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Life course socioeconomic status and DNA methylation in genes related to stress reactivity and inflammation: The multi-ethnic study of atherosclerosis

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Epigenetic changes, such as DNA methylation, have been hypothesized to provide a link between the social environment and disease development. The purpose of this study was to examine associations between life course measures of socioeconomic status (SES) and DNA methylation (DNAm) in 18 genes related to stress reactivity and inflammation using a multi-level modeling approach that treats DNAm measurements as repeat measures within an individual. DNAm and gene expression were assessed in purified monocytes for a random subsample of 1,264 non-Hispanic white, African-American, and Hispanic participants aged 55–94 from the Multi-Ethnic Study of Atherosclerosis (MESA). After correction for multiple testing, we found that low childhood SES was associated with DNAm in 3 stress-related genes (*AVP, FKBP5, OXTR*) and 2 inflammation-related genes (*CCL1, CD1D*), low adult SES was associated with DNAm in one stress-related gene (*AVP*) and 5 inflammation-related genes (*CD1D, F8, KLRG1, NLRP12, TLR3*), and social mobility was associated with DNAm in 3 stress-related genes (*AVP, FKBP5, OXTR*) and 7 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, NLRP12, PYDC1, TLR3*). In general, low SES was associated with increased DNAm. Expression data was available for 7 genes that showed a significant relationship between SES and DNAm. In 5 of these 7 genes (*CD1D, F8, FKBP5, KLRG1, NLRP12*), DNAm was associated with gene expression for at least one transcript, providing evidence of the potential functional consequences of alterations in DNAm related to SES. The results of this study reflect the biological complexity of epigenetic data and underscore the need for multi-disciplinary approaches to study how DNAm may contribute to the social patterning of disease.

Introduction

Numerous studies indicate that low socioeconomic status (SES) is associated with an increased risk of disease and death.[1] Alterations in stress response systems, including the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis, and inflammation have been hypothesized to be among the mechanisms contributing to socioeconomic disparities in health.[2] People with low SES have been shown to have alterations of stress response systems, as indicated by higher levels or altered diurnal patterns of stress hormones, including cortisol,[3] and catecholamines.[4] In addition, people with low SES tend to have higher levels of chronic inflammation.[5] However, the specific biologic mechanisms through which SES affects stress biology and inflammation are not well understood. Epigenetic changes in DNA methylation (DNAm), which give rise to mitotically heritable alterations in gene expression, have recently received attention as one of the biological pathways through which a range of environmental exposures could have sustained effects on many different health outcomes.[6-9] Because DNAm appears to be dynamic throughout the life course,[7,10] studies examining the epigenetic consequences of low SES have the potential to elucidate a modifiable molecular mechanism by which social factors become physically embodied.

Although a growing number of studies have begun to examine associations of SES over the life course with DNAm, findings have been inconsistent and limited by small sample sizes or restricted markers of DNAm. In a recent study

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examining a subset of 40 men from the 1958 British Birth Cohort Study, Borghol et al.[11] found that childhood SES showed stronger associations with DNAm profiles at age 45 than adult SES. Similarly, Lam et al.[12] found that early life SES, but not current SES, was associated with variable DNAm in a community cohort of 92 Canadian women and men aged 24–45. In a subset of 239 female and male participants aged 35–64 from the pSoBid cohort, McGuinness et al.[13] found that area-level socioeconomic deprivation during adulthood was associated with global hypomethylation. Tehranifar et al.[14] found that childhood family income was associated with global hypermethylation, whereas adult educational attainment was associated with global hypomethylation in a subset of 90 participants aged 38–46 from the New York Women's Birth Cohort. In contrast to these results, Subramanyam et al.[15] found no association between childhood SES or adult SES and global DNAm in 988 participants aged 45–84 from the Multi-Ethnic Study of Atherosclerosis. A handful of studies have investigated associations between SES and DNAm in infants and children. Perng et al.[16] found that maternal education was positively associated with global DNAm in boys (but not girls) in a subset of 568 children from the Bogota School Children Cohort. Obermann-Borst et al.[17] found that maternal education was associated with increased DNAm in a gene related to the insulin pathway in a sample of 120 children at 17 months of age; and Appleton et al.[18] found that maternal education was associated with decreased placental DNAm in a gene related to the inactivation of maternal cortisol in a study of 444 healthy newborns.

Variations in these findings in terms of what SES indicators were predictive of DNAm and in terms of the directionality of the associations may be due to differences in the definition of SES, differences in the populations studied, small sample sizes resulting in random variations, and differences in the DNAm measures used. Gene-specific DNAm studies may be more informative than studies of global DNAm, since gene-specific studies have the potential to identify specific biological pathways influenced by life course SES.

The purpose of this study was to examine associations between SES and gene-specific DNAm in a large, population-based sample of US adults with information on life course SES and state-of-the-art assessments of DNAm. We selected candidate genes based on the results of prior work in rodents, primates, and humans, which found that exposure to various psychosocial stressors was associated with DNAm in genes related to stress reactivity, including *AVP*,[19] *BDNF*,[20,21] *CRF*,[22] *FKBP5*,[23] *GR*,[24-29] *OXTR*,[30] and *SLC6A4*,[31-34] and inflammation, including *CD1D, CCL1, F8, IL8, KLRG1, LTA4H, NLRP12, PYDC1, SLAMF7, TLR1*, and *TLR3*.[35] Stress reactivity and inflammation have been hypothesized to mediate the impact of social circumstances on health and are therefore reasonable candidates for investigation of SES effects on DNAm processes. As shown in **Table 1**, we had data for 283 DNAm sites in the 18 genes selected for this study.

