

Unfavorable Socioeconomic Conditions in Early Life Presage Expression of Proinflammatory Phenotype in Adolescence

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Objective: Unfavorable socioeconomic status (SES) circumstances early in life are associated with heightened vulnerability to respiratory and cardiovascular diseases in adulthood. However, little is known about mechanisms underlying this phenomenon.

Methods: This study examined whether early-life SES predicts future activity of two genes involved in regulating inflammation. An ethnically diverse cohort of 136 adolescent females was enrolled in the study. SES was measured by home ownership. The messenger ribonucleic acid (mRNA) for glucocorticoid receptor (GR) and toll-like receptor 4 (TLR4) was quantified in peripheral blood leukocytes using real-time reverse transcriptase polymerase chain reaction (RT-PCR). **Results:** Three findings emerged: a) Years 2 to 3 of life were a critical period: the participants whose families owned homes during these childhood years showed higher GR mRNA and lower TLR4 mRNA during adolescence, a profile that suggests better regulation of inflammatory responses. b) These effects were not mediated through current economic circumstances, life stress, or health practices. c) Changes in SES during later years were unable to “undo” these effects. **Conclusions:** These findings suggest that unfavorable SES circumstances in the early years of life presage the expression of a proinflammatory phenotype in adolescence. To the extent that this proclivity toward inflammation persists over one’s lifespan it could explain the heightened incidence of respiratory and cardiovascular disease in low SES populations.

Key words: socioeconomic status, inflammation, glucocorticoid receptor, toll-like receptor 4, childhood, psychological stress.

GR = glucocorticoid receptor; **mRNA** = messenger ribonucleic acid; **RT-PCR** = reverse transcriptase polymerase chain reaction; **SES** = socioeconomic status; **TLR4** = toll-like receptor 4.

INTRODUCTION

Mounting evidence indicates that socioeconomic circumstances during the early years of life are an important determinant of later medical outcomes. To the extent that children are raised in low SES environments, their risk of developing respiratory infections, chronic obstructive pulmonary disease, myocardial infarction, and hemorrhagic stroke as adults increases proportionately (1–5). In most cases, these effects are independent of social standing in adulthood (3–5), suggesting that early SES is not simply acting as a proxy for social standing later in life.

To explain the mechanisms underlying these findings, researchers have advanced hypotheses regarding “critical periods” and “biological programming” (2,6,7). These models assume that unfavorable environmental circumstances during critical periods of development can “program” biological systems in a manner that persists across a person’s lifespan and accentuates one’s vulnerability to disease (2,6–8). For example, studies of early-life social exposures have shown that neonatal rodents handled daily exhibit diminished adrenocortical responses to stressful experience when they reach adulthood (9–12). This enhanced regulation of the hormonal response is attributable to epigenetic processes, such as demethylation of deoxyribonucleic acid (DNA) and acetylation of histone proteins that facilitate expression of the glucocorticoid receptor (GR) in hippocampal tissue (13–15). These epigenetic pro-

cesses bring about stable changes in genomic activity without structurally altering DNA. In rats, these changes persist into adulthood, showing that social experiences in early life can become genomically embedded for the long term.

The current project examines the possibility that SES in the early years of life operates in a similar fashion. We assessed the childhood SES circumstances of a cohort of healthy female adolescents as well as expression of genes that code for the GR and the toll-like receptor 4 (TLR4). These genes are centrally involved in regulating the inflammatory response and, as such, represent potential mechanisms linking childhood SES and future disease, especially in the respiratory, infectious, and cardiovascular domains. TLR4 acts as the primary receptor for endotoxin on leukocytes and, in doing so, functions as the interface between invading bacteria and the immune system. GR is the primary receptor for cortisol and has a wide range of biological activities. In the immune system, GR serves as a conduit for the anti-inflammatory signals of glucocorticoids; in the nervous system, it regulates behavioral and biological responses to stressful experience. Because childhood SES can help to establish psychosocial trajectories that persist over the long term, we also measured participants’ current SES circumstances, recent exposure to life stress, and patterns of health behavior, to evaluate whether they were more proximal determinants of GR and TLR expression. Finally, noting that economic conditions often change over the course of childhood, we examined whether upward or downward social mobility could “undo” the genomic influence of early SES.

METHODS

Participants

The sample consisted of 136 young women from greater Vancouver, BC, recruited through advertisements in local media. Participants were eligible for the project if they were 13 to 19 years old and fluent in English. They also had to be free of infectious disease for at least 2 weeks, and without chronic medical illness or major psychiatric disorders. Participants provided written informed consent. For those <18 years, separate consent was obtained from a parent. The protocol was approved by the University of British Columbia’s Research Ethics Board. Data collection took place between July 2004 and November 2006.

