Published in final edited form as:

J Pediatr. 2018 November ; 202: 150–156.e3. doi:10.1016/j.jpeds.2018.06.051.

Adverse Childhood Experiences, Epigenetic Measures, and Obesity in Youth

Joan Kaufman, PhD^{1,2,3}, Janitza L. Montalvo-Ortiz, PhD³, Hannah Holbrook, BA⁴, Kerry O'Loughlin, BA⁴, Catherine Orr, PhD⁴, Catherine Kearney, MA¹, Bao-Zhu Yang, PhD³, Tao Wang, PhD^{5,6}, Hongyu Zhao, PhD⁵, Robert Althoff, MD, PhD⁴, Hugh Garavan, PhD⁴, Joel Gelernter, MD^{3,7}, and James Hudziak, MD⁴

¹Center for Child and Family Traumatic Stress, Kennedy Krieger Institute

²Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD

³Department of Psychiatry, Yale University, New Haven, CT

⁴Vermont Center for Children, Youth, and Families, Department of Psychiatry, University of Vermont, Burlington, VT

⁵Department of Biostatistics, Yale University, New Haven, CT

⁶Department of Bioinformatics and Biostatistics, Shanghai Jiao Tong University, Shanghai, China

⁷Veterans Administration, West Haven, CT

Abstract

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*, *CxCI10*,

Reprint requests: Joan Kaufman, PhD, Research, Center for Child and Family Traumatic Stress, Kennedy Krieger Institute, Psychiatry, Johns Hopkins School of Medicine, 1750 E. Fairmount Ave, Second Floor, Baltimore, MD 21231. joan.kaufman@kennedykrieger.org.

The authors declare no conflicts of interest.

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.

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.

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.

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 extrafamilial 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 Methylation 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 heterogeneous 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, *H1D1*, *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.

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

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 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.

Acknowledgments

Supported by the Zanyvl and Isabelle Krieger Fund (to J.K.), the National Institutes of Health (R01MH098073 [to J.K. and J.H.], R01 MD011746–01 [to J.K.]); the National Center for Posttraumatic Stress Disorder—Veterans Affairs Connecticut (to J.G. and J.K.); the VA Cooperative Study #575B, Genomics of Posttraumatic Stress Disorder in Veterans (to J.G. and J.K.); and the Biological Sciences Training Program through 5T32 MH14276 (to J.M-O.).

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

Glossary

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

References

1. CDC. Adverse childhood experiences reported by adults—five states, 2009. *MMWR Morb Mortal Wkly Rep* 2010;59:1609–13. Reprinted in *JAMA*, 2011; 305(7):666–669. [PubMed: 21160456]
2. Kuehn BM. AAP: toxic stress threatens kids' long-term health. *JAMA* 2014;312:585–6. doi: 10.1001/jama.2014.8737. [PubMed: 25075798]
3. 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;301:2252–9. [PubMed: 19491187]
4. Anda RF, Croft JB, Felitti VJ, Nordenberg D, Giles WH, Williamson DF, et al. Adverse childhood experiences and smoking during adolescence and adulthood. *JAMA* 1999;282:1652–8. [PubMed: 10553792]
5. 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;57:953–9. [PubMed: 11015813]
6. Molnar BE, Buka SL, Kessler RC. Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. *Am J Public Health* 2001;91:753–60. [PubMed: 11344883]
7. Kaufman J Child abuse. Lewis' child and adolescent psychiatry: a comprehensive textbook 4th ed. Baltimore (MD): Lippincott Williams & Wilkins; 2007.
8. Burke NJ, Hellman JL, Brandon SG, Weems CF, Carrion VG. The impact of adverse childhood experiences on an urban pediatric population. *Child Abuse Negl* 2011;35:408–13. [PubMed: 21652073]
9. Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, 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;14:245–58. [PubMed: 9635069]
10. Dong M, Giles WH, Felitti VJ, Dube SR, Williams JE, Chapman DP, et al. Insights into causal pathways for ischemic heart disease: adverse childhood experiences study. *Circulation* 2004;110:1761–6. [PubMed: 15381652]
11. Romans S, Belaise C, Martin J, Morris E, Raffi A. Childhood abuse and later medical disorders in women. An epidemiological study. *Psychother Psychosom* 2002;71:141–50. [PubMed: 12021556]
12. Anda RF, Brown DW, Dube SR, Bremner JD, Felitti VJ, Giles WH. Adverse childhood experiences and chronic obstructive pulmonary disease in adults. *Am J Prev Med* 2008;34:396–403. [PubMed: 18407006]
13. Dube SR, Fairweather D, Pearson WS, Felitti VJ, Anda RF, Croft JB. Cumulative childhood stress and autoimmune diseases in adults. *Psychosom Med* 2009;71:243–50. [PubMed: 19188532]
14. Brown DW, Anda RF, Felitti VJ, Edwards VJ, Malarcher AM, Croft JB, et al. Adverse childhood experiences are associated with the risk of lung cancer: a prospective cohort study. *BMC Public Health* 2010;10:20. [PubMed: 20085623]
15. Yang B-Z, Zhang H, Ge W, Weder N, Douglas-Palumberi H, Perepletchikova F, et al. Child abuse and epigenetic mechanisms of disease risk. *Am J Prev Med* 2013;44:101–7. [PubMed: 23332324]
16. Zhang TY, Meaney MJ. Epigenetics and the environmental regulation of the genome and its function. *Annu Rev Psychol* 2010;61:439–66, C1–3. [PubMed: 19958180]
17. Bredy TW, Sun YE, Kobor MS. How the epigenome contributes to the development of psychiatric disorders. *Dev Psychobiol* 2010;52:331–42. doi:10.1002/dev.20424. [PubMed: 20127889]

