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Adverse Childhood Experiences, Epigenetic Measures, and Obesity in Youth

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Objective To determine if measures of adverse childhood experiences and DNA methylation relate to indices of obesity in youth.

Study design Participants were derived from a cohort of 321 8 to 15-year-old children recruited for an investigation examining risk and resilience and psychiatric outcomes in maltreated children. Assessments of obesity were collected as an add-on for a subset of 234 participants (56% female; 52% maltreated). Illumina arrays were used to examine whole genome epigenetic predictors of obesity in saliva DNA. For analytic purposes, the cohort analyzed in the first batch comprised the discovery sample (n = 160), and the cohort analyzed in the second batch the replication sample (n = 74).

Results After controlling for race, sex, age, cell heterogeneity, 3 principal components, and whole genome testing, 10 methylation sites were found to interact with adverse childhood experiences to predict cross-sectional measures of body mass index, and an additional 6 sites were found to exert a main effect in predicting body mass index ($P < 5.0 \times 10^{-7}$, all comparisons). Eight of the methylation sites were in genes previously associated with obesity risk (eg, *PCK2, CxCl10, BCAT1, HID1, PRDM16, MADD, PXDN, GALE)*, with several of the findings from the discovery data set replicated in the second cohort.

Conclusions This study lays the groundwork for future longitudinal studies to elucidate these mechanisms further and identify novel interventions to alleviate the health burdens associated with early adversity. *(J Pediatr 2018;202:150-6).*

Over the past 2 decades, there has been a growing appreciation of the role of adverse childhood experiences (ACEs) on the development of a range of negative health outcomes.[1-4] Child maltreatment and other ACE are nonspecific risk factors for multiple psychiatric disorders,[5-7] and several health risk behaviors, including smoking, overeating, and excessive alcohol and drug use.[4,8,9] Above and beyond the effect of these health risk behaviors, ACE predict a multitude of medical health problems later in life, including ischemic heart disease,[9-11] stroke,[9] respiratory problems,[12,13] diabetes,[9,11] and even cancer.[9,14]

There is preliminary evidence that childhood adversity may confer risk for this broad range of outcomes through epigenetic mechanisms.[15-17] Epigenetics refers to functionally relevant chemical modifications to the genome that do not involve a change in DNA nucleotide sequence.[18] These chemical modifications can alter gene activity and influence regulation of genes in response to changes in the environment, with epigenetic modifications induced by ACE reported frequently to persist into adulthood.[19]

DNA methylation is one of the most studied epigenetic mechanisms.[20] In a prior investigation, maltreated and control children were found to have significantly different methylation values at 2868 methylation sites.[15] The gene sets showing differential methylation between the maltreated and comparison children contained genes involved in biological processes relevant to psychiatric and substance use disorders (eg , neurogenesis, axonal guidance), heart disease (eg, cardiac development), stroke (development of blood vessel morphogenesis), respiratory disease (eg, interleukin regulation), diabetes (eg, leptin signaling), and cancer (eg, Wnt signaling, Notch signaling), as noted above, all medical illnesses that have been linked to adversity in youth.[9] That study, however, did not include any health outcomes data, and it is not known if these epigenetic differences were associated with health outcome measures.

ACEs	Adverse childhood experiences
BMI	Body mass index
GEEs	Generalized estimating equations
PC	Principal component
SES	Socioeconomic status
SNP	Single nucleotide polymorphism
Y-VACS	Yale-Vermont Adversity in Childhood Scale

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The goal of the current investigation was to determine if measures of ACE and DNA methylation predict cross-sectional indices of obesity in youth. Obesity was selected as the health outcome to examine as it is the medical problem associated with childhood adversity most apt to manifest in youth, and there is strong meta-analytic support for the association between child maltreatment and obesity.[21]

Methods

Participants were derived from a cohort of 321 children recruited between 2013 and 2016 as part of an investigation examining risk and resilience in maltreated children. Data were analyzed in 2017. The 321 children were from 235 families with several siblings and half siblings (range: 0-4 per family) included in the cohort. In the year prior to study enrollment, 37% of the children had an out-of-home placement because of reports of abuse or neglect. Another 15% of the children had prior allegations of maltreatment, and 48% of the sample was never referred to protective services. The children were from diverse socioeconomic status (SES) backgrounds (Hollingshead SES mean 3.34, SD 1.3, range 1-5), and as discussed further below, had experienced a broad range of childhood adversities.

The primary study was designed to assess predictors of mental health outcomes, with the assessment of obesity collected on a subset of the children as an add-on to the primary investigation. Child trauma, DNA methylation, and obesity measures were available on a total of 234 participants. The DNA data were analyzed in 2 batches; once at the end of the second year of recruitment, and once at the completion of the study. For analytic purposes, the cohort analyzed in the first batch was the discovery sample (n = 160), and the cohort analyzed in the second batch was the replication sample (n = 74). Table I depicts the demographic and clinical characteristics of the discovery and replication cohorts.