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Childhood Social Class

We had each participant complete SES measures with help from her mother or father. To obtain an accurate indicator of SES during childhood, we asked families to mark the participant's age at which they purchased their first home. Mounting evidence indicates that housing status is a robust marker of economic circumstances. It correlates with conventional indicators like income and assets, but housing status can be reported with a much higher degree of accuracy, especially when assessments are conducted retrospectively (3). We used these data to construct a series of binary variables reflecting whether the family owned versus rented their primary residence during each year of the participant's childhood (ages 0–12 years).

GR and TLR4 Gene Expression

A 2.5-ml blood sample was drawn through antecubital venipuncture into PAXgene blood ribonucleic acid (RNA) tubes (Qiagen Corporation, Toronto, Ontario). Total RNA was then extracted from bulk leukocytes using PAXgene blood RNA kits and frozen at -80°C until the end of the project. Expression of GR and TLR4 mRNA was quantified through real-time RT-PCR. RT-PCR reactions were carried out on an Applied Biosystems Prism 7000 Sequence Detection System, using commercially available one-step assays based on 5' nuclease activity of FAM-labeled TaqMan probes (Assay #Hs00152937 for TLR4; Hs00353740 for GR; Applied Biosystems, Foster City, CA). Threshold cycle (C_T) numbers were established using SDS 1.1 Relative Quantification Software. As an internal control, 18S mRNA levels were quantified in parallel with target genes (Assay #Hs99999901). The data were normalized using the ΔC_T method ($\Delta C_T = C_T \text{ target gene} - C_T \text{ internal control}$). Results are expressed as relative quantities of each target gene, calculated by subtracting each participant's ΔC_T value from the highest ΔC_T value in the distribution. Thus, higher relative quantities are indicative of greater expression of the GR and TLR4 genes. Because of technical difficulties with the assay, three participants were missing values for TLR4 mRNA.

Pathways Linking Childhood SES and Gene Expression

We explored several mechanisms that could underlie the associations between early-life SES and current expression of the GR and TLR4 genes. These mechanisms included current social class, life stress, and health practices. Current social class was assessed by reports of current ownership of primary residence (owned versus rented), automobile ownership (number of automobiles/driver), and perceived social standing of the family. The latter variable was derived from the MacArthur Scale of Subjective Status—Youth Version. Participants marked the rung on a 10-step ladder that corresponded to their perception of their family's standing in society (16).

To quantify stress, we administered modules of the UCLA Life Stress Interview (17). This semistructured interview assesses the degree of acute and chronic stress over the past 6 months. A trained interviewer asked a series of open-ended questions, and rated the level of chronic, ongoing stress on a 5-point scale. In the current analyses, we focused on chronic stress in the family and academic domains. The interview also yielded information regarding acute stressors (specific events with a discrete onset and offset). Impact ratings for acute events were made by team consensus on a 5-point scale. We used each child's most highly rated event as an indicator of acute stress in statistical analyses.

To obtain an indication of participants' current health practices, we assessed cigarette smoking, alcohol consumption, and overall adiposity. Smokers were defined as those who used cigarettes daily. Alcohol use was determined by counting the number of alcoholic drinks consumed during a typical week. We also assessed participants' height and weight using a balance-beam scale with a height rod and computed body mass index.

Statistical Analyses

Three major hypotheses were tested in the statistical analyses. 1) To determine whether SES during critical periods of childhood predict GR and TLR4 expression during adolescence, we constructed a series of hierarchical multiple regression equations in which relative quantities of each transcript were predicted from variables reflecting age at study entry and self-identified

ethnicity, variables reflecting social class at the time of study entry, and a binary variable reflecting whether the family owned versus rented their primary residence during each year of the participant's childhood. 2) Next, we examined whether life stress and health practices might explain the disparities in GR and TLR4 expression by including these factors before entering the own/rent variable during the identified critical period. 3) A final question concerned whether the genomic influence of early-life SES could be "undone" by upward mobility later in life. These analyses were also conducted as a series of hierarchical regressions. Variables included in each block were a) age and ethnicity; b) housing status during the identified critical period and an indicator of the family's current social standing (either housing status, automobile ownership, or perceived status); and c) an interaction term representing the product of housing during the critical period and one of the indicators of current social standing. We reasoned that if the influence of early social class can be undone, statistically significant interactions should be evident, indicating that mobility attenuates the impact of earlier SES on GR and TLR4 expression. For all statistical analyses, α was set to 0.05 and two-tailed tests of significance were performed. Values are expressed as mean \pm standard deviation unless otherwise noted.