18. Zhang H, Kranzler HR, Poling J, Gelernter J. Variation in the nicotinic acetylcholine receptor gene cluster CHRNA5-CHRNA3-CHRNA4 and its interaction with recent tobacco use influence cognitive flexibility. *Neuropsychopharmacology* 2010;35:2211–24. [PubMed: 20631687]
19. Nestler EJ. Epigenetic mechanisms in psychiatry. *Biol Psychiatry* 2009;65:189–90. [PubMed: 19146997]
20. Nestler E, Hyman SE, Malenka RC. *Molecular neuropharmacology: a foundation for clinical neuroscience* 2nd ed. New York (NY): McGraw-Hill Professional; 2008.
21. Danese A, Tan M. Childhood maltreatment and obesity: systematic review and meta-analysis. *Mol Psychiatry* 2014;19:544–54. [PubMed: 23689533]
22. Kaufman J Depressive disorders in maltreated children. *J Am Acad Child Adolesc Psychiatry* 1991;30:257–65. [PubMed: 2016230]
23. Kaufman J, Cicchetti D. The effects of maltreatment on school-aged children's socio-emotional development: assessments in a day camp setting. *Dev Psychol* 1989;25:516–24.
24. Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal J, et al. Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci USA* 2004;101:17316–21. [PubMed: 15563601]
25. Holbrook H, O'Loughlin K, Althoff R, Douglas-Palumberi H, Kaufman J, Hudziak J. The Yale-Vermont adversity in childhood scale: a quantitative approach to adversity assessment. *American Academy of Child and Adolescent Psychiatry's 61st Annual Meeting* San Diego, CA 2015.
26. Maksimovic J, Phipson B, Oshlack A. A cross-package Bioconductor workflow for analysing methylation array data. *F1000Res* 2016;5:1281. [PubMed: 27347385]
27. Fortin JP, Labbe A, Lemire M, Zanke BW, Hudson TJ, Fertig EJ, et al. Functional normalization of 450k methylation array data improves replication in large cancer studies. *Genome Biol* 2014;15:503. [PubMed: 25599564]
28. Jensen KP, Smith AH, Herman AI, Farrer LA, Kranzler HR, Sofuoglu M, et al. A protocadherin gene cluster regulatory variant is associated with nicotine withdrawal and the urge to smoke. *Mol Psychiatry* 2017;22:242–9. doi:10.1038/mp.2016.43. Epub Apr 12. [PubMed: 27067016]
29. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol* 2014;15:R31. [PubMed: 24495553]
30. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012;13:86. doi:10.1186/471-2105-13-86. [PubMed: 22568884]
31. Smith AK, Kilaru V, Klengel T, Mercer KB, Bradley B, Conneely KN, et al. DNA extracted from saliva for methylation studies of psychiatric traits: evidence tissue specificity and relatedness to brain. *Am J Med Genet B Neuropsychiatr Genet* 2015;168B:36–44. doi:10.1002/ajmg.b.32278. Epub 2014 Oct 29. [PubMed: 25355443]
32. Adkins RM, Krushkal J, Tykavsky FA, Thomas F. Racial differences in genespecific DNA methylation levels are present at birth. *Birth Defects Res A Clin Mol Teratol* 2011;91:728–36. [PubMed: 21308978]
33. Heyn H, Moran S, Hernando-Herraez I, Sayols S, Gomez A, Sandoval J, et al. DNA methylation contributes to natural human variation. *Genome Res* 2013;23:1363–72. [PubMed: 23908385]
34. Barfield RT, Almli LM, Kilaru V, Smith AK, Mercer KB, Duncan R, et al. Accounting for population stratification in DNA methylation studies. *Genet Epidemiol* 2014;38:231–41. [PubMed: 24478250]
35. Rakyen VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nat Rev Genet* 2011;12:529–41. [PubMed: 21747404]
36. Phipson B, Maksimovic J, Oshlack A. missMethyl: an R package for analyzing data from Illumina's HumanMethylation450 platform. *Bioinformatics* 2016;32:286–8. [PubMed: 26424855]
37. Huang RC, Garratt ES, Pan H, Wu Y, Davis EA, Barton SJ, et al. Genome-wide methylation analysis identifies differentially methylated CpG loci associated with severe obesity in childhood. *Epigenetics* 2015;10:995–1005. doi:10.1080/15592294.2015.1080411. [PubMed: 26646899]
38. Larsen LH, Angquist L, Vimalaswaran KS, Hager J, Viguerie N, Loos RJ, et al. Analyses of single nucleotide polymorphisms in selected nutrient-sensitive genes in weight-regain prevention: the