We used data on DNAm in purified monocytes from 1,264 community-dwelling women and men aged 55–94 from the Multi-Ethnic Study of Atherosclerosis (MESA) to examine the main study hypothesis that childhood SES, adult SES, and trajectories of SES from childhood to adulthood are associated with variation in DNAm in candidate genes related to stress reactivity and inflammation. From a life course perspective, exposure to low SES during a sensitive period, such as childhood, may have larger effects on health than exposure during a less sensitive developmental stage, such as midlife, while cumulative exposure to low SES may be more detrimental to health than exposure to low SES at one point in time.[36] Thus, we further hypothesized that childhood SES is more strongly associated with DNAm than

Table 1. Features of DNA Methylation Sites Examined

Gene	Pathway	# of Sites	Promoter Sites	Shore/Shelf Sites	Expression Data
AVP	Stress	12	Yes	Yes	1 transcript
BDNF	Stress	74	Yes	Yes	Not available
CRF	Stress	14	Yes	Yes	Not available
FKBP5	Stress	32	Yes	Yes	1 transcript
GR	Stress	34	Yes	Yes	3 transcripts
OXTR*	Stress	15	Yes	Yes	1 transcript
SLC6A4	Stress	13	Yes	Yes	Not available
CCL1	Inflammation	6	Yes	No	Not available
CD1D	Inflammation	15	Yes	Yes	1 transcript
F8	Inflammation	12	Yes	Yes	1 transcript
IL8	Inflammation	2	No	No	2 transcripts
KLRG1	Inflammation	4	Yes	No	1 transcript
LTA4H	Inflammation	8	Yes	Yes	1 transcript
NLRP12	Inflammation	11	Yes	No	3 transcripts
PYDC1	Inflammation	14	Yes	Yes	Not available
SLAMF7	Inflammation	4	Yes	No	1 transcript
TLR1	Inflammation	5	Yes	No	1 transcript
TLR3	Inflammation	8	Yes	No	Not available

*For *OXTR*, all non-shore/shelf sites are also non-promoter sites, and all shore/shelf sites are also promoter sites.

adult SES and that persistent low SES is more strongly associated with DNAm than downward social mobility, upward social mobility, or persistent high SES. In order to demonstrate the functional relevance of study results, we used data on gene expression to examine the secondary study hypothesis that DNAm in candidate genes found to be related to SES is associated with changes in gene expression levels.

Data and Methods

Data

MESA is a population-based longitudinal study designed to identify risk factors for the progression of subclinical cardiovascular disease (CVD).[37] Between July 2000 and August 2002, 6,814 non-Hispanic white, African-American, Hispanic, and Chinese-American women and men aged 45–84 without clinically apparent CVD were recruited from 6 regions in the US, including Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; St. Paul, MN; Chicago, IL; and Los Angeles County, CA. Each field center recruited from locally available sources, which included lists of residents, lists of dwellings, and telephone exchanges. Between April 2010 and February 2012 (corresponding to MESA Exam 5), DNAm and gene expression were assessed on a random subsample of 1,264 non-Hispanic white, African-American, and Hispanic MESA participants aged 55–94 y from the Baltimore, Forsyth County, New York, and St. Paul field centers who agreed to participate in an ancillary study examining the effects of DNAm on CVD. We excluded 33 respondents with missing data on one or more variables included in the final models (final n = 1,231). This study was approved by the Institutional Review Boards of all MESA field centers and the MESA Coordinating Center.

Measures

DNAm

Centralized training of technicians, standardized protocols, and extensive QC measures were implemented for collection, on-site processing, and shipment of MESA specimens, and routine calibration of equipment. The blood draw took place in the morning after a 12 h fast. Blood was collected in sodium heparin-containing Vacutainer CPT™ tubes (Becton Dickinson, Rutherford, NJ, USA), and monocytes were isolated using AutoMACs automated magnetic separation units (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocyte samples were consistently >90 % pure, based on flow cytometry analysis of 18 specimens. To avoid biases due to batch, chip, and position effects, a stratified random sampling technique was used to assign samples to chips and positions. The Illumina HumanMethylation450 BeadChip and HiScan reader were used to measure DNAm, and bead-level data were summarized in GenomeStudio. Quantile normalization was performed using the *lumi* package with default settings.[38] Quality control (QC) measures included checks for sex and race/ethnicity mismatches and outlier identification by multidimensional scaling plots. Criteria for elimination included: 'detected' DNAm levels in <90 % of MESA samples (detection *P*-value cut-off = 0.05), existence of a SNP within 10 base pairs of the target CpG site, overlap with a non-unique region, and 65 probes that assay highly-polymorphic single nucleotide polymorphisms (SNPs) rather than DNAm.[39] The final DNAm value for each site was computed as the M-value, the log ratio of the methylated to the unmethylated intensity.[40] CpG sites were assigned to genes according to Illumina annotation, which included sites in the promoter region, 5′ untranslated region, gene body, and 3′ untranslated region. We used Illumina annotation to determine whether CpG sites were in promoter regions (located up to 1500 bp upstream of the transcription start site; hereafter referred to as "promoter" sites), or were in CpG island shores or shelves (located up to 4,000 bp away from CpG island boundaries; hereafter referred to as "shore/shelf" sites). Chip and position effects were adjusted prior to analysis.

Gene expression

The Illumina HumanHT-12 v4 Expression BeadChip was used to measure gene expression, and initial background correction was conducted in GenomeStudio. QC analyses and bead type summarization were performed using the *beadarray* package.[41] The *limma* package was further used to estimate non-negative signal, perform quantile normalization and log transformation, eliminate control probes, and detect outliers. Criteria for elimination included: 'detected' expression levels in <10 % of MESA samples (detection *P*-value cut-off = 0.01), probes that contain a SNP, probes with low variance across samples (<10th percentile), or overlap with a non-unique region. A detailed description of the quantitation and data processing procedures used for DNAm and gene expression can be found in Liu et al.[42] Chip effects were adjusted prior to analysis.

Low Childhood SES

We used maternal education as an indicator of childhood SES.[43-45] At Exam 2, respondents reported the highest level of education completed by their mother. Response options were no schooling; some schooling but did not complete high school; high school degree; some college, but no college degree; college degree; and graduate or professional school. We created a dichotomous measure of maternal education (less than high school = 1; high school degree or more = 0).