Table I. Demographic and clinical characteristics of the sample

	Discovery cohort n = 160	Replication cohort n = 74	Statistic *P* value
Age, y	11.5 ± 1.9	10.7 ± 2.0	F (1) = 10.6 $P < .001$
Sex (% female/% male)	46% /54%	42%/58%	$\chi^2 = 0.13$ P = ns
Race (% European American)	88%	89%	$\chi^2 = 0.14$ P = ns
Y-VACS intrafamilial adversity score	18.4 ± 11.6	10.68 ± 9.7	Wald $\chi^2 = 28.3$ $P < .0001$
Y-VACS extrafamilial adversity score	5.6 ± 3.7	5.5 ± 3.0	Wald $\chi^2 = 0.06$ P = ns
Y-VACS total adversity score	23.7 ± 16.2	16.2 ± 11.2	Wald $\chi^2 = 20.3$ $P < .0001$
BMI	23.1 ± 6.1	19.8 ± 4.5	Wald $\chi^2 = 20.9$ $P < .0001$
Weight range: Overweight/obese	14% /17%	11%/3%	$\chi^2 = 15.8$ $P = .001$

Assessments from parents were collected during home visits and the majority of the child data for this study were collected at a day camp devised specifically for our research purposes, replicating a methodology used in previous investigations.[22-24] The institutional review boards at Yale University, University of Vermont, and Johns Hopkins Schools of Medicine approved this study. Prior to recruitment, an independent child advocate reviewed each case referred through protective services to determine that research participation was in the child's best interest. The child's parent or legal guardian provided informed consent and each child provided assent for study participation. Birth parent assent for child participation for children in state custody was obtained when clinically appropriate (eg, ongoing parent-child contact).

Multiple informants and data sources (eg, parents, children, protective services records) were used to obtain a best estimate of each child's adverse experiences. Permissions were secured to access state child protective services records from all study participants, and records were obtained for all children with prior protective services referrals (n = 122; 87 children recruited through protective services because of recent out-of-home placements and 35 children recruited through the community who were living with their biological families but had a history of prior protective services involvement). The data from these various sources were integrated and rated using the Yale-Vermont Adversity in Childhood Scale (Y-VACS) scoring procedures.[25] The Y-VACS assesses a range of intrafamilial (eg, physical abuse, witnessing domestic violence) and extrafamilial (eg, community violence, bullying, natural disasters) adversities, and generates scores that take into account severity and frequency of exposure. The Y-VACS generates a total adversity score, intrafamilial adversity score, extrafamilial adversity score, and individual item scores, with high inter-rater reliability reported in generating these scores.[25] As the children with and without prior protective services involvement did not differ on the extrafamial adversity scale of the Y-VACS (Wald $\chi^2 = .237$, df = 1, ns), children's scores on the intrafamilial adversity scale of the Y-VACS were used in subsequent analyses examining obesity risk.

The nurse at the research summer day camp program collected the measurements of height and weight which were used to calculate body mass index (BMI). The same scale was used for all children participating in the study.

Saliva specimens for DNA extraction were refrigerated within 2 hours of collection and DNA extracted using Puregene (Gentra, Minneapolis, Minnesota) kits. To prepare the specimens for the methylation study, 500 ng of genomic DNA were treated with bisulfite reagents included in the EZ-96 DNA methylation kit (Zymo Research, Orange, California) according to the manufacturer's protocol. Bisulfite-converted DNA samples were then used in the array-based DNA methylation assays.

The Illumina 450K Methylation BeadChip was used to analyze the DNA data of the discovery cohort (Illumina Inc, San Diego, California). Array-based epigenome-wide methylation analyses were completed at the Yale Center for Genome Analysis at Yale University using standard procedures. Quality control was conducted based on published methods.[26] Meth-

ylation sites with detection *P* value of >.001 were removed to ensure that only high-confidence probes were included. Probes were also excluded if they had a single nucleotide polymorphism (SNP) within the methylation site map to multiple places in the genome or to sex chromosomes. Methylation data were normalized using the functional normalization method in the "minfi" R package. This function uses internal control probes present on the array to control for between-array technical variation and outperforms other approaches.[27] A total of 456 513, (94% of sites) were left for subsequent analysis. The genomic inflation factor for the discovery cohort was 1.004, indicating that the epigenome wide analyses results are negligibly inflated and the results are unlikely due to spurious findings (**Figure 1**; available at www.jpeds.com). The script and methods used to generate the genomic inflation factor parallel the methods utilized by Gelernter et al.[28]

The Illumina Infinium Methylation EPIC (850K) BeadChip (Illumina, Inc) was used to assess DNA methylation in the replication cohort. Only the methylation sites that significantly predicted BMI in the children in the discovery cohort after controlling for whole genome testing ($P < 5.0 \times 10^{-7}$) were examined in the replication cohort. The 850K BeadChip was likewise processed at the Yale Center for Genome Analysis.

Because methylation values at methylation sites can be cell-type specific,[29] we conducted a cell composition estimation analysis using a modified version of the method by Housemann et al,[30,31] and estimated the relative proportion of each cell type (eg, CD34, CD14, and buccal cells) in the heterogenous peripheral saliva samples.

