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Neighborhood characteristics influence DNA methylation of genes involved in stress response and inflammation: The Multi-Ethnic Study of Atherosclerosis

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ABSTRACT

Living in a disadvantaged neighborhood is associated with poor health outcomes even after accounting for individual-level socioeconomic factors. The chronic stress of unfavorable neighborhood conditions may lead to dysregulation of the stress reactivity and inflammatory pathways, potentially mediated through epigenetic mechanisms such as DNA methylation. We used multi-level models to examine the relationship between 2 neighborhood conditions and methylation levels of 18 genes related to stress reactivity and inflammation in purified monocytes from 1,226 participants of the Multi-Ethnic Study of Atherosclerosis (MESA), a population-based sample of US adults. Neighborhood socioeconomic disadvantage, a summary of 16 census-based metrics, was associated with DNA methylation [False discovery rate (FDR) q-value ≤ 0.1] in 2 out of 7 stress-related genes evaluated (*CRF, SLC6A4*) and 2 out of 11 inflammation-related genes (*F8, TLR1*). Neighborhood social environment, a summary measure of aesthetic quality, safety, and social cohesion, was associated with methylation in 4 of the 7 stress-related genes (*AVP, BDNF, FKBP5, SLC6A4*) and 7 of the 11 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, NLRP12, SLAMF7, TLR1*). High socioeconomic disadvantage and worse social environment were primarily associated with increased methylation. In 5 genes with significant associations between neighborhood and methylation (*FKBP5, CD1D, F8, KLRG1, NLRP12*), methylation was associated with gene expression of at least one transcript. These results demonstrate that multiple dimensions of neighborhood context may influence methylation levels and subsequent gene expression of stress- and inflammation-related genes, even after accounting for individual socioeconomic factors. Further elucidating the molecular mechanisms underlying these relationships will be important for understanding the etiology of health disparities.

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Introduction

A growing body of literature has demonstrated that individual-level socioeconomic disadvantage is associated with alterations in DNA methylation, an epigenetic mechanism that influences the expression of genes.[1-4] In light of the increasing recognition of the influence of socioeconomic disadvantage on methylation, it is now critical to begin studying the impact of contextual level measures of disadvantage on epigenetic mechanisms. Previous research has demonstrated that living in disadvantaged neighborhoods is associated with worse health outcomes, even after accounting for individual-level socioeconomic status (SES), and that these effects may contribute to disparities in health.[5] This study extends individual-level studies of socioeconomic disadvantage to the investigation of the relationship between neighborhood-level characteristics and DNA methylation, contributing to the growing body of literature in the emerging field of social epigenomics.

Neighborhoods represent complex environments with unique cultural, physical, and economic attributes that impact health and disease.[6] Living in a disadvantaged neighborhood has been previously linked to an increased risk of mortality,[7] mental illnesses such as depression,[5,8] and a wide range of chronic diseases including cardiovascular disease, diabetes, obesity, and hypertension.[5] Neighborhood socioeconomic disadvantage may adversely affect health through its correlation with poor neighborhood physical conditions (e.g., decreased food availability, exposure to environmental toxicants, and poor aesthetic quality) and adverse social environments (e.g., lack of safety and low social cohesion).[9] It has long been known that these unfavorable neighborhood conditions can lead to chronic psychological distress,[9] which may result in accumulating "wear and tear" on physiologic

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systems of the body, such as the endocrine and inflammatory response systems.[10] Unfavorable conditions can also lead to less favorable health behaviors (e.g., reduced physical activity, lower quality diet),[5] which may also disrupt the endocrine and inflammatory response systems.[11,12] Indeed, people living in disadvantaged neighborhoods have altered patterns of stress-related hormones,[13,14] and inflammatory biomarkers.[15] However, the specific molecular mechanisms that link neighborhood conditions to altered physiologic profiles are not fully elucidated.

Epigenetic mechanisms (including DNA methylation, histone modification, and noncoding RNAs) influence gene expression levels without altering the underlying DNA sequence, thereby affecting the type and amount of protein products that are manufactured by the cell.[16] DNA methylation is a well-studied epigenetic mechanism that influences gene expression by modulating gene transcription.[17] DNA methylation levels may be dynamic throughout the life course, and there is evidence that DNA methylation is potentially reversible at some sites.[18] Previous studies have demonstrated a link between global or site-specific DNA methylation patterns and socially patterned stressors including low childhood SES,[1-4] low adult SES,[3, 4] perceived stress,[2] and neighborhood crime.[19] Differential DNA methylation patterns have also been demonstrated for a wide variety of physical and mental health indicators (e.g., cardiovascular disease,[20] inflammation,[21] cortisol levels,[2] and depression.)[22] In light of these associations, DNA methylation has been proposed as a mediator of the associations between social exposures and health.

Given that stress reactivity and inflammation may mediate the impact of neighborhood conditions on health, we examined neighborhood-level socioeconomic disadvantage and neighborhood social environment as predictors of DNA methylation levels in 18 genes related to stress reactivity and inflammation. We evaluated these relationships using DNA methylation measured from purified monocytes in a large, multi-ethnic, population-based sample of US adults. To evaluate the potential functional impact of neighborhood-associated differences in methylation, we also followed up significant results by examining whether methylation differences were associated with gene expression levels, and/or whether they were in regions containing predicted functional elements (i.e., enhancers or promoters for noncoding RNA).

Results

Sample characteristics

Characteristics of the sample are provided in Table 1. The mean age of the sample was 69.6 years, and approximately half of the respondents were female. Forty-seven percent were non-Hispanic white, 22% were African-American, and 31% were Hispanic. Just over half of the respondents (53%) experienced low childhood SES (mother did not complete high school), and 66% percent had low adult SES (did not complete a college degree). Respondents living in a neighborhood with greater socioeconomic disadvantage or a worse social environment were younger, were more likely to be female, Hispanic or African American, and were more likely to have low SES (both adult and childhood). Respondents who were in the oldest age category (86–95 years) were also more likely to have a worse neighborhood social environment, but not higher socioeconomic disadvantage. Mean neighborhood socioeconomic disadvantage was −0.32 [standard deviation (SD) = 1.11], with a range from −4.53 to 1.87. Mean neighborhood social environment was 0.48 (SD = 2.70), with a range from −6.32 to 7.94.

Table 1. Characteristics of the MESA sample (n = 1,226).