RESULTS

The sample's demographic, behavioral, and biological characteristics are displayed in Table 1. The sample was on average from a mid-to-high SES background. About 75% of the young women came from families who owned their current residences and, in most cases, there was an automobile for each person in the house who could drive. Nonetheless, there was considerable variability in SES, especially in terms of educational background. About 24% of the participants' fathers had completed a high school education or less. Thirty percent had completed some college training, whereas 27% had a 4-year college degree and the other 19% had a master's or doctoral degree. The values were similar for participants'

TABLE 1. Characteristics of the Sample ($n = 136$)

Variable	Mean \pm SD or %
Age at study entry	16.76 \pm 1.63
Ethnic background (White/Asian/other)	48.10%/39.80%/12.00%
Age when family purchased first residence	1.90 \pm 3.51
Father/mother with 4-year degree	46%/37%
Glucocorticoid receptor mRNA (relative quantity)	3.73 \pm 1.89
Toll-like receptor 4 mRNA (relative quantity)	6.02 \pm 2.74
Family household ownership at study entry	$n = 110$; 80.90%
Automobile ownership (automobiles/drivers)	0.85 \pm 0.47
Perceived social standing of family (1–10)	6.68 \pm 1.20
Most severe life event past 6 months (1–5)	1.93 \pm 0.90
Chronic family stress past 6 months (1–5)	2.70 \pm 0.78
Chronic academic stress past 6 months (1–5)	2.12 \pm 0.88
Daily cigarette smoker (yes/no)	$n = 3$; 2.30%
Consumption of alcohol (drinks/week)	1.37 \pm 4.99
Adiposity-body mass index (kg/m^2)	21.71 \pm 2.77

mRNA = messenger ribonucleic acid.

mothers: 23% had completed a high school education or less, 40% had completed some college training, 26% had a 4-year college degree, and 11% had an advanced degree.

Does a Critical Period Exist?

We found statistically significant associations between GR in adolescence and family housing status when participants were 1 to 4 years old (Table 2 and Figure 1). Participants whose families owned homes during these years exhibited significantly higher quantities of GR mRNA than participants whose families rented. The peak effects were at ages 1 to 2 years, when SES explained 5.2% to 6.2% of the variance in GR, or 50% more variance than it did at other times of childhood. At age 5 years, the association weakened to a trend, and remained that way until the age of 9 years. From ages 10 to 13 years, the family housing status was no longer a significant predictor of future GR. For descriptive purposes, mean and standard error (SE) values at each age are displayed in Table 3.

Housing status at the ages of 0 to 1 year was unrelated to TLR4 mRNA in adolescence. However, ages 2 to 3 years emerged as a potentially critical period for SES, during which ownership related negatively to expression of this gene. Participants whose families owned homes during these years exhibited lower relative quantities of TLR4 mRNA than participants whose families rented. The peak effect occurred when participants were 2 years old—SES accounted for 4.9% of the variance in TLR4, or 53% more than the next highest period. By the time participants reached the age of 4 years, housing status no longer predicted expression of this gene, and this continued throughout the remainder of childhood, with age 7 years being the only exception.

To confirm our results, we reconducted these analyses, this time entering housing status for each year of childhood simultaneously. This approach evaluates whether SES at each age predicts GR and TLR4 in adolescence, independent of other

years of life. Because housing status is stable over time, this is a statistically conservative approach. Nevertheless, it provided further evidence of a critical period around the ages of 1 to 2 years. In analyses with GR mRNA as the outcome, home ownership when the participant was 2 years old was a marginal predictor (unstandardized regression coefficient (B) = 1.76; SE = 1.03; $p < .09$), whereas none of the other years approached statistical significance ($p > .46$). For TLR4, home ownership when the participant were 1 and 2 years old was negatively related to mRNA in adolescence (B = -2.22; SE = 1.12; $p < .05$; B = -2.97; SE = 1.47; $p < .05$, respectively.) Housing status during other years of life was unrelated to TLR4 ($p > .13$).

Housing status is an imperfect indicator of SES because even some highly educated individuals, like students in the midst of graduate training, may rent homes until they launch formal careers. To determine whether the effects of renting are abrogated under these circumstances, we conducted additional regressions in which the interaction between housing status and parental education at age 2 was modeled. The results of these analyses were not consistent with such a scenario (p for interaction terms $> .65$). However, parental education was stable over time in our sample, so the education variable reflected both previous and current family SES, which may have diluted its ability to emerge as a buffer.

What Are the Underlying Mechanisms?