DNA methylation can vary by race or ethnicity.[32,33] To adjust for possible population stratification within the predominantly European American sample, we used a methylation-based principal component (PC) approach,[34] based on sets of methylation sites within 50 kb of SNPs using the 1000 Genomes Project variants with minor allele frequency of >0.1 following the Barfield et al method.[34] This method outperforms other methods that adjust for population stratification in methylation.[34]

Statistical Analyses

To control for familial correlations due to the inclusion of siblings in the cohort, generalized estimating equations (GEE) were used to examine the impact of intrafamilial adversity on BMI. Age, sex, and race were used as covariates in the GEE analysis (n = 234), and a separate GEE analysis was conducted with SES also included in the model for the subset of subjects with SES data (n = 175). In analyzing the methylation data for the discovery sample (https://cran.r-project.org/web/packages/lme4/index.html), linear mixed-effects models were used to examine the main effect of methylation M-values and intrafamilial childhood adversity scores and the interaction of these 2 variables using the "lme4" function in R software environment (R Foundation for Statistical Computing, Vienna, Austria). Familial correlations were modeled by assigning a random effect to each family. Potential confounding factors including age, sex, race, cell type (eg, CD14, CD34, buccal), and the first 3 PCs were also included in the model. To correct for multiple comparison testing, significant threshold for analyses was set to 5.0×10^{-7}, consistent with prior recommendations.[35] Only the methylation sites that significantly predicted BMI in the children in the discovery cohort after controlling for whole genome testing ($P < 5.0 \times 10^{-7}$) were examined in the replication cohort, with GEE used in these analyses with the same set of covariates listed above included in the models. The discovery cohort and replication samples were not analyzed together, as at present, methods do not exist to combine 450 K and 850 K data controlling for batch and BeadChip effects.

Results

The children in the cohort experienced a mean of 2.3 (SD = 2.3) intrafamilial adversities (range: 0-8), with 29% of the cohort reported to have experienced 4 or more intrafamilial adversities. **Table II** (available at www.jpeds.com) provides detailed information about the proportion of children to have experienced each ACE. Among the children in the maltreated cohort, 66% experienced physical abuse, 34% sexual abuse, 57% neglect, and 54% witnessed domestic violence. The Y-VACS scores of the discovery and replication cohorts are included in **Table I**. The higher intrafamilial adversity scores in the discovery cohort is likely attributable to the majority (82%) of the maltreated children with a recent out-of-home placement being recruited during the first 2 years of the study and included in the discovery cohort.

After controlling for age, sex, and race, the Y-VACS intrafamilial childhood adversity measure was a significant predictor of BMI (n = 234; $P < .0001$). Increased age ($P < .0001$) and African American ancestry ($P < .04$) also predicted greater BMI, but after accounting for the other measures in the model, sex ($P < .11$) was not a significant predictor of BMI. The Y-VACS intrafamilial childhood adversity score was still a significant predictor of BMI ($P < .004$) when SES was also included in the model, although these analyses were conducted on a smaller subset of subjects as SES data were missing for 25% of the sample, with missing SES data greatest for the most traumatized children— youth in out-of-home care who did not currently have any contact with their biological parents. Children who had high Y-VACS scores (n = 49), defined as 1 SD above the mean, were 16 times more likely to be obese than children who had low Y-VACS scores (n = 49), defined as 1 SD below the mean (percent obese: high Y-VACS: 16.3%; low Y-VACS: 1%, $\chi^2 = 17.6$, df = 3, $P < .001$).

After controlling for age, sex, race, cell heterogeneity, the first 3 PCs, and whole-genome testing, 6 methylation sites were found to exert a main effect in predicting BMI, and an additional 10 methylation sites were found to interact with the adverse childhood experiences measure to predict BMI (**Table III**). The direction of the interaction effects varied by site, with some methylation sites being stronger predictors of BMI in youth with low intrafamilial adversity scores (eg, *HID1, GALE)*, and other methylation sites being stronger predictors of BMI in youth with high intrafamilial adversity scores (eg, *PRDM16, PXDN)*. Eight of the 16 methylation sites identified in these analyses are in genes previously associated with obesity risk, and 3 were in intergenic regions.

Table III. Discovery cohort (n = 160): methylation sites that independently and in interaction with intrafamilial childhood adversity predicted BMI

Illumina ID	Gene symbol	Chr	Gene location	Methylation *P* value	Trauma *P* value	Interaction *P* value
cg10264529	***PCK2***	14	TSS1500	**7.53E-09**	ns	ns
cg14929207	*DHRS13*	17	TSS1500	**3.70E-08**	ns	ns
cg16110788		7	Intergenic (Enhancer)	**4.79E-08**	ns	ns
cg14855841	***CXCL10***	4	TSS1500	**7.59E-08**	ns	ns
cg26103104			Intergenic	**4.31E-07**	ns	ns
cg01555853	*KCNS3*	2	TSS200	**4.45E-07**	ns	ns
cg15990629	***BCAT1***	12	Body	ns	ns	**4.42E-09**
cg22806444	***HID1***	17	1st Exon (Regulatory)	ns	ns	**1.94E-08**
cg26764244	*GNG12*	1	TSS1500	ns	ns	**2.14E-08**
cg17489690	***PRDM16***	1	Body	ns	ns	**2.52E-08**
cg01507128		19	Intergenic	ns	ns	**5.55E-08**
cg16557308	*OSBPL9*	1	Promoter Associated	ns	ns	**6.27E-08**
cg18839416	*C1orf158*	1	TS1500	ns	ns	**6.34E-08**
cg05559960	***MADD***	11	TSS200	ns	ns	**6.60E-08**
cg24741066	***PXDN***	2	Body (Enhancer)	ns	ns	**2.66E-07**
cg26737766	***GALE***	1	Promoter Associated	ns	ns	**2.75E-07**

Chr, chromosome.
A linear mixed-effects model was used to examine association between methylation M-values, trauma (eg, intrafamilial childhood adversity), and the interaction between these 2 measures. The following covariates were included in the analysis: age, sex, race, cell type (eg, CD14, CD34, buccal), and the first 3 PCs. Bolded gene symbols are genes previously associated with risk for obesity.