	Full Sample (n, %)	Neighborhood Socioeconomic Disadvantage[a] Mean (SD)	Neighborhood Social Environment[a] Mean (SD)
Full sample	1226 (100)	−0.32 (1.11)	0.48 (2.70)
Age			
55–65 years	463 (38)	−0.18 (0.96)	0.80 (2.61)
66–75 years	397 (33)	−0.30 (1.16)	0.42 (2.71)
76–85 years	300 (24)	−0.47 (1.15)	0.05 (2.85)
86–95 years	66 (5)	−0.67 (1.46)	0.59 (2.16)
P-value		*0.0002*	*0.0022*
Gender			
Female	663 (52)	−0.24 (1.09)	0.78 (2.73)
Male	593 (48)	−0.40 (1.12)	0.16 (2.63)
P-value		*0.0142*	*0.000062*
Ethnicity			
Non-Hispanic white	581 (47)	−0.56 (1.18)	−0.72 (2.17)
African-American	263 (22)	−0.16 (0.98)	1.48 (2.61)
Hispanic	382 (31)	−0.05 (1.00)	1.61 (2.72)
P-value		*1.1E-12*	*1.1E-52*
Childhood Socioeconomic Status			
Low childhood SES (mother < high school)	653 (53)	−0.19 (1.05)	0.96 (2.76)
High childhood SES	573 (47)	−0.46 (1.16)	−0.069 (2.52)
P-value		*0.00002*	*1.6E-11*
Adult Socioeconomic Status			
Low adult SES (respondent < college)	815 (66)	−0.11 (0.93)	0.83 (2.73)
High adult SES	411 (34)	−0.74 (1.30)	−0.22 (2.49)
P-value		*1.5E-17*	*9.6E-11*

P-values were calculated using t-tests or one-way ANOVA.
[a]The mean and standard deviation of the neighborhood characteristic are provided for each demographic category. Higher (or less negative) values of neighborhood measures indicate more socioeconomic disadvantage or worse neighborhood social environment.

Neighborhood characteristics and DNA methylation

Neighborhood socioeconomic disadvantage

Neighborhood socioeconomic disadvantage had a statistically significant ($P < 0.05$) main effect or interaction with site type (promoter or CpG island shore/shelf) on methylation levels for 3 of the 7 stress-related genes (*AVP*, *CRF*, and *SLC6A4*) (Table 2) and 3 of the 11 inflammation-related genes (*F8*, *LTA4H*, and *TLR1*) (Table 3). Effect directions of statistically significant interactions varied across genes and site types (promoter or shore/shelf). To increase interpretation, we used model coefficients to estimate the mean M-values at each type of site for a respondent with "less neighborhood socioeconomic disadvantage" (mean neighborhood socioeconomic disadvantage minus one standard deviation) or "more neighborhood socioeconomic disadvantage" (mean neighborhood socioeconomic disadvantage plus one standard deviation). We then assessed whether the differences in these mean methylation levels were statistically significant (Table S2).

Neighborhood socioeconomic disadvantage was associated with methylation ($q \leq 0.10$) in at least one site type for 5 of the

Table 2. Regression of M-value on neighborhood socioeconomic disadvantage in stress pathway genes (n = 1,226).

	AVP		*CRF*		*SCL6A4*	
	β (SE)	*P*-value	*β* (SE)	*P*-value	*β* (SE)	*P*-value
Neighborhood Socioeconomic Disadvantage	−0.004 (0.004)	0.220	−0.001 (0.003)	0.752	0.001 (0.004)	0.795
Promoter	1.251 (0.003)	<2.0E-16	3.147 (0.012)	<2.0E-16	−3.255 (0.004)	<2.0E-16
Shore/Shelf	3.067 (0.007)	<2.0E-16	2.010 (0.009)	<2.0E-16	1.292 (0.004)	<2.0E-16
Neighborhood Socioeconomic Disadvantage * Promoter	−0.005 (0.003)	0.073	−0.031 (0.013)	0.014	−0.006 (0.004)	0.171
Neighborhood Socioeconomic Disadvantage * Shore/Shelf	0.020 (0.007)	0.004	0.021 (0.009)	0.015	0.017 (0.005)	0.0003

Regression models control for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Higher values indicate more neighborhood socioeconomic disadvantage. Asterisks (*) indicate interaction terms.

6 genes evaluated (all genes except for *LTA4H*). Q-values were modest, ranging from 0.01 to 0.10. Disadvantage was associated with increased methylation in non-promoter sites of *CRF, F8*, and *TLR1* (q = 0.03–0.10), shore/shelf sites of *AVP* and *SLC6A4* (q = 0.01–0.09), and non-shore/shelf sites of *F8* (q = 0.04), and it was associated with decreased methylation in non-shore/shelf sites of *CRF* (q = 0.07).

Neighborhood social environment

Neighborhood social environment had a statistically significant ($P < 0.05$) main effect or interaction with site type on methylation levels of 4 out of 7 stress-related genes (*AVP, BDNF, FKBP5*, and *SLC6A4*) (Table 4) and 7 out of 11 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, NLRP12, SLAMF7*, and *TLR1*) (Table 5). The estimated M-value for each site type by neighborhood social environment is presented in Table S3. Neighborhood social environment was associated with methylation level ($q \leq 0.10$) in at least one site type for all of the 11 genes evaluated, with Q-values as low as 1.1×10^{-05}. Social environment was associated with increased methylation in promoter sites of *AVP, CCL1, CD1D, F8, KLRG1*, and *SLAMF7* ($q = 2.1 \times 10^{-5}$–0.05), non-promoter sites of *AVP, BDNF, NLRP12*, and *TLR1* ($q = 1.0 \times 10^{-5}$–0.10), shore/shelf sites of *AVP, FKBP5, SLC6A4* ($q = 3.7 \times 10^{-5}$–0.06), and non-shore/shelf sites of *CD1D, F8, SLAMF7*, and *TLR1* (q = 0.0001–0.04). It was associated with decreased methylation in non-promoter sites of *CCL1* and *KLRG1* (q = 0.003–0.08), shore/shelves sites of *F8* (q = 0.04), and non-shore/shelf sites of *FKBP5* (q = 0.003).