Low Adult SES

At Exam 1, respondents reported the highest level of education they completed. Response options were no schooling; grades 1–8; grades 9–11; completed high school or GED; some college but no degree; technical school certificate; associate degree; bachelor's degree; and graduate or professional school. We created a dichotomous measure of respondent educational attainment (less than college = 1; college degree or more = 0).

SES Trajectories

We combined information on childhood and adult SES to create dummy variables for persistent low SES (low childhood SES = 1 and low adult SES = 1), upward social mobility (low

childhood SES = 1 and low adult SES = 0), and downward social mobility (low childhood SES = 0 and low adult SES = 1), with persistent high SES (low childhood SES = 0 and low adult SES = 0) as the reference category.

Analysis

The data structure consisted of multiple DNAm sites measured within each gene for each individual. In order to allow associations of SES with DNAm to vary across the different genes, we conducted analyses separately for each of the 18 genes examined. DNAm measures can be conceptualized as repeat measures within an individual. For this reason, we used a 2-level multilevel model,[46] to examine associations of individual-level SES with multiple measures of DNAm within each gene. This approach allowed us to account for correlations between DNAm levels within an individual by including a random intercept for each person. It also allowed inclusion of variables specific to each DNAm site. Given prior evidence that DNAm sites with particular characteristics (such as sites in the promoter region or in shore/shelves of CpG islands) may show different DNAm patterns in response to external influences than sites in other regions, we added indicator variables to specify the type of DNAm site, as well as SES-by-indicator interaction terms to allow the DNAm patterns to differ across types of DNAm sites. **Table S6** lists all DNAm probes examined, as well as information on the site type and associated gene. Models controlled for sex (female = 1), race/ethnicity (dummy variables for African-American and Hispanic, with non-Hispanic white as the reference category), age (in years), and residual sample contamination with non-monocytes (enrichment scores for neutrophils, B cells, T cells, and natural killer cells). See below for the model specification for any given gene:

$$Y_{ij} = \beta_0 + \eta_i + \beta_1 \cdot SES_i + \beta_2 \cdot Shore/Shelf_j + \beta_3 \cdot Promoter_j + \beta'_4 \cdot Covariates_i + \beta_5 \cdot SES_i \cdot Shore/Shelf_j + \beta_6 \cdot SES_i \cdot Promoter_j + \varepsilon_{ij}$$

Y_{ij}: The M-value for DNAm site j for individual i for the given gene.

SES_i: SES measure for individual i.

$ShoreShelf_j$: 'Shore/shelf' indicator for DNAm site j.

$Promoter_j$: 'Promoter' indicator for DNAm site j.

$Covariates_i$: Covariate measures for individual i.

η_i : Individual level random effect for individual i, $\eta_i \sim N\left(0,\ \sigma^2_{\text{individual}}\right)$.

ε_{ij}: Site-specific residual error term, $\varepsilon_{ij} \sim N\left(0,\ \sigma^2_{error}\right)$.

β_0: Intercept of the model.

β_1: Difference in the M-value by SES category.

β_2: Difference in the M-value between shore/shelf and non-shore/shelf DNAm sites.

β_3: Difference in the M-value between promoter and non-promoter sites.

β'_4: Vector of parameter estimates for covariates.

β_5: Difference in the SES effect on the M-value between shore/shelf and non-shore/shelf DNAm sites.

β_6: Difference in the SES effect on the M-value between promoter and non-promoter sites.

The multi-level modeling approach, which simultaneously examines associations of SES with all DNAm sites in a gene, is more parsimonious and makes more efficient use of the data than separate models for each site within a gene. It also allows statistical testing of whether associations differ systematically by characteristics of the site (e.g., promoter/non-promoter). In order to facilitate interpretation of results, for any multilevel model for a given gene that had a statistically significant ($P < 0.05$) main effect of SES or a statistically significant interaction between SES and site type, we used ESTIMATE statements in SAS to obtain mean M-values adjusted to the mean levels of all covariates in the model according to different categories of the SES variable (e.g., low childhood SES vs. high childhood SES) and site types (e.g., shore/shelf sites). The ESTIMATE statement was also used to determine the statistical significance of SES differences in mean M-values within promoter or shore/shelf categories. To adjust for multiple testing, we calculated the false discovery rate (FDR),[47] based on the *P*-value for the SES effect on DNAm within each site type. We applied a cutoff of $q < 0.2$ to indicate results that remained noteworthy after FDR correction.[48,49] A q-value of 0.2 indicates that an estimated 80% of significant findings are expected to be true positives.

For those genes that were found to have epigenetic variation associated with at least one measure of SES (FDR q-value < 0.2), we subsequently examined DNAm as a predictor of gene expression. Only 7 of the genes of interest had gene expression data available. Each of the 7 genes had only one transcript, except for *NLRP12*, which had 3 transcripts. Since the dependent variable was a single measure (transcript level) for 6 of the 7 genes, we used linear regression (rather than a multi-level model). We fit 2 models for each gene transcript. The reduced model included the core set of covariates (age, sex, race/ethnicity, and enrichment scores for neutrophils, B cells, T cells and natural killer cells) as the predictors. The full model added DNAm levels for all sites related to the target transcript as the predictors. A global likelihood ratio test was performed to compare the 2 models and test the hypothesis that at least one DNAm site was associated with gene expression level for the target transcript. For each gene, we also performed the global likelihood ratio test for each site type separately. See **Appendix S1** in the supplemental materials for a description of the workflow.