- DIOGENES study. *Am J Clin Nutr* 2012;95:1254–60. doi:10.3945/ajcn.111.016543. Epub 2012 Apr 4. [PubMed: 22492381]
39. Ishii M, Araki S, Goto M, Yamamoto Y, Kusuha K. CCL2 level is elevated with metabolic syndrome and CXCL10 level is correlated with visceral fat area in obese children. *Endocr J* 2016;63:795–804. Epub 2016 Jul 2. [PubMed: 27375180]
 40. Chen J, Meng Y, Zhou J, Zhuo M, Ling F, Zhang Y, et al. Identifying candidate genes for Type 2 Diabetes Mellitus and obesity through gene expression profiling in multiple tissues or cells. *J Diabetes Res* 2013;2013:970435. doi:10.1155/2013/970435. Epub 2013 Dec 26. [PubMed: 24455749]
 41. Weingarten A, Turchetti L, Krohn K, Kloting I, Kern M, Kovacs P, et al. Novel genes on rat chromosome 10 are linked to body fat mass, preadipocyte number and adipocyte size. *Int J Obes (Lond)* 2016;40:1832–40. doi:10.1038/ijo.2016.127. Epub Jul 27. [PubMed: 27460604]
 42. Kajimura S Promoting brown and beige adipocyte biogenesis through the PRDM16 pathway. *Int J Obes Suppl* 2015;5:S11–4. doi:10.1038/ijosup.2015.4. Epub Aug 4. [PubMed: 27152168]
 43. GeneCards. OSBPL9 Gene: Oxysterol Binding Protein Like 9 GeneCards Human Gene DataBase: Weizmann Institute of Science; 2017.
 44. Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, et al. Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. *Diabetes* 2011;60:2624–34. doi:10.3377/db11-0415. Epub 2011 Aug 26. [PubMed: 21873549]
 45. Bonaglia MC, Giorda R, Zanini S. A new patient with a terminal de novo 2p25.3 deletion of 1.9 Mb associated with early-onset of obesity, intellectual disabilities and hyperkinetic disorder. *Mol Cytogenet* 2014;7:53. doi:10.1186/755-8166-7-53. eCollection 2014. [PubMed: 25126114]
 46. Doco-Fenzy M, Leroy C, Schneider A, Petit F, Delrue MA, Andrieux J, et al. Early-onset obesity and paternal 2pter deletion encompassing the ACP1, TMEM18, and MYT1L genes. *Eur J Hum Genet* 2014;22:471–9. doi:10.1038/ejhg.2013.189. Epub Oct 16. [PubMed: 24129437]
 47. GeneCards. GALE Gene: UDP-Galactose-4-Epimerase Gene Cards: Human Gene Database: Weizmann Institute of Science; 2017.
 48. Demerath EW, Guan W, Grove ML, Aslibekyan S, Mendelson M, Zhou YH, et al. Epigenome-wide association study (EWAS) of BMI, BMI change and waist circumference in African American adults identifies multiple replicated loci. *Hum Mol Genet* 2015;24:4464–79. doi:10.1093/hmg/ddv161. Epub 2015 May 1. [PubMed: 25935004]
 49. Meeks KAC, Henneman P, Venema A, Burr T, Galbete C, Danquah I, et al. An epigenome-wide association study in whole blood of measures of adiposity among Ghanaians: the RODAM study. *Clin Epigenetics* 2017;9:103. doi:10.1186/s13148-017-0403-x. eCollection 2017. [PubMed: 28947923]
 50. Samblas M, Milagro FI, Mansego ML, Marti A, Martinez JA. PTPRS and PER3 methylation levels are associated with childhood obesity: results from a genome-wide methylation analysis. *Pediatr Obes* 2017;14:12224.
 51. Sayols-Baixeras S, Subirana I, Fernandez-Sanles A, Senti M, Lluís-Ganella C, Marrugat J, et al. DNA methylation and obesity traits: an epigenome-wide association study. The REGICOR study. *Epigenetics* 2017;3:1363951.
 52. Wahl S, Drong A, Lehne B, Loh M, Scott WR, Kunze S, et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature* 2017;541:81–6. doi:10.1038/nature20784. Epub 2016 Dec 21. [PubMed: 28002404]
 53. Samblas M, Milagro FI, Mansego ML, Marti A, Martinez JA. PTPRS and PER3 methylation levels are associated with childhood obesity: results from a genome-wide methylation analysis. *Pediatr Obes* 2018;13:149–58. doi:10.1111/ijpo.12224. Epub 2017 Jun 14. [PubMed: 28614626]
 54. Hodges E, Molaro A, Dos Santos CO, Thekkat P, Song Q, Uren PJ, et al. Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. *Mol Cell* 2011;44:17–28. [PubMed: 21924933]
 55. Yang J, Corces VG. Chromatin insulators: a role in nuclear organization and gene expression. *Adv Cancer Res* 2011;110:43–76. [PubMed: 21704228]