Methylation and gene expression

To identify potential functional effects of changes in methylation, we next evaluated whether the identified neighborhood-associated methylation differences were also associated with gene expression. Two stress-related genes (*AVP, FKBP5*) and 6 inflammation-related genes (*CD1D, F8, KLRG1, NLRP12, SLAMF7*, and *TLR1*) had methylation levels for at least one site type associated with at least one neighborhood characteristic (FDR q-value < 0.10) and had gene expression data available. As shown in Table S4, at least one methylation site was associated with gene expression in *FKBP5, CD1D, F8, KLRG1*, and 2 transcripts from *NLRP12* at the Bonferroni-corrected *P*-value of 0.005. Methylation was not associated with gene expression in *AVP, SLAMF7, TLR1*, or a third transcript of *NLRP12*.

For the 6 genes with a significant relationship between at least one methylation site and gene expression, we evaluated the strength of the methylation-gene expression association within each site type. As previously reported,[3] Table S5 shows that methylation in shore/shelf sites was significantly associated with gene expression for all genes that had methylation measured in this site type (*FKBP5, CD1D*, and *F8*). Significant associations were observed between methylation and gene expression in the promoter regions of 2 genes (*FKBP5* and *CD1D*), non-promoter regions of 4 genes (*FKBP5, F8, KLRG1*, and one transcript of *NLRP12*), and non-shore/shelf regions of 2 genes (*KLRG1* and 2 transcripts of *NLRP12*).

Integration of results from associations among neighborhood characteristics, methylation, and gene expression

We did not formally test for indirect effects of neighborhood characteristics on gene expression through methylation, nor did we examine the total effect of neighborhood on gene expression. However, as a step in determining the precise genes and corresponding site types through which neighborhood influences on methylation may impact proximal gene expression, we present effect directions for site types that demonstrated significant associations between i) neighborhood

Table 3. Regression of M-value on neighborhood socioeconomic disadvantage in inflammation pathway genes (n = 1,226).

	F8		*LTA4H*		*TLR1*	
	β (SE)	*P*-value	*β* (SE)	*P*-value	*β* (SE)	*P*-value
Neighborhood Socioeconomic Disadvantage	0.050 (0.018)	0.005	0.001 (0.003)	0.771	0.013 (0.004)	0.004
Promoter	−1.685 (0.008)	<2.0E-16	−2.608 (0.005)	<2.0E-16	−4.078 (0.007)	<2.0E-16
Shore/Shelf	1.351 (0.024)	<2.0E-16	2.430 (0.005)	<2.0E-16	—	—
Neighborhood Socioeconomic Disadvantage * Promoter	0.002 (0.008)	0.841	−0.008 (0.005)	0.082	−0.025 (0.007)	0.0003
Neighborhood Socioeconomic Disadvantage * Shore/Shelf	−0.061 (0.025)	0.012	0.010 (0.005)	0.045	—	—

Regression models control for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Higher values indicate more neighborhood socioeconomic disadvantage. Asterisks (*) indicate interaction terms.

Table 4. Regression of M-value on neighborhood social environment in stress pathway genes (n = 1,226).

	AVP		*BDNF*		*FKBP5*		*SLC6A4*	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Neighborhood Social Environment	0.001 (0.004)	0.707	0.005 (0.002)	0.009	−0.007 (0.002)	0.002	−0.0004 (0.004)	0.915
Promoter	−1.251 (0.003)	<2.0E-16	2.607 (0.002)	<2.0E-16	0.218 (0.003)	<2.0E-16	3.255 (0.004)	0.0001
Shore/Shelf	−3.067 (0.007)	<2.0E-16	0.928 (0.002)	<2.0E-16	−0.239 (0.003)	<2.0E-16	−1.292 (0.004)	<2.0E-16
Neighborhood Social Environment * Promoter	−0.015 (0.003)	7.6E-08	−0.004 (0.002)	0.028	−0.006 (0.003)	0.044	−0.001 (0.004)	0.685
Neighborhood Social Environment * Shore/Shelf	0.035 (0.007)	4.4E-07	−0.002 (0.002)	0.353	0.016 (0.003)	1.9E-07	0.011 (0.004)	0.011

Regression models control for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Higher values indicate worse neighborhood social environment. Asterisks (*) indicate interaction terms.

and methylation, and ii) methylation and gene expression (Table 6). For neighborhood socioeconomic disadvantage, there was only one site type in one gene (non-promoter sites in *F8*) that met both criteria. For these sites, more neighborhood disadvantage was associated with increased methylation, and the summary measure of methylation in these sites was associated with decreased gene expression. For neighborhood social environment, 5 genes had one site type that met both criteria (promoter region of *CD1D*, shore/shelf regions of *FKBP5* and *F8*, and non-promoter regions of *KLRG1* and *NLRP12*). For *CD1D* promoter sites, worse social environment was associated with increased methylation, which was associated with decreased gene expression. In *FKBP5*, worse social environment was associated with increased methylation in shore/shelf sites, which were associated with decreased gene expression; however, worse social environment was associated with decreased methylation in *F8* shore/shelf sites, and decreased methylation at these sites was associated with increased gene expression. In *NLRP12*, worse social environment was associated with increased methylation in non-promoter sites, which were associated with decreased gene expression; however, worse social environment was associated with decreased methylation in *KLRG1* non-promoter sites and subsequent decreased gene expression.

Bioinformatics characterization of functional elements

For genes that showed significant associations between non-promoter sites and at least one neighborhood characteristic, we evaluated whether the sites in the region contained predicted enhancers and/or promoters for noncoding RNA. Non-promoter sites in 3 genes (*CRF, F8*, and *TLR1*) were associated with neighborhood socioeconomic disadvantage, while non-promoter sites in 6 genes (*AVP, BDNF, CCL1, KLRG1, NLRP12*, and *TLR1*) were associated with neighborhood socioeconomic environment. Two of the 5 non-promoter sites in *AVP* and 7 of the 19 non-promoter sites in *BDNF* were located near enhancer elements. Two of 8 non-promoter sites in *F8* and one of 19 non-promoter sites in *BDNF* contained predicted promoters that were not located at the transcription start site of the gene of interest (or any nearby protein coding gene).

Supplemental epigenome-wide analysis

To assess whether the 18 genes related to stress reactivity and inflammation investigated in this analysis are among the most important genomic targets that show epigenetic differences with respect to neighborhood, we conducted epigenome-wide association analyses (EWAS) for neighborhood socioeconomic disadvantage and for neighborhood social environment. Table S6 shows the ranking within the EWAS results (top 1%, 2%, 5%, 10% and 25%) of each of the 283 methylation sites from the 18 genes. For neighborhood socioeconomic disadvantage and neighborhood social environment, methylation sites in *BDNF, CRF, FKBP5, NR3C1, OXTR*, and *NLRP12* all had at least one methylation site in the top 5% of EWAS results. Methylation sites in *SLC6A4, TLR1*, and *TLR3* were also in the top 5% for neighborhood socioeconomic disadvantage, and

Table 5. Regression of M-value on neighborhood social environment in inflammation pathway genes (n = 1,226).