Metacore software was used to identify biological processes associated with the top epigenome wide significant hits. This package accounts for the varying number of methylation sites per gene by assigning a prior probability for each gene based on gene length, followed by a modified hypergeometric test for over-representation of a gene set.[36] Methylation sites that had significance values of $P < 1.0 \times 10^{-5}$ were used in this analysis. To correct for multiple comparison testing, false discovery rate (FDR) was set at .05. Enrichment was seen in multiple muscle development and muscle cell proliferation processes, processes involved in responses to several different nutrients and glucocorticoids, as well as multiple processes relevant to translation and transcription. The top 50 significant gene ontology (GO) processes are included online only in **Table IV** (available at www.jpeds.com).

Of the 16 sites associated with obesity risk in the discovery cohort, 2 failed to pass quality control tests for analyses in the replication cohort; *KCNS3* (cg0155585) and *BCAT1* (cg15990629). As depicted in **Table V**, after controlling for age, sex, race, cell heterogeneity, and the first 3 PCs, the adversity measure and 4 of the methylation sites predicted BMI at nominal levels of significance, and the significance value associated with methylation in *PCK2* withstood Bonferroni correction for multiple comparisons ($P < .0031$). The interaction term of trauma with the methylation value at the methylation site in *GALE* also approached statistical significance in the replication sample.

There are multiple methylation sites contained on the 450K BeadChip for each of the genes identified as significant predictors of obesity. As depicted online in **Table VI** (available at www.jpeds.com), nominal levels of significance in predicting BMI were attained at 8 additional sites in these genes.

Given the replicated finding with *PCK2* across the 2 cohorts, follow-up mediation analyses were conducted using regression analyses and maintaining the demographic, cell heterogeneity, and PC covariates in the model. As depicted in **Figure 2**, after controlling for the relevant covariates, support for a mediation model was attained, with the effects of intrafamilial childhood adversities on indices of obesity found to be mediated by epigenetic changes in the *PCK2,* a gene implicated in obesity risk in a prior epigenome wide association study.[37]

Table V. Replication sample: methylation and trauma measures together predict BMI (n = 74)

ID	Gene symbol	Methylation *P* value	Trauma *P* value	Interaction *P* value
cg10264529	*PCK2*	.003	.02	ns
cg16110788		.03	.03	ns
cg26103104		.02	.03	ns
cg22806444	*HID1*	.02	.04	ns
cg26737766	*GALE*	.11	.04	.066 +

The covariates age, sex, race, cell type (CD14, CD34, buccal), and the first 3 PCs to account for population stratification were included in all analyses. The direction of the associations between BMI and the methylation markers was the same in the discovery and replication cohorts at each methylation site, except cg26103104.

Discussion

In the current investigation, ACEs predicted cross-sectional assessments of BMI in the children. Children who had high intrafamilial adversity scores, defined as 1 SD above the mean, were 16 times more likely to be obese than children who had low intrafamilial adversity scores, defined as 1 SD below the mean. The findings of this investigation are consistent with the results of a recent meta-analysis of 41 studies examining the association of child maltreatment and obesity.[21] The authors of that meta-analysis concluded that child maltreatment is a potentially modifiable risk factor for obesity, and future

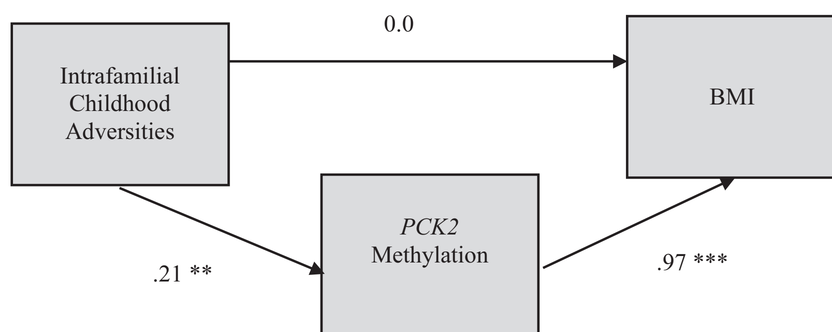

Figure 2. Indirect effects of intrafamilial childhood adversities on BMI through methylation of *PCK2*. After controlling for the relevant demographic factors (eg, age, sex, race), cell heterogeneity measures (eg, CD34, CD14, and buccal cells), and the first 3 PCs, support for a mediation model was attained, with the effects of intrafamilial childhood adversities on indices of obesity found to be mediated by epigenetic changes in the *PCK2,* a gene implicated in obesity risk in a prior epigenome wide study. ** = $P < .01$; *** = $P < .0001$.

research should clarify the mechanisms through which child maltreatment affects obesity risk.

The present study suggests child maltreatment may confer risk for obesity through epigenetic mechanisms. Among the subjects in the discovery data set, after controlling for age, sex, race, cell heterogeneity, 3 PCs, and whole-genome testing, 10 methylation sites were found to interact with the ACE measure to predict cross-sectional assessments of BMI, and an additional 6 sites were also found to exert a main effect in predicting BMI.