Having found that early-life SES predicts expression of the GR and TLR4 genes in adolescence, we next examined whether life stress or health practices were more proximal determinants of this phenomenon. As shown in Table 4, the effects of early-life SES were not mediated through disparities in life stress at study entry. Participants whose families owned homes by age 2 years continued to exhibit significantly higher quantities of GR mRNA and lower quantities of TLR4 mRNA despite adjustment for life stress. A primary reason for this

TABLE 2. Hierarchical Regression of SES During Childhood and Expression of GR and TLR4 mRNA During Adolescence ($n = 136$)

Child's Age	%Rent/%Own	GR B	SE B	ΔR^2	p	TLR-4 B	SE B	ΔR^2	p
0	46/54	0.58	0.34	.020	<.10	-0.59	0.54	.010	<.28
1	35/65	0.99	0.36	.052	<.01	-0.82	0.49	.023	<.10
2	29/71	1.13	0.37	.062	<.01	-1.38	0.56	.049	<.02
3	25/75	0.89	0.41	.034	<.03	-1.17	0.56	.032	<.05
4	23/77	0.84	0.41	.030	<.04	-1.09	0.61	.026	<.08
5	20/80	0.76	0.44	.021	<.09	-1.15	0.66	.025	<.09
6	19/81	0.81	0.45	.023	<.08	-1.04	0.66	.020	<.12
7	18/82	0.91	0.47	.025	<.07	-1.35	0.68	.032	<.05
8	17/83	0.92	0.47	.027	<.06	-1.27	0.70	.027	<.07
9	16/84	1.03	0.50	.029	<.05	-0.99	0.75	.011	<.26
10	15/85	0.86	0.52	.020	<.10	-0.80	0.77	.009	<.31
11	13/87	0.78	0.55	.015	<.16	-0.67	0.82	.006	<.42
12	12/88	0.51	0.59	.005	<.39	-0.05	0.85	.001	<.96
13	8/92	0.09	0.72	.001	<.91	0.07	1.04	.001	<.95

GR = glucocorticoid receptor; TLR4 = toll-like receptor 4; mRNA = messenger ribonucleic acid; B = unstandardized regression coefficient; SE B = standard error of B; ΔR^2 = unique variance explained in outcome.

Values in table are from regression equations in which relative quantities of mRNA were predicted from age, ethnicity, social class at study entry, and whether the family owned versus rented their primary residence each year of the participant's childhood.

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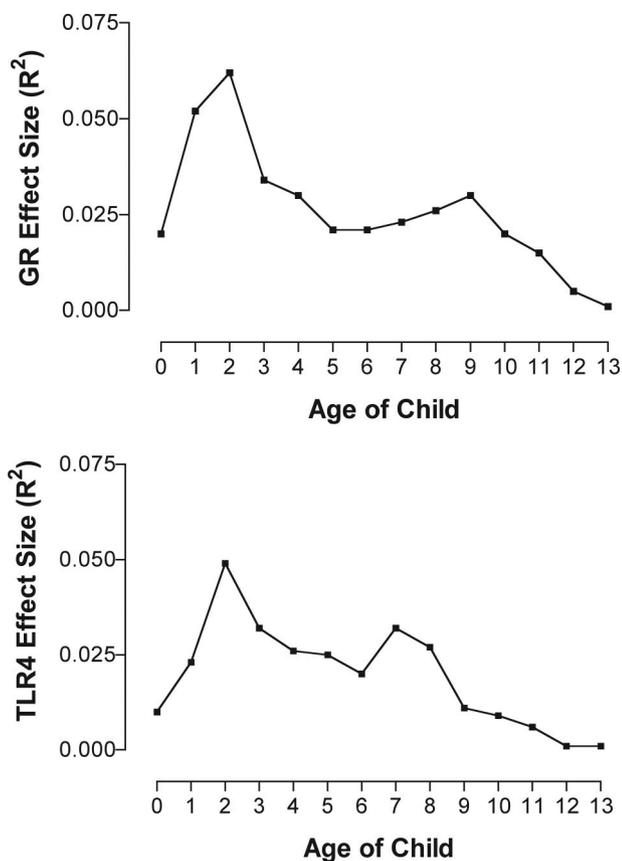


Figure 1. The impact of socioeconomic (SES) circumstances varies across childhood. Findings indicate that from the participants ages 1 to 10 years, there are statistically significant associations between housing status and future expression of glucocorticoid receptor mRNA. Participants whose families owned homes during these years express higher relative quantities of glucocorticoid receptor (GR) messenger ribonucleic acid (mRNA) during adolescence than participants whose families were renting (upper panel). For toll-like receptor 4 (TLR4), years 2 to 3 of life emerges as a critical period for SES. Participants whose families owned homes during these years express lower relative quantities of TLR4 mRNA during adolescence than participants whose families rented (lower panel). In both panels, the R^2 value on the y axis reflects the amount of variance in transcript uniquely explained by SES for that age, after statistical adjustment for age at study entry, self-reported ethnicity, and current economic circumstances.

may be that participants whose families owned versus rented when they were aged 2 years were similar in terms of current chronic and acute stress, $t < 1.00$; $p > .33$.