Results

Descriptive statistics are shown in **Table 2**. Just over half of the sample (53%) experienced low childhood SES, as defined by maternal education less than high school. Sixty-seven percent of respondents did not complete a college degree and were classified as having low adult SES. Regarding SES trajectories, 41% experienced persistent low SES, 12% experienced upward mobility, 25% experienced downward mobility, and 22% experienced persistent high SES. Approximately half of the sample (51%) was female. Forty-seven percent of respondents were non-Hispanic

Table 2. Descriptive Statistics (n = 1,231)

		Childhood SES		Adult SES		SES Trajectories			
	Full Sample	Low	High	Low	High	Persistent Low	Upward Mobility	Downward Mobility	Persistent High
Socioeconomic Status (SES)									
Low Childhood SES (1 = mom <high school)	0.53	1.00	0.00	0.62	0.36	1.00	1.00	0.00	0.00
Low Adult SES (1 ≤ college)	0.67	0.78	0.54	1.00	0.00	1.00	0.00	1.00	0.00
Persistent Low SES	0.41	0.78	0.00	0.62	0.00	1.00	0.00	0.00	0.00
Upward Mobility	0.12	0.22	0.00	0.00	0.36	0.00	1.00	0.00	0.00
Downward Mobility	0.25	0.00	0.54	0.38	0.00	0.00	0.00	1.00	0.00
Persistent High SES	0.22	0.00	0.46	0.00	0.64	0.00	0.00	0.00	1.00
Female	0.51	0.55	0.48	0.56	0.42	0.59	0.38	0.51	0.44
Non-Hispanic white	0.47	0.32	0.65	0.36	0.70	0.26	0.53	0.53	0.78
African-American	0.21	0.22	0.21	0.23	0.18	0.22	0.21	0.25	0.15
Hispanic	0.31	0.46	0.15	0.41	0.13	0.52	0.25	0.22	0.06
Age	69.55 (9.35)	70.64 (9.37)	68.31(9.18)	69.78(9.33)	69.09(9.40)	70.50(9.29)	71.12(9.68)	66.61(9.28)	67.97(9.07)

Note: Means with standard deviations in parentheses are shown for continuous variables, and proportions are shown for categorical variables.

white, 21% were African-American, and 32% were Hispanic. Mean age was 69.55 y.

Dna methylation

As shown in **Table 3**, there was a statistically significant ($P<0.05$) main effect of low childhood SES and/or a statistically significant interaction between childhood SES and site type on DNAm in 4 out of 7 stress-related genes (*AVP, BDNF, FKBP5*, and *OXTR*) and 3 out of 11 inflammation-related genes (*CCL1, CD1D*, and *F8*). As shown by the statistically significant interactions between childhood SES and site type (promoter or shore/shelf), associations varied across site types. To facilitate the interpretation of results, we used model coefficients to estimate (1) the mean M-value in each site type (shore/shelf, non-shore/shelf, promoter, and non-promoter) by childhood SES and (2) whether the difference in DNAm by childhood SES within each site type was statistically significant (see **Table S1** for estimated M-values by childhood SES and site type). In *AVP*, low childhood SES was associated with increased DNAm in shore/shelf sites ($P = 0.04$, q = 0.17) and non-promoter sites ($P = 0.05$, q = 0.19) and decreased DNAm in non-shore/shelf sites ($P = 0.0001$, q = 0.004) but was not associated in promoter sites. In *FKBP5*, low childhood SES was associated with increased DNAm in shore/shelf sites ($P = 0.03$, q = 0.13) but was not associated in other site types. In *OXTR*, low childhood SES was associated with increased DNAm in non-shore/shelf, non-promoter sites ($P = 0.02$, q = 0.10) but not in shore/shelf, promoter sites ($P = 0.30$; q = 0.39) [*Note that for OXTR, all non-shore/shelf sites are also non-promoter sites, and all shore/shelf sites are also promoter sites*]. In *CCL1*, low childhood SES was associated with increased DNAm in promoter sites ($P = 0.009$, q = 0.07) and decreased DNAm in non-promoter sites ($P = 0.04$, q = 0.16). In *CD1D*, low childhood SES was associated with increased DNAm in promoter sites ($P = 0.002$, q = 0.02) but not in other site types. The results for *BDNF* and *F8* were no longer noteworthy after FDR correction.

Table 4 shows the results for adult SES. There was a statistically significant ($P<0.05$) main effect of low adult SES and/or a statistically significant interaction between adult SES and site type on DNAm in 2 out of 7 stress-related genes (*AVP* and *SLC6A4*) and 5 out of 11 inflammation-related genes (*CD1D, F8, KLRG1, NLRP12*, and *TLR3*). **Table S2** presents the estimated M-value in each site type (shore/shelf, non-shore/shelf, promoter, and non-promoter) by adult SES. In *AVP*, low adult

Table 3a. Regression of M-Value on Childhood SES in Stress Pathway Genes (n = 1,231)

	Stress Pathway											
	AVP			*BDNF*			*FKBP5*			*OXTR**		
	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value
Low Childhood SES	−0.017	0.007	0.020	0.006	0.004	0.148	−0.001	0.004	0.877	0.025	0.010	0.015
Promoter	1.263	0.004	<0.0001	−2.602	0.003	<0.0001	−0.211	0.004	<0.0001	—	—	—
Shore/Shelf	3.040	0.010	<0.0001	−0.926	0.003	<0.0001	0.230	0.004	<0.0001	3.315	0.016	<0.0001
SES*Promoter	−0.022	0.006	0.0002	−0.009	0.004	0.020	−0.014	0.006	0.020	—	—	—
SES*Shore/Shelf	0.052	0.014	0.0002	−0.003	0.004	0.419	0.017	0.006	0.007	−0.047	0.022	0.033

*For OXTR, all non-shore/shelf sites are also non-promoter sites, and all shore/shelf sites are also promoter sites.
Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Low childhood SES is a dichotomous variable, where 1 = mom < high school.

Table 3b. Regression of M-Value on Childhood SES in Inflammation Pathway Genes (n = 1,231)

	Inflammation Pathway								
	CCL1			*CD1D*			*F8*		
	Beta	**SE**	***P*-value**	**Beta**	**SE**	***P*-value**	**Beta**	**SE**	***P*-value**
Low Childhood SES	−0.028	0.013	0.037	0.0005	0.005	0.924	0.048	0.031	0.122
Promoter	−1.710	0.011	<0.0001	−2.678	0.010	<0.0001	−1.704	0.011	<0.0001
Shore/Shelf	—	—	—	4.653	0.006	<0.0001	1.401	0.035	<0.0001
SES*Promoter	0.052	0.016	0.0009	0.048	0.015	0.0017	0.033	0.015	0.027
SES*Shore/Shelf	—	—	—	−0.006	0.008	0.463	−0.087	0.048	0.069

Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Low childhood SES is a dichotomous variable, where 1 = mom < high school.

