06521673	0.001966007	NS	0.085889227	NS	5'UTR
cg18849621	0.001721291	NS	0.097944081	NS	Promoter_Associated
cg03857453	0.283607981	NS	0.119954731	NS	Body
cg16586394	0.003905033	NS	0.136234215	NS	Body
cg08818984	0.096333071	NS	0.169543704	NS	Promoter_Associated
cg00629244	0.338032142	NS	0.203578753	NS	Promoter_Associated
cg20753294	0.214111138	NS	0.209441883	NS	1stExon;5'UTR
cg18068240	0.053022202	NS	0.229798544	NS	Promoter_Associated
cg13648501	0.0000691	Significant	0.303157839	NS	Promoter_Associated
cg06952416	0.011228949	NS	0.344343845	NS	Promoter_Associated
cg15910486	0.003149538	NS	0.359899641	NS	Promoter_Associated
cg12466613	0.00389258	NS	0.38332975	NS	TSS1500
cg21702128	0.0000107	Significant	0.430251048	NS	Promoter_Associated
cg14558428	0.079998106	NS	0.445535838	NS	Promoter_Associated
cg17860381	0.000588729	Significant	0.477987609	NS	Promoter_Associated
cg25535999	0.008783015	NS	0.491526029	NS	Body
cg04111177	0.000000219	Significant	0.522112775	NS	Promoter_Associated
cg18998365	0.279758979	NS	0.531264979	NS	Promoter_Associated
cg24026230	0.046826622	NS	0.557433876	NS	Promoter_Associated

TABLE S1 Continued

NR3C1 Sites[c]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	CpG Location
cg26720913	0.231614305	NS	0.584930345	NS	Promoter_Associated
cg18019515	0.051751654	NS	0.624242757	NS	Promoter_Associated
cg07528216	0.069531579	NS	0.627095119	NS	5′UTR
cg18146873	0.00262041	NS	0.674449501	NS	1stExon;5′UTR
cg07589972	0.015769704	NS	0.686431242	NS	TSS1500
cg06968181	0.953625703	NS	0.743809595	NS	Promoter_Associated
cg23273257	0.023373748	NS	0.754958769	NS	3′UTR
cg08845721	0.136636243	NS	0.817967173	NS	5′UTR
cg27107893	0.15671811	NS	0.819298735	NS	Body
cg15645634	0.0000446	Significant	0.835603366	NS	Promoter_Associated
cg18484679	0.013710222	NS	0.850485782	NS	Body
cg17342132	0.863237026	NS	0.860313386	NS	Promoter_Associated
cg19457823	0.025924253	NS	0.867711695	NS	Body
cg16335926	0.843628055	NS	0.871456264	NS	Promoter_Associated
cg27345592	0.004833828	NS	0.886590373	NS	5′UTR
cg17617527	0.110096996	NS	0.932152634	NS	5′UTR
cg06613263	0.04847934	NS	0.934776774	NS	Body
cg26464411	0.003539604	NS	0.948562712	NS	Promoter_Associated
cg10847032	0.117169097	NS	0.952378569	NS	Promoter_Associated
cg27122725	0.135298329	NS	0.953684151	NS	Promoter_Associated

FKBP5 site[d]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	CpG Location
cg08915438	0.301765811	NS	0.019567564	NS	Body
cg08636224	0.212124561	NS	0.027355404	NS	5′UTR
cg00862770	0.981001487	NS	0.050908687	**NS**	Promoter Associated
cg06087101	0.927864506	NS	0.087863315	NS	Body
cg00610228	0.0000979	Significant	0.09667896	NS	Promoter Associated
cg25114611	0.0000424	Significant	0.124552096	NS	Promoter Associated
cg16052510	0.246977086	NS	0.182948128	NS	Body
cg17085721	0.107141894	NS	0.206640507	NS	5′UTR
cg14642437	0.069897722	NS	0.263292328	NS	Promoter Associated
cg03591753	0.153801721	NS	0.296422536	NS	5′UTR
cg07485685	0.947515722	NS	0.319104319	NS	Promoter Associated
cg19226017	0.290309624	NS	0.363997377	NS	Promoter Associated
cg00130530	0.980905893	NS	0.394927091	NS	Promoter Associated
cg07843056	0.094112971	NS	0.465704551	NS	Promoter Associated
cg01294490	0.070552234	NS	0.492693295	NS	Promoter Associated
cg03546163	0.067252111	NS	0.504722822	NS	5′UTR
cg00052684	0.296682857	NS	0.565458655	NS	5′UTR
cg06937024	0.003010829	NS	0.569006171	NS	Promoter Associated
cg20813374	0.264103036	NS	0.578380658	NS	Promoter Associated
cg10913456	0.591837481	NS	0.600712636	NS	Promoter Associated
cg18726036	0.000560558	Significant	0.601827263	NS	3′UTR
cg17030679	0.237980508	NS	0.604358676	NS	Promoter Associated
cg10300814	0.32407941	NS	0.617369297	NS	Body
cg14284211	0.100769754	NS	0.625299877	NS	Body
cg07061368	0.053756916	NS	0.640872399	NS	5′UTR

TABLE S1 Continued

FKBP5 site[d]	Maltreated vs. Control Group Differences		Predictor of Depression Severity Score		CpG Location
	Uncorrected Significance	Bonferroni Correction	Uncorrected Significance	Bonferroni Correction	
cg19014730	0.024598834	NS	0.64924611	NS	5'UTR
cg23416081	0.051070684	NS	0.656993852	NS	5'UTR
cg08586216	0.213968836	NS	0.664922547	NS	5'UTR
cg02665568	0.002103952	NS	0.739132841	NS	Body
cg15929276	0.31636485	NS	0.744679253	NS	5'UTR
cg00140191	0.121851127	NS	0.848214121	NS	Promoter Associated
cg11845071	0.044082904	NS	0.849839854	NS	Promoter Associated
cg07633853	0.0000327	Significant	0.882618756	NS	Body
cg16012111	0.162688928	NS	0.951652076	NS	Promoter Associated

Note: The 450K BeadChip does not include any CpG sites in the promoter region of SLC6A4, so a test for replication with prior research findings was not feasible. Bolded text reflects significant findings after correcting for whole-genome testing. NS = not significant.

[a]Bonferroni significance set at 0.003 for SLC6A4 (16 CpG sites); significance after controlling for demographic factors.

[b]Bonferroni significance set at 0.00065 for BDNF (77 CpG sites); significance after controlling for demographic factors.

[c]Bonferroni significance set at 0.001 for NR3C1 (41 CpG sites); significance after controlling for demographic factors.

[d]Bonferroni significance set at 0.0015 for FKBP5 (34 CpG sites); significance after controlling for demographic factors.