A similar pattern of findings emerged for health practices. Despite adjustments for cigarette smoking, alcohol consumption, and body mass, owing a home when the participant was aged 2 years continued to predict significantly higher GR mRNA and lower TLR4 mRNA. Again, this was likely a result of the fact that current cigarette smoking, alcohol consumption, and body mass were similar in participants whose families rented versus owned when the participants were aged 2 years, $t < 1.00$; $p > .83$.

Estimates of explained variance can be optimistic when the ratio of predictors to participants in a regression equation grows high. To evaluate whether this was occurring in our data, we reconducted the mediator analyses, this time including only a single mechanism in each equation. (This meant

seven predictors in each model, with a total of 136 participants in the sample.) These analyses yielded R^2 estimates for early SES that were nearly identical to those obtained in the more complex models presented in Table 4. Hence, it does not seem to be the case that the multiple predictors in these models were leading to upwardly biased estimates of the impact of early-life SES.

Can the Impact of Early-Life SES Be Undone?

The final analyses concerned whether the genomic influence of early social class could be “undone” by upward mobility later in life. The results of our analyses were inconsistent with this scenario. For GR mRNA, there was no evidence that SES at the age of 2 years interacted with current social standing, regardless of whether it was indexed by housing status, automobile ownership, or perceived family status. (Estimated statistics for these interaction terms were $B = -0.16$; $SE = 0.81$; $p > .85$; $B = 0.37$; $SE = 0.38$; $p > .34$; and $B = 0.10$; $SE = 0.38$; $p > .76$, respectively.) A similar pattern of findings was evident for TLR4 mRNA; current social standing did not significantly interact with housing status at the age of 2 years. (The estimated statistics for the interaction terms were $B = -0.13$; $SE = 1.15$; $p > .91$; $B = 0.02$; $SE = 0.54$; $p > .98$; and $B = 0.55$; $SE = 0.47$; $p > .24$, respectively.) Representative examples of these findings are displayed in Figure 2.

DISCUSSION

Despite evidence that early-life SES contributes to morbidity and mortality in adulthood, little is known about the biological mechanisms that are responsible for this phenomenon. This project examined whether SES in early life predicts future activity of two genes involved in regulating inflammation. Its findings suggest that, to the extent that a child is raised in a higher SES environment, she will exhibit greater quantities of GR mRNA and lower quantities of TLR4 mRNA during adolescence, regardless of what her family’s economic circumstances are currently. These findings provide some of the first evidence that in human beings, early-life social exposures can program future activity of biological systems, and do so at the level of the genome. Furthermore, these findings suggest biological mechanisms for the epidemiologic data linking unfavorable economic circumstances in early life with heightened vulnerability to respiratory, infectious, and cardiovascular morbidity in adulthood (3,4).

Critical-period analyses demonstrated that the importance of SES varied across childhood. The strongest evidence of a critical period emerged for age 2 years, when SES predicted increased GR and decreased TLR4 mRNA, with effect sizes that were markedly greater relative to other epochs (25% to 5000%). This pattern of findings is consistent with previous research on the impact of childhood SES, which shows that family SES at this age is a powerful determinant of vulnerability to respiratory infection in adulthood (3). Children’s immune systems mature during the first 6 to 12 months of life, and in the years that follow, patterns of responsivity become

TABLE 3. Descriptive Statistics for GR and TLR-4 mRNA According to Housing Status Each Year of Childhood ($n = 136$)

Child's Age	GR				TLR4			
	Owners, Mean	Owners, SE	Renters, Mean	Renters, SE	Owners, Mean	Owners, SE	Renters, Mean	Renters, SE
0	3.99	0.22	3.42	0.24	5.73	0.29	6.05	0.34
1	4.06	0.20	3.03	0.28	5.52	0.31	6.21	0.41
2	4.05	0.19	2.90	0.31	5.58	0.27	6.50	0.44
3	3.94	0.19	3.05	0.34	5.66	0.26	6.39	0.49
4	3.92	0.18	3.08	0.36	5.70	0.26	6.30	0.50
5	3.88	0.18	3.12	0.39	5.69	0.26	6.42	0.54
6	3.88	0.18	3.08	0.39	5.72	0.25	6.34	0.55
7	3.89	0.18	2.97	0.41	5.66	0.25	6.58	0.56
8	3.88	0.18	2.97	0.43	5.68	0.25	6.50	0.57
9	3.89	0.18	2.95	0.42	5.73	0.25	6.42	0.64
10	3.85	0.18	2.99	0.47	5.75	0.25	6.37	0.66
11	3.83	0.18	3.05	0.50	5.78	0.25	6.24	0.70
12	3.79	0.18	3.28	0.54	5.83	0.25	5.94	0.76
13	3.73	0.17	3.65	0.58	5.85	0.25	5.66	0.96

GR = glucocorticoid receptor; TLR4 = toll-like receptor 4; mRNA = messenger ribonucleic acid; SE = standard error.