SES was associated with increased DNAm in shore/shelf sites ($P = 0.0003$, q = 0.007) and non-promoter sites ($P = 0.002$, q = 0.03) and decreased DNAm in non-shore/shelf sites ($P < 0.0001$, q = 0.003) but was not associated in promoter sites. Results for *SLC6A4* were no longer noteworthy after FDR correction. In *CD1D*, low adult SES was associated with increased DNAm in promoter sites ($P = 0.002$, q = 0.03) and decreased DNAm in non-promoter sites ($P = 0.04$, q = 0.17), but not in other site types. In *F8*, low adult SES was associated with decreased DNAm in shore/shelf sites ($P < 0.0001$, q = 0.0009) and non-promoter sites ($P = 0.01$, q = 0.07) and increased DNAm in non-shore/shelf sites ($P < 0.0001$, q = 0.003) and promoter sites ($P = 0.02$, q = 0.13). Low adult SES was associated with increased DNAm in the non-promoter sites of *KLRG1* ($P = 0.04$, q = 0.17), *NLRP12* ($P = 0.002$, q = 0.02), and *TLR3* ($P = 0.02$, q = 0.11) but not in promoter sites. These 3 genes had no shore/shelf sites.

Finally, there was a statistically significant ($P < 0.05$) main effect of SES trajectories and/or a statistically significant interaction between SES trajectories and site type on DNAm in 4 out of 7 stress-related genes (*AVP, BDNF, FKBP5*, and *OXTR*) and 7 out of 11 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, NLRP12, PYDC1*, and *TLR3*) (see **Table 5**). **Table S3** presents the estimated M-value in each site type (shore/shelf, non-shore/shelf, promoter, and non-promoter) by SES trajectory. In *AVP* persistent low SES and downward social mobility were associated with increased DNAm in shore/shelf sites ($P = 0.0001$, q = 0.003 and $P = 0.0007$, q = 0.01, respectively); persistent low SES, upward social mobility, and downward social mobility were associated with increased DNAm in non-promoter sites ($P = 0.0003$, q = 0.007; $P = 0.03$, q = 0.14; $P = 0.0008$, q = 0.01, respectively); and persistent low SES was associated with decreased DNAm in non-shore/shelf sites ($P < 0.0001$, q = 0.0009). Results for *BDNF* were no longer noteworthy after FDR correction. In *FKBP5*, persistent low SES was associated with increased DNAm in shore/shelf sites ($P = 0.03$, q = 0.15) but not in other site types. In *OXTR*, persistent low SES ($P = 0.02$, q = 0.12) and upward mobility ($P = 0.004$, q = 0.04) were associated with increased DNAm in non-shore/shelf, non-promoter sites but not in shore/shelf, promoter sites. In *CCL1*, persistent low SES was associated with increased DNAm in promoter sites ($P = 0.003$, q = 0.03), but not in other site types. In *CD1D*, persistent low SES ($P < 0.0001$, q = 0.003), upward social mobility ($P = 0.04$, q = 0.13), and downward social mobility ($P = 0.04$; q = 0.16) were associated with increased DNAm in promoter sites, and persistent low SES was also associated with increased DNAm in non-shore/shelf sites ($P = 0.01$, q = 0.09). In *F8*, persistent low SES was associated with decreased DNAm in shore/shelf sites ($P = 0.001$, q = 0.02) and increased DNAm in non-shore/shelf sites ($P = 0.0009$, q = 0.01) and promoter sites ($P = 0.02$, q = 0.11), while upward social mobility was associated with increased DNAm in shore/shelf sites ($P = 0.02$, q = 0.11) and non-promoter sites ($P = 0.02$, q = 0.11). In *KLRG1*, persistent low SES was associated with increased DNAm in non-promoter sites ($P = 0.05$, q = 0.19), while downward social mobility was associated with increased DNAm in promoter sites ($P = 0.04$, q = 0.16). For *NLRP12*, both persistent low SES and downward social mobility were associated with increased DNAm in non-promoter sites ($P = 0.002$, q = 0.03 and $P = 0.02$, q = 0.11, respectively). In *PYDC1*, upward social mobility was associated with increased DNAm in shore/shelf sites ($P = 0.05$, q = 0.20), but not in other site types. In *TLR3*, downward social mobility was associated with increased DNAm in non-promoter sites ($P = 0.004$, q = 0.04), but not in promoter sites.

Table 4a. Regression of M-Value on Adult SES in Stress Pathway Genes (n=1,231)

	Stress Pathway					
	AVP			*SLC6A4*		
	Beta	**SE**	***P*-value**	**Beta**	**SE**	***P*-value**
Low Adult SES	−0.020	0.008	0.008	0.0003	0.007	0.965
Promoter	1.265	0.005	<0.0001	−3.247	0.007	<0.0001
Shore/Shelf	3.018	0.011	<0.0001	1.279	0.007	<0.0001
SES*Promoter	−0.022	0.006	0.0005	−0.012	0.008	0.162
SES*Shore/Shelf	0.074	0.014	<0.0001	0.020	0.009	0.026

Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Low adult SES is a dichotomous variable, where 1 ≤ college.