	CCL1		*CD1D*		*F8*		*KLRG1*		*NLRP12*		*SLAMF7*		*TLR1*	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
Neighborhood Social Environment	−0.025 (0.007)	0.0003	0.008 (0.003)	0.001	0.061 (0.017)	0.0003	−0.018 (0.008)	0.023	0.017 (0.005)	0.001	−0.003 (0.006)	0.631	0.023 (0.005)	2.1E-05
Promoter	1.684 (0.008)	<2.0E-16	2.652 (0.008)	<2.0E-16	1.685 (0.008)	<2.0E-16	0.499 (0.009)	<2.0E-16	−0.162 (0.008)	<2.0E-16	4.934 (0.008)	<2.0E-16	4.078 (0.007)	0.256
Shore/Shelf	—	—	−4.650 (0.004)	<2.0E-16	−1.351 (0.024)	<2.0E-16	—	—	—	—	—	—	—	—
Neighborhood Social Environment* Promoter	0.049 (0.008)	2.7E-10	0.032 (0.007)	0.001	0.016 (0.007)	0.031	0.034 (0.009)	0.0001	−0.016 (0.008)	0.042	0.029 (0.008)	0.001	−0.019 (0.007)	0.010
Neighborhood Social Environment* Shore/Shelf	—	—	−0.014 (0.004)	1.5E-05	−0.084 (0.023)	0.0003	—	—	—	—	—	—	—	—

Regression models control for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). Higher values indicate worse neighborhood social environment. Asterisks (*) indicate interaction terms.

Table 6. Directions of effect for site types that demonstrated significant associations between 1) neighborhood and methylation, and 2) methylation and gene expression (n = 1,226).

Gene	Pathway	Neighborhood Characteristic	Site Type	Transcript	Effect Direction (Neighborhood-Methylation)[a]	Effect Direction (Methylation-Gene Expression)[b]
FKBP5	Stress	Social Environment	Shore/Shelf	ILMN_1778444	+	−
CD1D	Inflammation	Social Environment	Promoter	ILMN_1719433	+	−
F8	Inflammation	Socioeconomic Disadvantage	Non-Promoter	ILMN_1675083	+	−
	Inflammation	Social Environment	Shore/Shelf	ILMN_1675083	−	−
KLRG1	Inflammation	Social Environment	Non-Promoter	ILMN_1658399	−	+
NLRP12	Inflammation	Social Environment	Non-Promoter	ILMN_1716105	+	−

Results are only shown for genes with site types that demonstrated significant associations between 1) at least one neighborhood characteristic and methylation (Tables S2 and S3), and 2) methylation and gene expression (Table S5). No genes had non-shore/shelf sites that met both of these criteria.

[a]Overall direction of effect of more neighborhood socioeconomic disadvantage or worse neighborhood social environment on methylation (see Tables S2 and S3). Regression models control for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for each of 4 major blood cell types (neutrophils, B cells, T cells, and natural killer cells). (+) = more disadvantageous/worse neighborhood characteristic is associated with increased methylation, (−) = more disadvantageous/worse neighborhood characteristic is associated with decreased methylation.

[b]Direction of effect of the β coefficient for the summary methylation measure, for models with significant association between at least one methylation site and gene expression in the global likelihood ratio test (see Table S5). Regression 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). (+) = increased methylation is associated with increased gene expression, (−) = increased methylation is associated with decreased gene expression.

methylation sites in *CCL1, CD1D, KLRG1, SLAMF7* were in the top 5% for neighborhood social environment. The top 5% of EWAS results for neighborhood socioeconomic disadvantage were enriched for stress reactivity genes ($P = 0.035$) but not inflammation genes, while the top 1% and 2% of EWAS results for neighborhood social environment were enriched for inflammation genes ($P = 0.012$ and 0.009, respectively) (Table 7).

Discussion

As the field of social epigenomics expands, it has become apparent that social context is a potent influencer of DNA methylation over the life course. The effects of individual-level SES have been demonstrated previously, and this study shows that neighborhood-level context also impacts the epigenome, even after accounting for individual-level SES. This implies that DNA methylation patterns in the stress and inflammatory pathways may be responsive to both neighborhood-level interventions as well as individual-level interventions. A deeper understanding of the biologic mechanisms that contribute to neighborhood effects on health has the potential to shed light on the etiology and causal mechanisms driving health disparities.

In this study, we evaluated 2 neighborhood-level indicators: neighborhood socioeconomic disadvantage and neighborhood social environment. We found that after adjustment for individual childhood and adult SES, neighborhood socioeconomic disadvantage was associated with methylation in genes related to stress and inflammation (corresponding to 43% and 27% of the stress and inflammation-related genes evaluated, respectively). Neighborhood social environment was associated with methylation in a larger number of both stress-related (57%) and inflammation-related (64%) genes evaluated. These results indicate that both neighborhood conditions may influence the stress and inflammation pathways substantially, but that they appear to operate through at least partially different mechanisms.

Table 7. Enrichment of DNA methylation sites from 18 stress reactivity and inflammation genes in top results from epigenome-wide analyses for neighborhood characteristics (n = 1,226).

Neighborhood Characteristic	Top Percentage of EWAS Results	Enrichment for Stress Reactivity Genes (*P*-value[a])	Enrichment for Inflammation Genes (*P*-value[a])
Socioeconomic Disadvantage	1%	0.309	0.591
	2%	0.347	0.834
	5%	0.035	0.656
Social Environment	1%	0.858	0.012
	2%	0.980	0.009
	5%	0.506	0.077

Stress reactivity genes include 194 methylation sites in 7 genes. Inflammation genes include 89 methylation sites in 11 genes.

[a]*P*-value from one-sided Fisher's Exact Test for enrichment of stress reactivity/inflammation genes in top epigenome-wide association study (EWAS) results.