Values are estimated marginal means from analysis of variance after controlling for age, ethnicity, and social class at study entry.

TABLE 4. Pathways Linking SES During Early Life and GR and TLR-4 Expression During Adolescence ($n = 136$)

	GR				TLR4			
	B	SE B	ΔR^2	p	B	SE B	ΔR^2	p
Model 1—current SES								
Age	0.17	0.15	.017	<.12	-0.32	0.15	.037	<.04
Race	0.31	0.25	.011	<.22	-0.82	0.35	.044	<.03
Current housing status	0.12	0.43	.001	<.78	-0.53	0.61	.006	<.40
Automobile ownership	-0.41	0.17	.030	<.02	0.50	0.24	.036	<.04
Perceived family status	0.16	0.16	.007	<.31	-0.22	0.22	.008	<.32
Home ownership, age 2 years	1.13	0.37	.062	<.01	-1.38	0.56	.049	<.02
Cumulative model			.132	<.01			.125	<.01
Model 2—life stress								
Age	0.17	0.11	.017	<.12	-0.35	0.15	.044	<.03
Race	0.27	0.26	.008	<.30	-0.78	0.36	.040	<.04
Current housing status	0.10	0.43	.001	<.82	-0.57	0.61	.007	<.36
Automobile ownership	-0.40	0.17	.039	<.02	0.44	0.24	.029	<.07
Perceived family status	0.20	0.16	.011	<.21	-0.31	0.23	.016	<.18
Major life event	0.06	0.19	.001	<.74	-0.43	0.27	.022	<.12
Chronic family stress	0.30	0.22	.012	<.18	-0.20	0.32	.003	<.58
Chronic academic stress	0.16	0.21	.001	<.43	-0.23	0.28	.006	<.40
Home ownership, age 2 years	1.11	0.37	.060	<.01	-1.13	0.55	.036	<.05
Cumulative model			.148	<.01			.145	<.01
Model 3—health practices								
Age	0.17	0.11	.017	<.12	-0.33	0.15	.040	<.04
Race	0.27	0.26	.008	<.30	-0.73	0.37	.033	<.05
Current housing status	0.10	0.43	.001	<.82	-0.45	0.62	.005	<.47
Automobile ownership	-0.39	0.17	.037	<.03	0.49	0.24	.035	<.05
Perceived family status	0.14	0.16	.005	<.38	-0.21	0.22	.008	<.35
Body mass index	-0.03	0.06	.001	<.66	0.03	0.08	.001	<.74
Cigarette smoking	0.54	0.85	.003	<.53	-1.30	1.54	.006	<.41
Alcohol consumption	-0.04	0.03	.009	<.26	0.04	0.04	.007	<.37
Home ownership, age 2 years	1.12	0.38	.061	<.01	-1.25	0.55	.043	<.03
Cumulative model			.145	<.02			.179	<.01

SES = socioeconomic status; GR = glucocorticoid receptor; TLR4 = toll-like receptor 4; B = unstandardized regression coefficient; SE B = standard error of B; ΔR^2 = unique variance explained in outcome.

established through microbial and allergic exposures (18,19). Our data suggest that low SES operates most potently during this period of immune-system priming, and we speculate that

it does so in a way that favors the emergence of a proinflammatory phenotype. To the extent that a proclivity toward inflammation persists over the lifespan, it would increase