56. Cotnoir-White D, Laperriere D, Mader S. Evolution of the repertoire of nuclear receptor binding sites in genomes. *Mol Cell Endocrinol* 2011;334:76–82. [PubMed: 21056084]
57. Oelsner KT, Guo Y, To SB, Non AL, Barkin SL. Maternal BMI as a predictor of methylation of obesity-related genes in saliva samples from preschool-age Hispanic children at-risk for obesity. *BMC Genomics* 2017;18:57. doi:10.1186/s12864-016-3473-9. [PubMed: 28068899]
58. Souren NY, Tierling S, Fryns JP, Derom C, Walter J, Zeegers MP. DNA methylation variability at growth-related imprints does not contribute to overweight in monozygotic twins discordant for BMI. *Obesity (Silver Spring)* 2011;19:1519–22. doi:10.038/oby.2010.353. Epub 1 Jan 27. [PubMed: 21273997]
59. Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and other environmental factors. *Adv Genet* 2010;71:3–39. doi:10.1016/B978-0-12-380864-6.00001-8. [PubMed: 20933124]
60. Cao-Lei L, Dancause KN, Elgbeili G, Massart R, Szyf M, Liu A, et al. DNA methylation mediates the impact of exposure to prenatal maternal stress on BMI and central adiposity in children at age 13(½) years: Project Ice Storm. *Epigenetics* 2015;10:749–61. doi: 10.1080/15592294.2015.1063771. [PubMed: 26098974]
61. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci USA* 2008;105:17046–9. doi:10.1073/pnas.0806560105. Epub 2008 Oct 27. [PubMed: 18955703]
62. Veenendaal MV, Costello PM, Lillycrop KA, de Rooij SR, van der Post JA, Bossuyt PM, et al. Prenatal famine exposure, health in later life and promoter methylation of four candidate genes. *J Dev Orig Health Dis* 2012;3:450–7. doi:10.1017/S2040174412000396. [PubMed: 25084298]

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.