Table 4b. Regression of M-Value on Adult SES in Inflammation Pathway Genes (n = 1,231)

	Inflammation Pathway														
	CD1D			*F8*			*KLRG1*			*NLRP12*			*TLR3*		
	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value	Beta	SE	*P*-value
Low Adult SES	−0.002	0.005	0.759	0.123	0.033	0.0002	0.029	0.015	0.043	0.036	0.011	0.0017	0.016	0.007	0.019
Promoter	−2.688	0.011	<0.0001	−1.721	0.012	<0.0001	−0.496	0.015	<0.0001	0.193	0.013	<0.0001	−2.593	0.008	<0.0001
Shore/Shelf	4.660	0.007	<0.0001	1.500	0.040	<0.0001	—	—	—	—	—	—	—	—	—
SES*Promoter	0.053	0.015	0.0005	0.053	0.015	0.0006	−0.005	0.019	0.773	−0.045	0.016	0.006	0.003	0.010	0.762
SES*Shore/Shelf	−0.016	0.009	0.074	−0.218	0.050	<0.0001	—	—	—	—	—	—	—	—	—

Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Low adult SES is a dichotomous variable, where 1 ≤ college.

Gene expression

In order to demonstrate the potential functional relevance of the results described above, we examined associations between DNAm and gene expression in genes where (1) at least one measure of SES was associated with DNAm (FDR q-value < 0.2) and (2) gene expression data was available, including 3 stress-related genes (*AVP, FKBP5*, and *OXTR*) and 4 inflammation-related genes (*CD1D, F8, KLRG1*, and *NLRP12*). As shown in **Table S4**, DNAm was associated with gene expression in *FKBP5* (ILMN_1778444, $P = 0.0002$), *CD1D* (ILMN_1719433, $P<0.0001$), *F8* (ILMN_1675083, $P < 0.0001$), *KLRG1* (ILMN_1658399, $P < 0.0001$), and 2 transcripts from *NLRP12* (ILMN_1716105, $P < 0.0001$; ILMN_1758735, $P = 0.001$), even after applying a Bonferroni-corrected *P*-value of 0.006 (0.05/9 tests). DNAm was not associated with gene expression in *AVP* (ILMN_1811443, $P = 0.227$), *OXTR* (ILMN_1804929, $P = 0.641$), or a third transcript of *NLRP12* (ILMN_1739815, $P = 0.309$).

For each gene that showed a significant relationship between DNAm and gene expression, we evaluated whether the relationship was driven by a particular type of site and determined the direction of effect of DNAm on gene expression by site type. **Table S5** shows that DNAm in shore/shelf sites was significantly associated with gene expression for all genes that had DNAm measured in shore/shelf sites (*FKBP5, CD1D*, and *F8*). For the majority of significant sites, as DNAm increased, gene expression decreased (i.e., there was a negative direction of effect). For genes that showed a significant relationship between DNAm in promoter regions and gene expression (*FKBP5* and *CD1D*), there was again a negative direction of effect for the majority of significant sites. Directions of effect for significant sites were less consistent in non-shore/shelf and non-promoter sites.

Discussion

Epigenetic studies have the potential to elucidate biological mechanisms by which social conditions are physically embodied. Building on the results of recent animal and human studies, we used data from the population-based Multi-Ethnic Study of Atherosclerosis to examine associations between life course SES and DNAm in 18 genes related to stress reactivity and inflammation.

Table 5a. Regression of M-value on SES Trajectories for Stress Pathway Genes (n = 1,231)

	Stress Pathway							
	AVP		*BDNF*		*FKBP5*		*OXTR*†	
	Beta(SE)	*P*-value	Beta(SE)	*P*-value	Beta(SE)	*P*-value	Beta(SE)	*P*-value
Persistent Low SES*	−0.026(0.010)	0.010	0.008(0.006)	0.146	−0.004(0.005)	0.427	0.032(0.014)	0.020
Upward Mobility*	0.006(0.012)	0.626	0.008(0.007)	0.240	−0.0003(0.007)	0.996	0.047(0.016)	0.004
Downward Mobility*	−0.006(0.010)	0.568	0.004(0.005)	0.519	−0.005(0.005)	0.324	0.019(0.013)	0.155
Promoter	1.275 (0.006)	<0.0001	−2.601(0.004)	<0.0001	−0.217(0.007)	<0.0001	—	—
Shore/Shelf	3.003(0.013)	<0.0001	−0.925(0.004)	<0.0001	0.225(0.008)	<0.0001	3.337(0.023)	<0.0001
Persistent Low SES*Promoter	−0.036(0.008)	<0.0001	−0.011(0.005)	0.045	−0.008(0.008)	0.321	—	—
Upward Mobility*Promoter	−0.028(0.010)	0.0072	−0.009(0.007)	0.196	−0.004(0.012)	0.747	—	—
Downward Mobility*Promoter	−0.023(0.009)	0.0071	−0.002(0.006)	0.750	0.013(0.009)	0.135	—	—
Persistent Low SES*Shore/Shelf	0.102(0.018)	<0.0001	−0.002(0.005)	0.659	0.022(0.008)	0.007	−0.072(0.029)	0.012
Upward Mobility*Shore/Shelf	0.044(0.025)	0.081	−0.011(0.006)	0.066	0.017(0.012)	0.132	−0.055(0.040)	0.162
Downward Mobility*Shore/Shelf	0.068(0.019)	0.0003	−0.002(0.006)	0.688	0.008(0.009)	0.350	−0.040(0.031)	0.206

*The reference category is persistent high SES.

†For OXTR, all non-shore/shelf sites are also non-promoter sites, and all shore/shelf sites are also promoter sites.

Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells).