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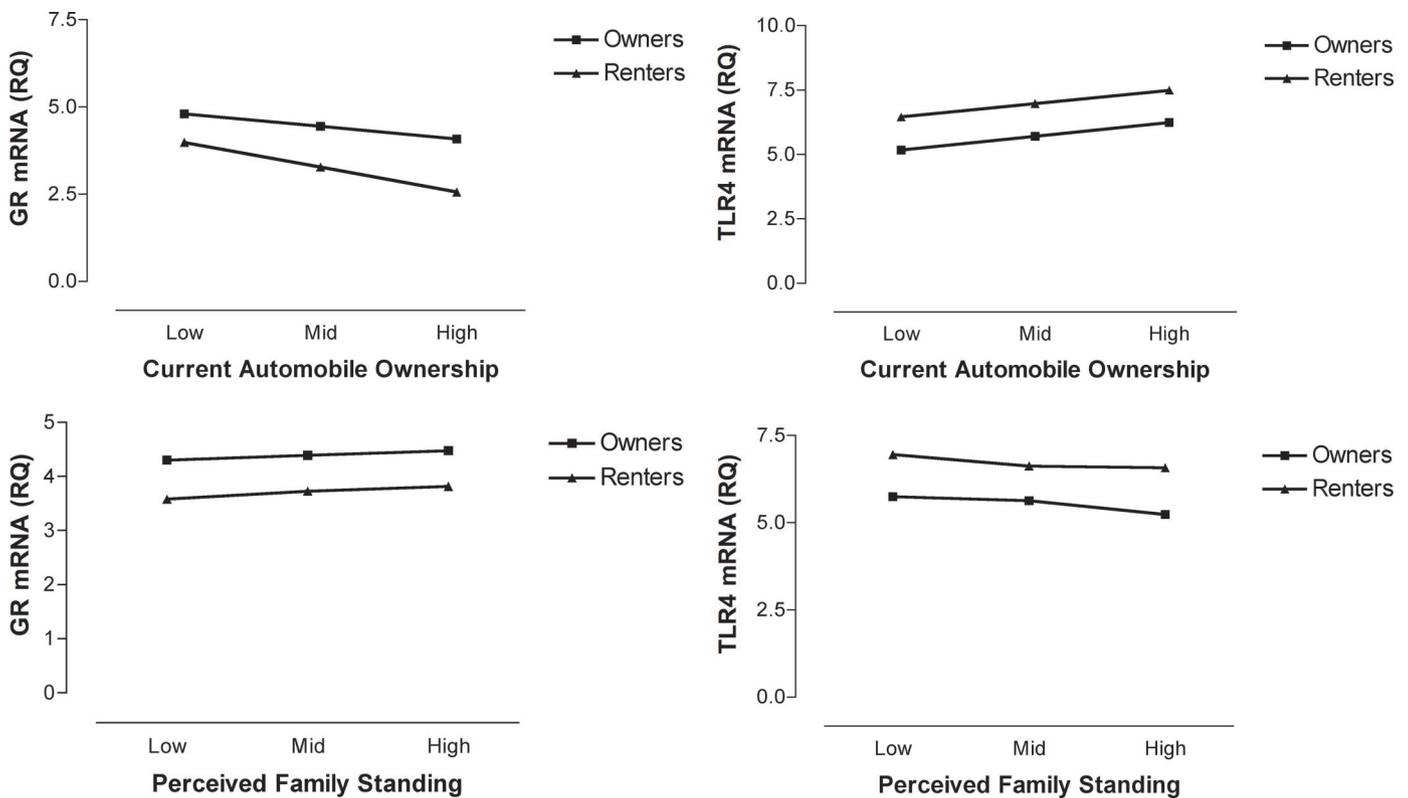


Figure 2. The genomic influence of early-life socioeconomic status (SES) is not “undone” by later mobility in a person’s economic circumstances. Participants whose families owned their homes when they were aged 2 years showed higher relative quantities of glucocorticoid receptor (GR) messenger ribonucleic acid (mRNA) during adolescence than participants whose families rented at that time. These disparities persist regardless of current family SES, as indexed by ownership of automobiles (top left) and perceived family standing (bottom left). An identical pattern is evident for expression of toll-like receptor 4 mRNA, suggesting the influence of early-life SES on this gene cannot be undone. This is true regardless of whether current family standing is measured as ownership of automobiles (top right) or perceived family standing (bottom right). RQ = relative quantity of mRNA.

morbidity and mortality due to diseases involving dysregulation of inflammation such as chronic obstructive pulmonary disease and manifestations of cardiovascular disease (8,20,21).

The TLR4 and GR genes are centrally involved in regulating inflammation. TLR4 is a pattern-recognition molecule expressed primarily by monocytes and serves as a coreceptor for lipopolysaccharide (LPS) on the surface of Gram-negative bacteria (22). Persons who express greater quantities of TLR4 are vulnerable to conditions involving excessive inflammation, as seen by the increased prevalence of carotid atherosclerosis among those with the Asp299Gly wild-type, which amplifies receptor signaling and accentuates cytokine responses to LPS (23). The TLR4 activities are counterregulated through ligation of the GR. Inflammatory cytokines activate the hypothalamo-pituitary-adrenal axis, leading to cortisol secretion (24), which binds to GR; this complex regulates the inflammatory response by inhibiting activities of the transcription factor nuclear factor- κ B. When GR signaling in leukocytes becomes deficient, NF κ B activity flourishes, leading to prolonged and excessive immune responses as well as vulnerability to inflammatory conditions (25,26). These observations suggest that a phenotype comprised of increased TLR4 and decreased GR would display exaggerated inflammatory responses when exposed to infectious or traumatic stimuli.