Eight of the methylation sites identified in the discovery data set are in genes previously associated with obesity risk or functionally related to relevant biological indices. Specifically, *PCK2* encodes an enzyme in mitochondria involved in glucose metabolism. It is mainly expressed in liver, pancreas, intestine fibroblasts and is involved in the insulin signaling pathway, with genetic variation in *PCK2* previously found to predict individual differences in response to dietary interventions.[38] *CXCL10* encodes a chemokine, with values of *CXCL10* found to correlate significantly with measures of visceral fat area in obese children.[39] The protein product of *BCAT1* catalyzes the first reaction in the catabolism of the essential branched chain amino acids leucine, isoleucine, and valine and has been identified as a candidate risk gene for obesity.[40] *HIDI* is associated with body fat mass regulation, preadipocyte number and adipocyte size in rats.[41] The *PRDM16* protein product is involved in the differentiation of brown adipose tissue, and the *PRDM16* transcriptional pathway has been identified as promising for the development of novel therapeutic interventions for the treatment of obesity and obesity-related diseases.[42] *OSBPL9* encodes a member of the oxysterol-binding protein family, a group of intracellular lipid receptors.[43] *MADD* variants are implicated in type 2 diabetes and have been associated with fasting proinsulin levels in genome wide association studies.[44] *PXDN* deletions have been associated with early onset obesity,[45,46] and *GALE* encodes UDP-galactose-4-epimerase which catalyzes 2 distinct but analogous reactions with important metabolic consequences.[47] Although the exact methylation sites identified in the current investigation have not been reported in prior epigenome-wide studies of obesity risk,[37,48-53] methylation of other methylation sites in 4 of the genes identified in this study (eg, *PCK2, MADD, PRDM16*, *BCAT1*) were reported in the prior investigations.[37,48-50,53]

Three of the significant methylation sites identified in the discovery data set were in intergenic regions. Many methylation islands in intergenic regions are enriched for factor binding sites and are involved in the 3-dimensional organization of the genome and gene regulation.[54,55] Transcription factor binding sites and chromatin insulators within intergenic regions are believed to mediate intra- and interchromosomal interactions, affecting gene expression at both proximal and distal locations,[55] and there are numerous instances where intergenic genetic variation is associated with disease risk.[56]

Results of the gene ontology analysis conducted in the discovery cohort with the top epigenome wide significant hits ($P < 1.0 \times {}^{10-}5$) found enrichment in multiple biologically relevant processes. Muscle development and muscle cell proliferation processes were enriched among the methylation sites with the highest association with BMI. Processes involved in responses to several different nutrients (eg, vitamin A, vitamin D, iron) and glucocorticoids, as well as multiple processes relevant to translation and transcription were also enriched in the top epigenome significant findings.

In terms of the replication analyses, even though the replication sample was small (n = 74) and had a lower prevalence of obesity and history of childhood adversities, several of the findings from the discovery data set were also significant predictors of BMI in the second cohort. The most robust of the findings involved *PCK2,* with this result withstanding Bonferroni correction for multiple comparisons. In addition, follow-up analyses with *PCK2* provided support for a mediation model in which the effects of intrafamilial childhood adversities on indices of obesity were found to be mediated by epigenetic changes in *PCK2*.

Although prior literature indicates that DNA methylation levels in saliva are similar to those in peripheral blood, skin

fibroblasts, and buccal swab DNA, it may not reflect the epigenome of adipose tissue, muscle, pancreas, gastrointestinal system, or the pituitary—tissues most relevant in obesity.[57,58] Additional limitations of the current investigation include small size of the replication cohort, use of the 450K BeadChip, which does not include methylation sites at the most replicated previously identified obesity genetic risk SNPs, limiting the test of these genes in the current investigation, use of the 850K platform in the replication cohort, the lack of genetic variant and gene expression data, the absence of food intake and exercise data,[59] failure to assess prenatal factors known to affect methylation and obesity risk,[60-62] and the cross-sectional nature of the study which limits inferences that can be made regarding the causal consequences of the methylation findings.

The data reported in this article suggest adverse childhood experiences may confer risk for health problems through epigenetic mechanisms. Although epigenetic modifications are frequently long lasting, they can sometimes be reversed. This study lays the groundwork for future longitudinal clinical and translational studies to further elucidate these mechanisms and identify novel prevention and treatment interventions to alleviate the health burden associated with early adversity. ■