Neighborhood social environment may be particularly influential on the DNA methylation patterning of the inflammatory pathway, given that it was significantly associated with approximately 2 thirds of the inflammation-related genes examined. Neighborhood social environment may also have an overall stronger influence on the stress and inflammation pathways than neighborhood socioeconomic disadvantage, as indicated by a much larger number of significant site type-specific associations across the genes examined (21 vs. 7 associations for social environment and disadvantage, respectively, Tables S2 and S3). The strength of association in both stress-related and inflammation-related genes was also markedly stronger for site type-specific associations with neighborhood social environment compared with neighborhood socioeconomic disadvantage. This result is consistent with a previous study in MESA participants that found that another biomarker of stress, telomere length, was associated with neighborhood social environment but not neighborhood disadvantage.[23] This may be because neighborhood disadvantage is a census-based summary measure that captures neighborhood composition, and thus may be a poor proxy for the causally relevant neighborhood variables.[5] In contrast, measures of neighborhood social environment specifically capture neighborhood contextual factors that are theoretically and empirically linked to stress biomarkers and chronic disease.

Although the relationship between neighborhood conditions and DNA methylation varied across genes and site types, several major patterns emerged. Most striking was the general

increase in DNA methylation associated with higher neighborhood socioeconomic disadvantage and social environment. Of the 7 significant site type-specific associations between neighborhood socioeconomic disadvantage and DNA methylation, all but one linked increased DNA methylation to higher disadvantage (Table S2). Analogously, all but 4 of the 21 significant relationships between DNA methylation and neighborhood social environment indicated more DNA methylation in the presence of a worse social environment (Table S3). Increases in DNA methylation related to higher socioeconomic disadvantage tended to be concentrated in shore/shelf and non-promoter sites, while those related to worse social environment were observed in all 4 site types including the promoter region of one stress-related gene (*AVP*) and 5 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, SLAMF7*).

The effects of methylation on proximal gene expression may be influenced by the type of site, proximity to the transcription start site of the gene, and the presence of other nearby epigenetic marks. For example, methylation of CpG islands in promoter regions tends to be associated with decreased gene expression, but methylation in other gene regions, such as the gene body, is more likely to be associated with increased transcription.[24] However, the relationship between all of these factors and the actual functional effect of methylation at a particular site is quite complicated.[25] A study in fibroblast cells recently showed that the methylation site most highly correlated with gene expression was in the promoter region for only about 25% of genes, and in the gene body for 30% of genes.[26] Of the promoter region methylation sites that were correlated with gene expression, approximately 33% showed the opposite direction of correlation than expected (that is, a positive correlation between methylation and gene expression).

Since gene expression data was not available for all of the genes of interest, and because change in expression of the gene most proximal to a given methylation site is only one possible mechanistic consequence of disadvantage-associated changes in methylation, we did not focus primarily on the relationship between neighborhood disadvantage and gene expression. However, we incorporated gene expression data in supplemental analyses to help inform potential mechanisms of action. To this end, we assessed relationships between methylation and gene expression (when available) within site types that demonstrated an association between a neighborhood characteristic and methylation to extrapolate potential effect directions of neighborhood on gene expression through methylation of the sites of interest. For those sites, increased methylation at promoter sites (*CD1D*), and shore/shelf sites (*FKBP5* and *F8*), and some non-promoter sites (*F8* and *NLRP12*) was associated with decreased gene expression. However, increased methylation in non-promoter sites was associated with increased gene expression in one gene (*KLRG1*). It is important to note that more disadvantaged/worse neighborhood was not always associated with increased methylation (it was associated with decreased methylation in *F8* shore/shelf sites and *KLRG1* non-promoter sites).

While the potential impact of these neighborhood-associated changes in gene expression on health and disease is speculative, we note that changes in gene expression for several of the genes implicated in this study have been linked to health outcomes. *CD1D* encodes a protein that presents antigens to invariant natural killer T cells (iNKT), and mice lacking this protein have a higher risk of developing autoimmune diabetes.[27] Links between iNKT cells and other metabolic diseases involving inflammation (such as obesity and type 2 diabetes), as well as the influence of *CD1D* in these disease processes, is now being explored. *NLRP12* encodes an inflammasome-associated protein in myeloid/monocyte cells that suppresses inflammatory response. Loss of function mutations in this gene have been shown to associate with autoinflammatory symptoms including fever, myalgia, and elevated serum inflammatory markers, and the role of this gene (and other genes in the *NLRP* family) in other metabolic diseases is an active area of research.[28] *KLRG1* encodes a marker of natural killer cells and immunosenescent T cells, which is increased in the peripheral blood of humans with persistent viral infections, as well as spinal fluid (but not peripheral blood) of people with spondyloarthritis and rheumatoid arthritis.[29] It is also a marker of T cell activation that is associated with accelerated atherosclerosis in mice.[30] *F8* encodes a prothrombotic acute-phase protein that increases in response to inflammatory cytokines. Increased *F8* protein in the context of this study may simply be a marker of increased inflammatory markers in the bloodstream (such as IL-1, IL-6, IL-8, or TNF-α). *FKBP5* encodes a protein regulated by corticosteroids and decreases the binding affinity of the glucocorticoid receptor (*GR*, or *NR3C1*), and increased expression of the *FKBP5* protein in the brain has been robustly associated with major depression.[31] The directions of effect for *KLRG1* and *FKBP5* that we inferred in this study are opposite of the expected directions based on previous literature. This may be due to differences in tissue type examined (e.g., brain vs. blood), the populations under study (clinical samples vs. population-based samples), and/or the reflection of biologic feedback loops (for example, sustained stress may eventually lead to downregulation of stress response biomarkers).

Neighborhood-associated differences in DNA methylation may modify expression in proximal genes, but could also have other functional consequences. For example, these methylation sites may be in genomic regions that contain enhancer elements or promoters for noncoding RNA. We found that neighborhood socioeconomic disadvantage was associated with non-promoter sites in one gene, *F8*, which had 2 sites located near a potential noncoding RNA promoter region. Neighborhood social environment was associated with non-promoter sites in 2 genes that were near enhancers (*AVP* and *BDNF*) and/or potential noncoding RNA promoter regions (*BDNF*). For both genes, the predicted noncoding RNA promoters were within CpG island boundaries. Although this feature has historically been associated with ubiquitous ("housekeeping") expression of RNA, approximately half of the predicted FANTOM promoters in CpG islands demonstrated cell type-specific expression and were near cell type-specific enhancers.[32]