Table 5b. Regression of M-value on SES Trajectories for Inflammation Pathway Genes (n = 1,231)

	Inflammation Pathway													
	CCL1		*CD1D*		*F8*		*KLRG1*		*NLRP12*		*PYDC1*		*TLR3*	
	Beta (SE)	***P* -value**	**Beta (SE)**	***P* -value**	**Beta (SE)**	***P* -value**	**Beta (SE)**	***P* -value**	**Beta (SE)**	***P* -value**	**Beta (SE)**	***P* -value**	**Beta (SE)**	***P* -value**
Persistent Low SES*	−0.028(0.017)	0.101	0.0009(0.007)	0.901	1.127(0.042)	0.002	0.037(0.019)	0.049	0.043(0.014)	0.002	−0.002(0.010)	0.845	0.015(0.009)	0.102
Upward Mobility*	−0.028(0.024)	0.252	0.004(0.008)	0.608	−0.040(0.055)	0.467	0.003(0.023)	0.890	0.010(0.019)	0.596	−0.006(0.012)	0.632	0.012(0.011)	0.256
Downward Mobility*	−0.002(0.018)	0.907	0.002(0.007)	0817	0.078(0.046)	0.087	0.020(0.019)	0.298	0.035(0.015)	0.017	−0.005(0.009)	0.623	0.027(0.009)	0.004
Promoter	−1.718(0.015)	<0.0001	−2.704(0.014)	<0.0001	−1.717(0.016)	<0.0001	−0.497(0.019)	<0.0001	0.200(0.017)	<0.0001	1.094(0.011)	<0.0001	−2.585(0.010)	<0.0001
Shore/Shelf	—	—	4.662(0.009)	<0.0001	1.460(0.051)	<0.0001	—	—	—	—	2.476(0.013)	<0.0001	—	—
Persistent Low SES*Promoter	0.063(0.020)	0.002	0.082(0.020)	<0.0001	0.063(0.020)	0.002	−0.016(0.023)	0.484	−0.052(0.020)	0.011	−0.024(0.014)	0.083	−0.004(0.013)	0.732
Upward Mobility *Promoter	0.044(0.028)	0.123	0.046(0.024)	0.055	−0.012(0.024)	0.616	0.003(0.032)	0.917	−0.020(0.027)	0.457	−0.032(0.017)	0.059	−0.023(0.018)	0.205
Downward Mobility *Promoter	0.012(0.022)	0.577	0.048(0.020)	0.018	0.025(0.023)	0.259	0.016(0.026)	0.537	−0.051(0.023)	0.024	−0.012(0.015)	0.442	−0.006(0.014)	0.672
Persistent Low SES *Shore/Shelf	—	—	−0.018(0.011)	0.093	−0.221(0.063)	0.0005	—	—	—	—	0.027(0.016)	0.080	—	—
Upward Mobility *Shore/Shelf	—	—	−0.007(0.015)	0.627	0.110(0.082)	0.182	—	—	—	—	0.046(0.021)	0.029	—	—
Downward Mobility *Shore/Shelf	—	—	−0.018(0.012)	0.139	−0.111(0.070)	0.114	—	—	—	—	0.029(0.017)	0.085	—	—

*The reference category is persistent high SES.

Note: Models control for age, sex, race/ethnicity, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells).

After correction for multiple testing, we found that SES was associated with DNAm in 10 of these genes. Analysis of gene expression data provided evidence of the potential transcriptional consequences of DNAm in 5 out of 7 genes (*FKBP5, CD1D, F8, KLRG1*, and *NLRP12*), where one or more measures of SES were associated with DNAm and we had data on gene expression. In general, we found that low SES was associated with increased DNAm, but many of the observed associations varied according to site type. In several cases, we found *opposite* associations in promoter vs. non-promoter sites and shore/shelf vs. non-shore/shelf sites. Consistent with previous studies,[12,50,51] we found that DNAm in promoter sites was primarily inversely associated with gene expression. We also found this relationship in shore/shelf sites. However, we observed both positive and negative correlations between DNAm and gene expression in other site types, which is consistent with previous studies that have challenged the assumption that DNAm is inversely associated with gene expression.[42,52] The results of this study reflect the biological complexity of epigenetic data and underscore the need for multidisciplinary approaches to study how DNAm may contribute to the social patterning of disease.

We found that childhood SES was associated with DNAm in approximately the same number of stress- and inflammation-related genes (3 stress and 2 inflammation), whereas adult SES was primarily associated with DNAm in inflammation-related genes (5 inflammation-related genes vs. 1 stress-related gene). These results suggest that low SES during childhood and adulthood could potentially operate through different biological pathways. Overall, the strongest results were found for one stress-related gene (*AVP*) and one inflammation-related gene (*CD1D*), which were associated with all 3 measures of SES (childhood SES, adult SES, and SES trajectories from childhood to adulthood). Notably, while we observed numerous differences in DNAm patterns for respondents who experienced persistent low SES compared to those with persistent high SES, we observed fewer differences between those who experienced upward social mobility and those with high SES throughout the life course. Although this finding requires replication in other studies, it suggests that the negative consequences of low childhood SES for adult health and well-being could potentially be reduced by providing opportunities for upward social mobility through education.

There are a number of pathways through which SES could affect DNAm. One possible pathway involves exposure to psychosocial stressors. Low SES is associated with increased exposure to stressful life events and chronic strains, as well as decreased access to material and psychosocial resources that can buffer the negative impact of stress on health.[53] For example, children in low SES families are more likely than their higher SES counterparts to be exposed to chronic and acute stressors, such as harsh, inconsistent parenting, family conflict, housing instability, and neighborhood violence,[54] while low SES adults are more likely to be exposed to financial stress, relationship difficulties, divorce, and violent victimization.[55] Low socioeconomic status may also induce stress in children and adults via social subordination.[56] Rodent and primate studies examining various stress exposures, including restraint stress, social defeat, and maternal separation, have found associations of these exposures with DNAm in genes that encode proteins involved in functioning of the HPA axis, including *AVP*,[19] *BDNF*,[20,21] *CRF*,[22] *FKBP5*,[23] *GR*,[24-26] and *SLC6A4*.[31,34] Similarly, previous research in humans has found that a range of stress-related exposures, including childhood abuse, in-utero exposure to maternal depression, acute stress exposure (the Trier Social Stress Test), and history of major depressive disorder, are associated with alterations in DNAm in stress-related genes, such as *GR*,[27-29] *OXTR*,[30] and *SLC6A4*,[32,33] while exposure to post-traumatic stress disorder (PTSD) is associated with DNAm in a number of genes related to inflammation, including *CD1D, CCL1, F8, IL8, KLRG1, LTA4H, NLRP12, PYDC1, SLAMF7, TLR1*, and *TLR3*.[35] In addition to stress exposure, SES may be linked to DNAm through other pathways, such as negative affect, household or occupational exposure to carcinogens and pathogens, and diet and physical activity.[53] More work is needed to disentangle the social, environmental, psychological, and behavioral mechanisms underlying associations between SES and DNA methylation.