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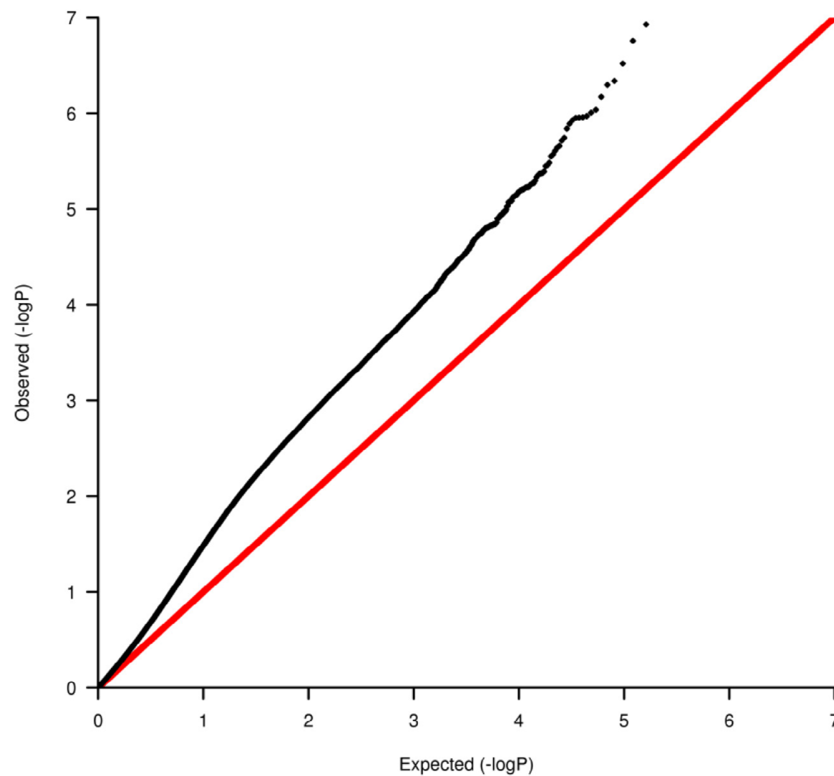

Figure 1. Quantile-quantile (Q-Q) plots. The figure above depicts the Q-Q plots for the *P* values of the association. The genomic inflation factor for the discovery cohort was 1.004, indicating that the epigenome wide analyses results are negligibly inflated and the results are unlikely due to spurious findings.

Table II. Proportion of children who experienced each adversity

	Discovery cohort n = 160	Replication cohort n = 74
Intrafamilial adversities		
Psychological abuse	70 (44%)	10 (13%)
Physical abuse	77 (48%)	4 (5%)
Sexual abuse	40 (25%)	2 (3%)
Neglect	59 (37%)	11 (15%)
Parent separation/death	85 (53%)	10 (14%)
Domestic violence	59 (37%)	8 (11%)
Caregiver substance abuse	43 (27%)	6 (8%)
Suicidality in immediate family	34 (24%)	7 (9%)
Caregiver criminal behaviour	30 (19%)	5 (7%)
Extrafamilial adversities		
Community violence	29 (18%)	5 (7%)
Bullying	25 (16%)	5 (7%)
War/terrorism	5 (3%)	0 (0%)
Nonfamilial sexual assault	24 (15%)	2 (3%)
Accident	72 (45%)	37 (50%)
Natural disaster	30 (19%)	16 (22%)
Health-related trauma	35 (22%)	7 (10%)
Fire	16 (10%)	8 (11%)
Death of loved one (nonimmediate family member)	94 (59%)	44 (60%)

As noted in the manuscript, the majority (71/87, 82%) of the maltreated children who were recruited after a recent out-of-home placement were recruited during the first 2 years of the study, accounting for the greater rate of adversity in the discovery cohort.