In our previous MESA study, we evaluated the influence of childhood and adult SES on the same 18 genes examined here.[3] We found that childhood SES, adult SES, and/or social mobility influenced methylation in 3 stress-related (*AVP, FKBP5*, and *OXTR*) and 7 inflammation-related genes (*CCL1, CD1D, F8, KLRG1, NLRP12, PYDC1, TLR3*). In several instances, effects of SES on methylation were also observed for neighborhood

characteristics, even after adjusting for both childhood and adult SES. Specifically, the same significant DNA methylation differences were found for adult SES and both neighborhood characteristics in shore/shelf sites in *AVP* and non-shore/shelf sites in *F8*. Consistent effects were also observed between adult SES and neighborhood socioeconomic environment at promoter sites in *CDID* and *F8*, non-promoter sites in *AVP* and *NLRP12*, and shore/shelf sites in *F8*. Significant methylation differences with opposite directions of effect were only found in non-promoter sites in *F8* (opposite between adult SES and neighborhood socioeconomic disadvantage) and *KLRG1* (opposite between adult SES and neighborhood social environment). Consistent DNA methylation differences for adult SES and neighborhood social environment that were also associated with gene expression include promoter sites in *CD1D*, non-promoter sites in *NLRP12*, and shore/shelf sites in *F8*.

Of note, the total number of associations observed between neighborhood social environment and DNA methylation was greater than the number of associations observed between neighborhood socioeconomic disadvantage or any of the individual-level SES measures[3] and DNA methylation. In addition to the DNA methylation associations that neighborhood social environment shares with adult SES, there are also associations in additional genes (*BDNF, FKBP5, SLC6A4, CCL1, SLAMF7*, and *TLR1*) even though we used a more conservative FDR q-value for considering findings noteworthy in this study (q-value < 0.1) compared with our SES study (q-value < 0.2). This indicates that just as has been observed for biomarkers of stress,[13-15,23] methylation patterning at genes related to stress reactivity and inflammation genes are influenced not only by SES but also by the neighborhood context. The results of this study suggest that worse neighborhood social environment and, to some extent, low neighborhood SES may also lead to additional changes in methylation beyond those patterned by individual-level SES.

Only a small number of other studies have evaluated the impact of neighborhood context on methylation. A study of 99 African American women from the Family and Community Health Study (FACHS) found that methylation of sites within a CpG island in the serotonin transporter gene (*SLC6A4*, or *5-HTT*) was a mediator of an interaction between *SLC6A4* genetic variants and neighborhood crime on depression.[19] Our study also showed that neighborhood context, including both SES and social environment (which includes neighborhood safety), were associated with *SLC6A4* methylation. However, the association we observed was within shore/shelf sites, while the association in FACHS was observed for sites in a CpG island (non-shore/shelf sites). A study of 34 African American men found that methylation at the *IL-6* promoter was associated with childhood trauma, but not childhood exposure to neighborhood violence.[33] Our study did not evaluate *IL-6*, but we did not find any relationship between neighborhood context and methylation of another interleukin gene, *IL-8*.

To our knowledge, this was the first study to examine the relationship between neighborhood context and methylation in a large, multi-ethnic, population-based cohort. The rich neighborhood measures in this study allowed us to evaluate both objective (census-based) and subjective (survey-based) metrics, including perceptions of neighborhood features from members of the community that were not MESA participants. We used a repeated measures modeling approach to efficiently use the multiple measures of DNA methylation data and reduce the multiple testing burden compared with modeling each site separately. This method also allowed the evaluation of systematic differences in the associations by site type. A further strength of this study was the examination of DNA methylation, followed by gene expression, in a single cell type rather than whole blood, which is a mixture of white blood cell types that differ in their methylation and gene expression patterns.

Since this study was limited to only 18 genes, further investigation will be necessary to identify relationships between neighborhood context and other stress- and inflammation-related genes, as well as genes involved other biologic pathways. A second limitation is that the study sample consisted of relatively healthy older adults, which may have led to survival bias. Replication of findings, particularly in young or more representative older samples, will be a necessary next step. A third limitation is that our adjustment for individual-level variables was imperfect. For example, dichotomous variables for childhood and adult SES may not have completely controlled for all SES-related influences on DNA methylation. There may also have been residual confounding by individual-level health behaviors that are associated with neighborhood measures and influence DNA methylation. In addition, while our repeated measures approach has several advantages, it also imposes assumptions on the similarity of effects across DNA methylation sites of the same type. Due to data sparsity, we were also not able to evaluate site type on a more fine-grained level (for example, analyzing shores and shelves separately).

Finally, although we observed statistically significant relationships between neighborhood and DNA methylation, the methylation changes were small in magnitude. For neighborhood-associated DNA methylation changes (FDR $q < 0.1$, Tables S2 and S3), the difference in percent methylation between participants living in "better" or "worse" neighborhoods ($\pm$ 1 SD from mean neighborhood characteristic) ranged from 0.2% to 2.2% (calculated by converting M-values to β values). For example, for non-shore/shelf sites of the *F8* gene, participants in neighborhoods with worse social environments had average β values of 0.47 and those in better neighborhoods had less DNA methylation ($\beta = 0.45$). It is not yet clear whether these relatively small differences in DNA methylation will ultimately translate to measurable differences in health and disease. Despite these limitations, this study identified multiple effects of neighborhood context on DNA methylation. Future extensions of this work should include evaluating DNA methylation as a mediator of neighborhood effects on health outcomes, such as cardiovascular disease, mental illness, and autoimmune disease, as well as health biomarkers such as levels of cortisol and inflammatory biomarkers. Since genetic variation has been shown to shape methylation patterning[34] as well as interact with neighborhood to influence health outcomes,[19] another important area of research is evaluating the influence of genetic factors on relationships between neighborhood, DNA methylation, and health.

Neighborhood context may substantially influence DNA methylation levels and subsequent gene expression levels of stress- and inflammation-related genes, even after accounting

for individual socioeconomic factors. There is also evidence that different neighborhood-level attributes, such as socioeconomic status and social environment, may be biologically embedded through methylation patterning of some shared genes, but that they also likely influence biologic pathways such as stress reactivity and inflammation independently. By showing epigenetic modifications in stress and inflammation-related genes, our findings suggest that stress and inflammation may be important pathways linking neighborhoods to health. Elucidating the molecular mechanisms underlying the relationships between neighborhood, DNA methylation, and gene expression will contribute to reducing health disparities by developing a more thorough understanding of disease etiology and thus facilitating the design of more effective interventions (including both neighborhood level and individual level interventions), and the identification of at-risk subgroups.

