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

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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

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 a 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

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

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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