Table IV. Enrichment analysis report

#	Enrichment by gene ontology processes				BMI_10-5CpG_genenames_beta			
#	Processes	Total	*P* value	Min FDR	*P* value	FDR	In data	Network objects from active data
1	Modulation by virus of host morphology or physiology	69	6.065E-05	2.508E-02	6.065E-05	2.508E-02	3	TYSY, RXRA, RXR
2	Response to vitamin A	73	7.179E-05	2.508E-02	7.179E-05	2.508E-02	3	TYSY, RXRA, RXR
3	Response to nutrient	441	1.099E-04	2.508E-02	1.099E-04	2.508E-02	5	TYSY, RXRA, RXR, SRF, IP10
4	Response to vitamin D	89	1.295E-04	2.508E-02	1.295E-04	2.508E-02	3	RXRA, RXR, IP10
5	Synapse maturation	16	1.409E-04	2.508E-02	1.409E-04	2.508E-02	2	NF-I, NFIA
6	Modification by symbiont of host morphology or physiology	92	1.429E-04	2.508E-02	1.429E-04	2.508E-02	3	TYSY, RXRA, RXR
7	Response to vitamin	245	1.459E-04	2.508E-02	1.459E-04	2.508E-02	4	TYSY, RXRA, RXR, IP10
8	Positive regulation of translational initiation by iron	17	1.596E-04	2.508E-02	1.596E-04	2.508E-02	2	RXRA, RXR
9	Gland development	776	1.695E-04	2.508E-02	1.695E-04	2.508E-02	6	NF-I, TYSY, RXRA, RXR, RLTPR, SRF
10	Regulation of translational initiation by iron	18	1.794E-04	2.508E-02	1.794E-04	2.508E-02	2	RXRA, RXR
11	Ureter development	19	2.004E-04	2.547E-02	2.004E-04	2.547E-02	2	NF-I, NFIA
12	Secretory columnal luminar epithelial cell differentiation involved in prostate glandular acinus development	21	2.458E-04	2.756E-02	2.458E-04	2.756E-02	2	RXRA, RXR
13	Coronary vasculature development	113	2.624E-04	2.756E-02	2.624E-04	2.756E-02	3	RXRA, RXR, SRF
14	Modification of morphology or physiology of other organism	293	2.890E-04	2.756E-02	2.890E-04	2.756E-02	4	TYSY, RXRA, RXR, IP10
15	Angiogenesis involved in coronary vascular morphogenesis	23	2.957E-04	2.756E-02	2.957E-04	2.756E-02	2	RXRA, RXR
16	Regulation of branching involved in prostate gland morphogenesis	25	3.501E-04	3.010E-02	3.501E-04	3.010E-02	2	RXRA, RXR
17	Visceral serous pericardium development	26	3.791E-04	3.010E-02	3.791E-04	3.010E-02	2	RXRA, RXR
18	Negative regulation of pri-miRNA transcription from RNA polymerase II promoter	27	4.091E-04	3.010E-02	4.091E-04	3.010E-02	2	NF-I, SRF
19	Peroxisome proliferator activated receptor signaling pathway	27	4.091E-04	3.010E-02	4.091E-04	3.010E-02	2	RXRA, RXR
20	Tube development	957	5.230E-04	3.656E-02	5.230E-04	3.656E-02	6	NF-I, NFIA, TYSY, RXRA, RXR, SRF
21	Prostate glandular acinus development	32	5.761E-04	3.835E-02	5.761E-04	3.835E-02	2	RXRA, RXR
22	Carboxylic acid transport	383	7.923E-04	4.553E-02	7.923E-04	4.553E-02	4	SLC16A13, RXRA, SAT-1, RXR
23	Epithelial cell differentiation involved in prostate gland development	38	8.132E-04	4.553E-02	8.132E-04	4.553E-02	2	RXRA, RXR
24	Transcription from RNA polymerase II promoter	1046	8.354E-04	4.553E-02	8.354E-04	4.553E-02	6	NF-I, NFIA, RXRA, RXR, PHRF1, SRF
25	Columnar/cuboidal epithelial cell differentiation	168	8.355E-04	4.553E-02	8.355E-04	4.553E-02	3	TYSY, RXRA, RXR
26	Organic acid transport	394	8.804E-04	4.553E-02	8.804E-04	4.553E-02	4	SLC16A13, RXRA, SAT-1, RXR
27	Cardiac muscle cell differentiation	178	9.874E-04	4.553E-02	9.874E-04	4.553E-02	3	RXRA, RXR, SRF
28	Midgut development	42	9.933E-04	4.553E-02	9.933E-04	4.553E-02	2	RXRA, RXR
29	Cardiac muscle cell proliferation	43	1.041E-03	4.553E-02	1.041E-03	4.553E-02	2	RXRA, RXR
30	Response to glucocorticoid	414	1.058E-03	4.553E-02	1.058E-03	4.553E-02	4	TYSY, RXRA, RXR, PPCKM
31	Retinoic acid receptor signaling pathway	44	1.090E-03	4.553E-02	1.090E-03	4.553E-02	2	RXRA, RXR
32	Viral genome replication	44	1.090E-03	4.553E-02	1.090E-03	4.553E-02	2	NF-I, NFIA
33	Negative regulation of macromolecule biosynthetic process	2005	1.093E-03	4.553E-02	1.093E-03	4.553E-02	8	NF-I, NFIA, TYSY, RXRA, RXR, LAG3, SET8, SRF
34	Immortalization of host cell by virus	1	1.110E-03	4.553E-02	1.110E-03	4.553E-02	1	TYSY
35	Striated muscle cell proliferation	45	1.140E-03	4.553E-02	1.140E-03	4.553E-02	2	RXRA, RXR
36	Ventricular cardiac muscle cell differentiation	47	1.243E-03	4.675E-02	1.243E-03	4.675E-02	2	RXRA, RXR
37	Modification of morphology or physiology of other organism involved in symbiotic interaction	195	1.284E-03	4.675E-02	1.284E-03	4.675E-02	3	TYSY, RXRA, RXR
38	Muscle structure development	760	1.326E-03	4.675E-02	1.326E-03	4.675E-02	5	KCRS, RXRA, RXR, SRF, IP10
39	Negative regulation of transcription from RNA polymerase II promoter	1154	1.391E-03	4.675E-02	1.391E-03	4.675E-02	6	NF-I, NFIA, RXRA, RXR, SET8, SRF
40	Positive regulation of translational initiation	50	1.406E-03	4.675E-02	1.406E-03	4.675E-02	2	RXRA, RXR
41	Pericardium development	50	1.406E-03	4.675E-02	1.406E-03	4.675E-02	2	RXRA, RXR
42	Response to corticosteroid	452	1.463E-03	4.675E-02	1.463E-03	4.675E-02	4	TYSY, RXRA, RXR, PPCKM
43	Protein homooligomerization	454	1.487E-03	4.675E-02	1.487E-03	4.675E-02	4	CCDC88C, RXRA, RXR, Kv9.3
44	Response to selenium ion	52	1.519E-03	4.675E-02	1.519E-03	4.675E-02	2	RXRA, RXR
45	Cardiac chamber morphogenesis	207	1.524E-03	4.675E-02	1.524E-03	4.675E-02	3	RXRA, RXR, SRF
46	Negative regulation of cellular biosynthetic process	2113	1.538E-03	4.675E-02	1.538E-03	4.675E-02	8	NF-I, NFIA, TYSY, RXRA, RXR, LAG3, SET8, SRF
47	Muscle cell proliferation	53	1.578E-03	4.686E-02	1.578E-03	4.686E-02	2	RXRA, RXR
48	Muscle organ development	466	1.636E-03	4.686E-02	1.636E-03	4.686E-02	4	KCRS, RXRA, RXR, IP10
49	Cartilage development	213	1.654E-03	4.686E-02	1.654E-03	4.686E-02	3	NF-I, TYSY, SRF
50	Monocarboxylic acid transport	214	1.676E-03	4.686E-02	1.676E-03	4.686E-02	3	SLC16A13, RXRA, RXR

FDR, false discovery rate.