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.

Table I.

Demographic and clinical characteristics of the sample

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

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 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 <i>P</i> value	Trauma <i>P</i> value	Interaction <i>P</i> value
cg10264529	<i>PCK2</i>	14	TSS1500	7.53E-09	ns	ns
cg14929207	<i>DHRS13</i>	17	TSS1500	3.70E-08	ns	ns
cg16110788		7	Intergenic (Enhancer)	4.79E-08	ns	ns
cg14855841	<i>CXCL10</i>	4	TSS1500	7.59E-08	ns	ns
cg26103104			Intergenic	4.31E-07	ns	ns
cg01555853	<i>KCNK3</i>	2	TSS200	4.45E-07	ns	ns
cg15990629	<i>BCAT1</i>	12	Body	ns	ns	4.42E-09
cg22806444	<i>HDI</i>	17	1 st Exon (Regulatory)	ns	ns	1.94E-08
cg26764244	<i>GNG12</i>	1	TSS1500	ns	ns	2.14E-08
cg17489690	<i>PRDM16</i>	1	Body	ns	ns	2.52E-08
cg01507128		19	Intergenic	ns	ns	5.55E-08
cg16557308	<i>OSBPL9</i>	1	Promoter Associated	ns	ns	6.27E-08
cg18839416	<i>C1orf158</i>	1	TS1500	ns	ns	6.34E-08
cg05559960	<i>MADD</i>	11	TSS200	ns	ns	6.60E-08
cg24741066	<i>PXDN</i>	2	Body (Enhancer)	ns	ns	2.66E-07
cg26737766	<i>GALE</i>	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.

Table IV.

Enrichment analysis report

Enrichment by gene ontology processes				BMI 10–5CpG genenames beta				Network objects from active data
#	Processes	Total	P value	Min FDR	P value	FDR	In 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 columnar luminal 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

Enrichment by gene ontology processes				BMI_10-5CpG_genenames_beta				Network objects from active data	
#	Processes	Total	P value	Min FDR	P value	FDR	In data		
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-1, NF1A	
33	Negative regulation of macromolecule biosynthetic process	2005	1.093E-03	4.553E-02	1.093E-03	4.553E-02	8	NF-1, NF1A, 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-1, NF1A, 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, K9.3	
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-1, NF1A, 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-1, TYSY, SRF	
50	Monocarboxylic acid transport	214	1.676E-03	4.686E-02	1.676E-03	4.686E-02	3	SLC16A13, RXRA, RXR	

Table V.

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

ID	Gene symbol	Methylation <i>P</i> value	Trauma <i>P</i> value	Interaction <i>P</i> value
cg10264529	<i>PCK2</i>	.003	.02	ns
cg16110788		.03	.03	ns
cg26103104		.02	.03	ns
cg22806444	<i>HID1</i>	.02	.04	ns
cg26737766	<i>GALE</i>	.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.

Table VI.

Additional methylation sites in replicated genes that predicted BMI

Ilmn ID	UCSC_Ref	Regulatory_Feature	beta	Rintrafa	betaRintrafa
cg10264529	PKC2		7.53E-09	0.224387393	0.010497497
cg09378756	PKC2		0.27985477	0.5733348026	0.028519211
cg14089728	PKC2	Promoter_Associated	0.069079566	0.51270066	0.044400055
cg15412728	PKC2	Promoter_Associated	0.235420241	0.786173809	0.08317027
cg15467148	PKC2	Promoter_Associated	0.130683774	0.626457756	0.303843673
cg18599124	PKC2	Promoter_Associated	0.253946953	0.763249148	0.025101018
cg20017797	PKC2	Promoter_Associated	0.130769156	0.784854292	0.893846413
cg22454119	PKC2	Promoter_Associated	0.114150643	0.747082866	0.005328352
cg23112188	PKC2	Promoter_Associated	0.879932822	0.620458559	0.024862955
cg23204518	PKC2	Promoter_Associated	0.91842871	0.710926936	0.120762948
cg26402828	PKC2	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
cg034443162	C17orf28		0.619154034	0.708902821	0.57843624
cg035533897	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

Ilmn ID	UCSC_Ref	Regulatory_Feature	beta	Rintrafa	betaRintrafa
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
cg034441514	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	0.1622226526
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

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

UCSC, University of California, Santa Cruz.

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.