Further analyses indicated that early-SES findings were not mediated through disparities in participants’ current social standing, recent exposure to life stress, or smoking, drinking, and adiposity. Despite the fact that some families experienced upward social mobility across childhood, improving economic conditions did not appear to attenuate the genomic impact of early SES. If early-life SES did not operate by sorting participants into their current environmental circumstances, then how might it have influenced expression of the TLR4 and GR genes many years into the future? We speculate that the early childhood represents a sensitive period during which social and physical exposures become embedded in a person’s biology (2,6,7). Although the mechanisms underlying this embedding process have not yet been established, an intriguing program of research in animals suggests that epigenetic modifications could be responsible (13,14). These are stable changes in the pattern of gene expression, brought about through methylation of DNA and acetylation of histone proteins, that can arise early in life and persist through adulthood. Epigenetic processes represent a critical pathway through which genomic activities are regulated by environmental circumstances (27). We argue that children who grow up in unfavorable SES environments are exposed to detrimental social and physical conditions, such as unstable, conflictual

families, suboptimal childcare, neighborhood violence, residential crowding, inadequate nutrition, infectious diseases, chemical pollutants, parental smoking, and other toxins. These social and physical “pollutants” may get biologically embedded through epigenetic mechanisms and result in long-lasting changes in genomic activity that are difficult (or impossible) to reverse. In future research, this hypothesis should be evaluated by measuring the extent of cytosine methylation in the promoters of the GR and TLR4 genes (or the way in which these genes are packaged in nucleosomes, which can facilitate or suppress transcription of mRNA).

Several important limitations of this study must be recognized. Because reports of early-life SES were collected retrospectively, our findings could have been shaped by distorted recall. We view this as unlikely because home ownership is salient enough that most people report it accurately in hindsight (3). Distorted reports would also increase random error and, in doing so, reduce the amount of statistical power to detect associations. Nonetheless, there are limitations to using home ownership as a measure of SES, including the categorical nature of this variable and the possibility that the meaning of home ownership varies across families who come from different regions or cultures. However, other traditional measures of SES, such as parent education and family income, were not appropriate as lifetime SES measures. Parent education has a high degree of stability over time and would not have allowed us to disentangle effects of SES at different periods of a child’s life. Family income presents recall challenges, in that parents find it difficult to accurately report on income each year over a 20-year period.

Because of the observational and retrospective design of this study, we cannot ascertain whether disparities in SES actually triggered disparities in gene expression. Unmeasured variables may have contributed to this pattern of associations, and patterns of genomic activity may have predated disparities in social standing. Even if early-life SES did operate in causal fashion, our study was not optimally designed for identifying the critical period at which biological embedding occurred. Another important limitation of the study is the small number of participants enrolled. Special caution is warranted in interpreting our findings during later childhood and early adolescence, as only a small fraction of the families were renting their homes during this time. Thus, even small changes in the composition of the renter cohort could result in pronounced statistical differences. The small cohort also may have limited our power to detect “undoing” effects; however, plots of these findings provide little indication that such effects are present. Finally, because the sample consisted entirely of young women, we cannot be certain whether its findings generalize to men. Future research must evaluate this issue.

To overcome these limitations and substantiate the conclusions of our work, future projects with more rigorous methodology will be necessary. Ideally, these projects will be multiwave, prospective, birth-cohort studies in which potential confounders and mediators are exhaustively measured. Our study was limited in this respect, especially with regard to

chemical, nutritional, allergic, and infectious exposures. It also will be important for this research to incorporate serologic and functional indicators of inflammation, because although our findings suggest that low SES favors the emergence of an inflammatory phenotype, we do not have the immunologic functioning data to substantiate this claim.

Despite all these limitations, this study offers novel insights into why SES during the early years of life is an important determinant of later respiratory and cardiovascular outcomes. Previous research exploring mechanisms has linked unfavorable childhood economic circumstances with diminished pulmonary function and symptoms of metabolic syndrome (28–30). The current findings build on this work in several important respects. First, they identify a critical period (ages 2–3 years) during which social standing appears to influence the long-term function of biological systems. Second, they suggest that these effects cannot be “undone” by upward mobility in social and economic spheres. Finally, they provide some of the first empirical evidence in humans that childhood SES may produce biological embedding that occurs at the level of the genome. With further research, scientists may soon be able to identify the mechanisms underlying this embedding process in humans and articulate their consequences for long-term patterns of morbidity and mortality.

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