The results of this study show some similarities to previous research. For example, 7 out of 8 prior studies in this area found evidence of an association between at least one measure of life course SES and DNAm,[11-14,16-18] which is consistent with the results of this study. Similar to Borghol et al.,[11] who found little overlap in DNAm patterns associated with childhood and adult SES, we found that childhood SES was associated with DNAm in *AVP, FKBP5, OXTR, CCL1, CD1D*, and *F8*, while adult SES was associated with DNAm in a somewhat different set of genes, including *AVP, CD1D, F8, KLRG1, NLRP12*, and *TLR3*. In contrast to some prior studies, which concluded that early life SES was more consequential than adult SES for DNAm,[11,12] the results of this study suggest that low childhood SES and low adult SES are associated with DNAm in the same number of genes. Differences in study findings may reflect methodological differences, including the operationalization of life course SES and/or the methods used to analyze DNAm.

Strengths, Limitations, and Directions for Future Research

To our knowledge, the current study was the largest to ever examine associations between SES and DNAm. Most prior research in this area has been conducted in small, homogeneous samples, which limits power and generalizability. Other strengths include the use of purified monocytes rather than a mixture of peripheral blood cells, the analysis of gene-specific rather than global DNAm, the availability of data on childhood and adult SES, the use of multi-level models to examine DNAm at the gene level, and the use of a multi-ethnic sample. In addition, the availability of gene expression data allowed us to examine the potential functional consequences of alterations in DNAm related to SES.

Despite these strengths, this study had several limitations. First, we used a candidate-gene approach. Genes were selected based on the results of prior animal and human studies examining stress exposure and DNAm. The candidate genes encode

proteins that contribute to the regulation of the HPA axis and inflammation. Given that chronic stress exposure has been linked to dysregulation of the physiologic stress response,[3,4] as well as increased inflammation,[5] many studies have focused on genes involved in these 2 pathways. The candidate-gene approach limited the number of tests performed but may have underestimated the extent to which life course SES is associated with DNAm. Epigenome-wide studies in large, population-based studies like MESA are needed to determine the consequences of low SES for DNAm throughout the genome, but high levels of replication will be necessary. In the future, the availability of additional studies with data on DNAm and gene expression in monocytes will facilitate the discovery and replication of psychosocial predictors of genome-wide DNAm and expression. Promising strategies, such as bump hunting to identify differentially methylated regions,[57] have been proposed and should be examined in future work.

Next, the models presented here assume that the correlation structure for CpG sites is invariant across site types within genes. However, prior research has shown that the correlation structure for CpG sites differs according to site type.[58] Thus, in sensitivity analyses, we included random slopes for promoter and/or shore/shelf, depending on the site type(s) available within each gene (see **Table 1** for more information about the characteristics of CpG sites within each of the 18 candidate genes). For *AVP*, the main effects of childhood SES, adult SES, and persistent low SES from childhood to adulthood were no longer significant at the $P < 0.05$ level after adding random slopes for promoter and shore/shelf. Overall, however, we found that results for the remaining 17 genes were robust to model specification.

Another potential limitation of this study was the use of DNAm and gene expression data from peripheral blood cells. Associations between stressful life circumstances, such as low childhood or adult SES, and health are likely to be mediated by psychological processes. Although recent evidence suggests that peripheral tissues have utility in epidemiologic studies due to the high correlation ($r = 0.66$) between DNAm in blood and brain tissue in some sites (even in sites that have markedly different quantitative levels of DNAm between the 2 tissues), DNAm levels are tissue-specific.[59] Thus, it would be preferable, but not practical, to examine DNAm in brain tissue as well as blood cells.

Another limitation is that we dichotomized measures of childhood and adult SES, which may have resulted in a loss of information, and we only examined one component of socioeconomic status. In addition to education, income, occupation, and wealth are important markers of SES. While future studies should consider how multiple aspects of SES influence DNAm, some prior research suggests that education is more strongly associated with health outcomes than other indicators of SES.[60] This could be due to the fact that education is a foundational measure of SES (i.e., income, occupation, and wealth tend to follow from education) and/or the fact that education shapes non-material resources that promote health, such as the sense of personal control, and develops skills and abilities that can be used to solve a variety of problems, including problems related to health.[60]

Finally, although we observed a number of statistically significant differences in DNAm according to SES, these differences were small in magnitude. Changes in DNAm that were noteworthy after FDR correction (shown in **Tables S1–S3**) ranged from 0.1% to 2.6% (calculated by converting M-values to Beta-values according to the formula in Du et al.).[40] For example, in the *F8* gene, the difference between DNAm levels for low adult SES vs. high adult SES was 1.2% in shore/shelf sites and 2.4% in non-shore/shelf sites. It is not yet clear whether these differences have measureable effects on biological processes related to health. More work is needed to determine what constitutes a meaningful increase or decrease in DNAm. Furthermore, studies that examine associations among socioeconomic status, DNAm, gene expression, and health outcomes are needed to provide a full test of the hypothesis that changes in DNAm contribute to socioeconomic health disparities. As noted by Borghol et al.,[11] this will ultimately require a better understanding of genetic influences on complex diseases, such as cardiovascular disease and diabetes, that disproportionately affect individuals with low SES.

Conclusions

This study found that low SES was associated with DNAm in several genes related to stress reactivity and inflammation. To the extent that DNAm patterns influence gene expression, these findings could help explain why low SES is associated with excess morbidity and mortality. However, our study also illustrates the complexity of these associations, since the presence and directionality of associations were not always consistent across genes or types of DNAm sites. More work is needed to better synthesize this complexity and to understand whether and how these differences in DNAm affect health and contribute to profound differences in health by SES across multiple health outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

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