Table VI. Additional methylation sites in replicated genes that predicted BMI

Ilmn ID	UCSC_Ref	Regulatory_Feature	beta	Rintrafa	betaRintrafa
cg10264529	PCK2		7.53E-09	0.224387393	0.010497497
cg09378756	PCK2		0.27985477	0.573348026	0.028519211
cg14089728	PCK2	Promoter_Associated	0.069079566	0.51270066	0.044400055
cg15412728	PCK2	Promoter_Associated	0.235420241	0.786173809	0.08317027
cg15467148	PCK2	Promoter_Associated	0.130683774	0.626457756	0.303843673
cg18599124	PCK2	Promoter_Associated	0.253946953	0.763249148	0.025101018
cg20017797	PCK2	Promoter_Associated	0.130769156	0.784854292	0.893846413
cg22454119	PCK2	Promoter_Associated	0.114150643	0.747082866	0.005328352
cg23112188	PCK2	Promoter_Associated	0.879932822	0.620458559	0.024862955
cg23204518	PCK2	Promoter_Associated	0.91842871	0.710926936	0.120762948
cg26402828	PCK2	Promoter_Associated	0.575684999	0.629630835	0.117913307
cg22806444	C17orf28		0.01231404	0.14989789	1.94E-08
cg00806198	C17orf28		0.93848168	0.662796003	0.325379181
cg02910913	C17orf28		0.019788616	0.851725304	0.599933481
cg02971219	C17orf28		0.502069316	0.858404572	0.926408345
cg03154277	C17orf28		0.409905078	0.702229156	0.809669773
cg03443162	C17orf28		0.619154034	0.708902821	0.57843624
cg03553897	C17orf28		0.560073058	0.809326097	0.458254573
cg05865011	C17orf28		0.575394141	0.714386769	0.4533703
cg07430967	C17orf28		0.869814807	0.784834206	0.127374153
cg08118042	C17orf28		0.366762261	0.652592968	0.069566382
cg08449860	C17orf28		0.076589066	0.718542144	0.333185956
cg10907148	C17orf28		0.374014637	0.788391716	0.821145073
cg12349832	C17orf28		0.736324051	0.669589407	0.122706656
cg13258989	C17orf28		0.987813718	0.754331951	0.726390523
cg13745142	C17orf28		0.424505282	0.715517995	0.092671424
cg13751265	C17orf28		0.513649469	0.711737244	0.240326202
cg13923669	C17orf28		0.403168024	0.841460761	0.519914853
cg17713161	C17orf28		0.266090603	0.467955285	0.077845135
cg18909235	C17orf28		0.536785314	0.805901352	0.593164502
cg20653144	C17orf28		0.636954817	0.732283889	0.800346799
cg20935945	C17orf28		0.878879606	0.525065067	0.107795851
cg21404063	C17orf28		0.694186282	0.761037329	0.94855794
cg24428040	C17orf28		0.212666083	0.618930153	0.2845851
cg26737766	GALE	Promoter_Associated	0.093330626	0.531049042	2.75E-07
cg00988350	GALE	Promoter_Associated	0.910762261	0.700646975	0.155178001
cg03441514	GALE	Promoter_Associated	0.140169638	0.719064082	0.256553291
cg04975205	GALE	Promoter_Associated	0.936582679	0.728230197	0.729488336
cg06902898	GALE		0.54783286	0.811498399	0.162226526
cg07291005	GALE	Promoter_Associated_	0.308069403	0.726319948	0.174036904
cg08733957	GALE		0.474868631	0.711729523	0.282496223
cg10498717	GALE		0.923735355	0.737527332	0.089257897
cg10961323	GALE		0.116414025	0.697266947	0.466426283
cg11410649	GALE		0.023572638	0.391003777	0.041192457
cg12130907	GALE		0.176216704	0.920303841	0.224545139
cg12275652	GALE	Promoter_Associated	0.862038702	0.84825002	0.20564886
cg13180787	GALE	Promoter_Associated	0.450415584	0.658193289	0.09690108
cg13391456	GALE	Promoter_Associated	0.335938736	0.845397753	0.79768066
cg13580783	GALE		0.043911036	0.635057866	0.158267601
cg15988345	GALE		0.270596868	0.725994747	0.767709152
cg21523719	GALE		0.612729187	0.79712088	0.503888169
cg22041707	GALE		0.78238677	0.814194736	0.185582746
cg22857999	GALE	Promoter_Associated	0.91062492	0.765938181	0.819766378
cg24409107	GALE	Promoter_Associated	0.517157447	0.722857112	0.593508859
cg24448013	GALE	Promoter_Associated	0.841150938	0.723758644	0.797507359
cg24454698	GALE		0.416277666	0.524580134	0.094275019
cg25203007	GALE	Promoter_Associated_	0.746106303	0.614525399	0.072719981
cg25549791	GALE		0.461533973	0.761174261	0.884385127
cg27448574	GALE	Promoter_Associated	0.192541489	0.885561915	0.327866691

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Among the additional CpG sites included on the BeadChip in the replicated genes found to predict BMI in the children, traditional levels of significance in predicting BMI were attained at 8 sites. None of the sites survived corrections for multiple comparisons.