Materials and methods

Study sample

MESA is a population-based longitudinal study designed to identify risk factors for the progression of subclinical cardiovascular disease (CVD).[21] A total of 6,814 non-Hispanic white, African-American, Hispanic, and Chinese-American women and men aged 45–84 without clinically apparent CVD were recruited between July 2000 and August 2002 from the following 6 regions in the US: 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, including lists of residents, lists of dwellings, and telephone exchanges. During MESA Exam 5 (between April 2010 and February 2012), DNA methylation 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 methylation on CVD. We excluded 38 respondents with missing data on one or more variables included in the final models (final n = 1,226). This study was approved by the Institutional Review Boards of all MESA field centers, the MESA Coordinating Center, and the University of Michigan.

Gene selection

Consistent with a previous investigation of individual-level SES and methylation in the MESA cohort,[3] our study evaluated methylation levels of 18 genes related to stress reactivity or inflammation that had been previously shown in humans and/or animals to have methylation levels associated with psychosocial stress exposure. Genes related to stress reactivity included *AVP*,[35] *BDNF*,[36,37] *CRF*,[38] *FKBP5*,[39] *GR*,[40-45] *OXTR*,[46] and *SLC6A4*,[47-50] and genes related to inflammation included *CD1D, CCL1, F8, IL8, KLRG1, LTA4H, NLRP12, PYDC1, SLAMF7, TLR1*, and *TLR3*.[51] Table S1 provides descriptive information for each of the 18 genes evaluated in this study, including the total number of methylation sites, the number of methylation sites in promoter regions and in CpG island shore or shelf regions, and the availability of gene expression data.

Measures

DNA methylation

A detailed description of the quantitation and data processing procedures used for DNA methylation and gene expression can be found in Liu et al.[25] Briefly, blood was drawn in the morning after a 12 h fast. Monocytes were isolated using AutoMACs automated magnetic separation units (Miltenyi Biotec, Bergisch Gladbach, Germany) and were consistently >90% pure. Samples were plated using a stratified random sampling technique to reduce bias due to batch, chip, and position effects. Methylation was measured using the Illumina HumanMethylation450 BeadChip, and bead-level data were summarized in GenomeStudio. Quantile normalization was performed using the *lumi* package with default settings.[52] Quality control measures included checks for sex and race/ethnicity mismatches and outlier identification by multidimensional scaling plots. Criteria for elimination included: 'detected' methylation 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, and overlap with a non-unique region. 65 probes that assay highly-polymorphic single nucleotide polymorphisms (SNPs) rather than methylation were also excluded.[53] The methylation level for each site was computed as the M-value, the log ratio of the methylated to the unmethylated signal intensity.[54] Chip and position effects were adjusted before analysis.

CpG sites were annotated using Illumina-provided annotation files.[55] Specifically, CpG sites were assigned to genes using the UCSC database, which included sites in the promoter region, 5′ untranslated region, gene body, and 3′ untranslated region. The UCSC database was also used to determine whether CpG sites were in promoter regions (located up to 1,500 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). CpG sites near informatically-predicted enhancer elements were determined by the ENCODE Consortium, and low- or high-density CpG regions associated with FANTOM4 promoters were identified by the FANTOM consortium.

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.[56] 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 (< 10^{th} percentile), or overlap with a non-unique region. Chip effects were adjusted before analysis.

Socioeconomic status

We used maternal educational attainment as an indicator of childhood SES.[57, 58] 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 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).

Adult SES was indicated by adult educational attainment. 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).

Neighborhood characteristics

Neighborhood socioeconomic disadvantage scores for each neighborhood were created based on a principal components analysis of 16 census-tract level variables from the 2000 US Census. These variables reflect dimensions of education, occupation, income and wealth, poverty, employment, and housing. The neighborhood socioeconomic disadvantage score is the weighted sum of the following 6 standardized variables, which accounted for 49% of the variance and loaded on the first factor: percent in census tract with a bachelor's degree; percent with a managerial/professional occupation; percent with a high school education; median home value; median household income; and percent with household income greater than $50,000 per year. Higher values on the scale indicate greater neighborhood socioeconomic disadvantage.

Neighborhood social environment is the sum of standardized conditional empirical Bayes estimate (CEB) scales for aesthetic quality, safety, and social cohesion (which, at low levels, may serve as stressors). Information on neighborhood social environment was obtained from questionnaires administered to MESA participants and to an auxiliary sample of other neighborhood residents in the New York site.[59] Responses were aggregated across respondents in census tracts to create neighborhood-level measures of aesthetic quality, safety, and social cohesion. The CEB estimates are more reliable than the census-tract crude means because they borrow information from other census tracts in cases where the sample size per tract is very small. In addition, the CEB estimates adjust for important factors in survey response, including site, participant sex and age, and survey type (MESA or auxiliary sample).

Respondents were asked to report their levels of agreement on a 5-point scale (1 = strongly agree to 5 = strongly disagree) to statements pertaining to neighborhood aesthetic quality, safety, and social cohesion. Scales for each measure were created by summing responses to all statements that pertain to the measure of interest and reverse coded as necessary so that higher values indicate worse negative neighborhood social environment (lower aesthetic quality, less safety, or lower social cohesion). The statements for aesthetic quality were: (1) there is a lot of trash and litter on the street in my neighborhood; (2) there is a lot of noise in my neighborhood; and (3) my neighborhood is attractive. The statements for safety were: (1) I feel safe walking in my neighborhood day or night; and (2) violence is a problem in my neighborhood. The statements for social cohesion were: (1) people around here are willing to help their neighbors; (2) people in my neighborhood generally get along with each other; (3) people in my neighborhood can be trusted; and (4) people in my neighborhood share the same values. Cronbach's α for the scales were 0.67 for aesthetic quality, 0.64 for safety, and 0.72 for social cohesion.

Since neighborhood conditions may operate across the life course to affect health, we were interested in evaluating the long-term exposure to neighborhood conditions. For all neighborhood measures, we used the cumulative average of the measure across all available MESA examinations (maximum of 5 exams, representing the approximately 10 y timespan from baseline to exam 5).

Statistical analysis

Analysis of the relationship between neighborhood characteristics and DNA methylation was conducted analogously to our previous investigation of individual-level SES and methylation in the MESA cohort,[3] and was performed using SAS (SAS Institute Inc., Cary, NC, USA) and R.[60] Analyses for each of the 18 genes were conducted separately, to allow for differential effects of neighborhood conditions across genes. Since methylation measures within a small (200–400 kb) genomic region may be intercorrelated,[61] we conceptualized methylation measures within each gene as repeated measures for each individual. Using 2-level models with a random intercept for each person, we tested for associations between neighborhood conditions and multiple measures of methylation, accounting for the intercorrelation between methylation measures within a person. Since respondents living in a particular neighborhood may be more similar than those in different neighborhoods, we examined the possibility of a 3-level model that accounted for shared neighborhoods across respondents. However, when we added neighborhood as a random intercept, the models failed to converge because the average number of respondents per neighborhood was low (mean = 2.5; 56% of neighborhoods contained only one respondent).

Since prior evidence points to differential effects of external stimuli on methylation sites with particular characteristics (such as sites in the promoter region or in shore/shelves of CpG islands), we included indicator variables to specify the type of site as well as neighborhood-by-indicator interaction terms, which allowed neighborhood effects on methylation to differ across site types. Information about each of the methylation sites examined is provided as supplemental material in Needham et al. (2015).[3]

Regression models controlled for sex, race/ethnicity, age, childhood SES, adult SES, and residual sample contamination with non-monocytes (enrichment scores for neutrophils, B cells, T cells, and natural killer cells). The model specification

for a given gene is provided below:

$$Y_{ij} = \beta_0 + \eta_i + \beta_1 \cdot Neighborhood_i + \beta_2 \cdot Promoter_j + \beta_3 \cdot Shore/Shelf_j + \beta_4' \cdot Covariates_i + \beta_5 \cdot Neighborhood_i \cdot Promoter_j + \beta_6 \cdot Neighborhood_i \cdot Shore/Shelf_j + \epsilon_{ij}$$

Y_{ij}: M-value for methylation site j for individual i for the given gene.
$Neighborhood_i$: Neighborhood condition for individual i.
$Promoter_j$: 'Promoter' indicator for methylation site j.
$Shore/Shelf_j$: 'Shore/shelf' indicator for methylation site j.
$Covariates_i$: Covariate measures (individual-level sociodemographic variables and residual sample contamination with non-monocytes) for individual i.
η_i: Individual level random intercept for individual i, $\eta_i \sim N(0,\ \sigma^2{}_{individual})$.
ϵ_{ij}: Site-specific residual error term, $\epsilon_{ij} \sim N(0,\ \sigma^2{}_{error})$.
β_0: Intercept of the model.
β_1: Change in the M-value for each 1-unit increase in neighborhood condition.
β_2: Difference in the M-value between promoter and non-promoter methylation sites.
β_3: Difference in the M-value between shore/shelf and non-shore/shelf methylation sites.
β_4': Vector of parameter estimates for covariates.
β_5: Difference in the neighborhood effect on the M-value between promoter and non-promoter methylation sites.
β_6: Difference in the neighborhood effect on the M-value between shore/shelf and non-shore/shelf methylation sites.

To facilitate interpretation of the statistically significant study results ($P < 0.05$ for the main effect of neighborhood condition or the interaction between neighborhood condition and site type), we next calculated mean M-values that were adjusted to the mean levels of all covariates in the model for specific values of each neighborhood condition (e.g., mean + 1 SD and mean - 1 SD of neighborhood socioeconomic disadvantage) and site type (e.g., promoter sites). This also allowed us to assess the statistical significance of neighborhood-related differences in mean M-values within promoter or shore/shelf categories. We calculated the false discovery rate (FDR)[62] based on the P-value for the neighborhood effect on methylation within each site type to account for multiple testing. We applied a cutoff of $q \leq 0.1$ to indicate results that remained noteworthy after FDR correction.

For genes with an association between at least one neighborhood condition and methylation in at least one site type ($q \leq 0.1$), we evaluated whether methylation was associated with gene expression. Gene expression data was available for 8 of the 11 genes of interest, with a single transcript available for all but one of the genes (*NLRP12*, which had 3 transcripts). Since most of the genes had only one transcript, we used linear regression rather than a multi-level model. A global likelihood ratio test was used to assess whether at least one methylation site was associated with the gene expression level of the target transcript, after controlling for age, sex, race/ethnicity, childhood SES, adult SES, and enrichment scores for the 4 major blood cell types (neutrophils, B cells, T cells and natural killer cells). The model specification for a given gene is provided below. The null hypothesis is that $M_1,\ldots,M_k = 0$. Significance was declared at the Bonferroni corrected P-value of 0.005 (0.05/10 tests).

$$Y_i = \beta_0 + \beta_1 \cdot M_1 + \beta_2 \cdot M_2 + \ldots + \beta_p \cdot M_p + \beta'_{p+1} \cdot Covariates_i + \epsilon_i$$

Y_i: Gene expression value for individual i.
M_k: M-value of methylation site k for a given gene for individual i, $k = 1,\ldots,p$.
$Covariates_i$: Covariate measures (individual-level sociodemographic variables and residual sample contamination with non-monocytes) for individual i.
ϵ_i: Residual error term, $\epsilon_i \sim N(0,\ \sigma^2{}_{error})$.
β_0: Intercept of the model.
β_k: Change in gene expression value for site k, $k = 1,\ldots, p$
β'_{p+1}: Vector of parameter estimates for covariates.

We then conducted analogous global likelihood ratio tests stratified by site type for each gene to identify which type of sites had methylation associated with gene expression. Significance was declared at the Bonferroni-corrected P-value of 0.002 (0.05/21 tests). For each site type with a significant global test, we evaluated the direction of effect for methylation on gene expression. First, we calculated the sum of the M-values across all of the sites within site type, then we regressed this summary methylation measure onto gene expression using the same adjustment variables as above and noted the sign of the β coefficient for the methylation measure.

Supplemental epigenome-wide analysis

For each methylation site, we ran a linear regression model with the site as the outcome variable and regressed it onto the neighborhood characteristic (neighborhood socioeconomic disadvantage or neighborhood social environment) and the same adjustment variables as we used in the gene-based models: sex, race/ethnicity, age, childhood SES, adult SES, and residual sample contamination with non-monocytes (enrichment scores for neutrophils, B cells, T cells, and natural killer cells). For each of the methylation sites from the 18 candidate genes, we characterized the EWAS ranking (top 1%, 2%, 5%, etc.) by P-value of the β coefficient for the neighborhood characteristic. We then performed one-sided Fisher's exact tests to assess whether the top results from EWAS were enriched for methylation sites in the 7 stress reactivity and 11 inflammation genes.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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