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Transcriptomic analyses of black women in neighborhoods with high levels of violence

Meggan J. Lee[a], **Clare C. Rittschof**[b], **Andrew J. Greenlee**[c], **Kedir N. Turi**[d], **Sandra L. Rodriguez-Zas**[e], **Gene E. Robinson**[f], **Steven W. Cole**[g], **Ruby Mendenhall**[a,h,*]

[a]Department of Sociology, University of Illinois, Urbana-Champaign, 702 South Wright St, Champaign, IL 61820, USA

[b]Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, KY 40546, USA

[c]Department of Urban and Regional Planning, University of Illinois, Urbana-Champaign, 611 Taft Drive, Champaign, IL 61820, USA

[d]Center for Asthma Research, Vanderbilt University School of Medicine, 1215 21ts Ave South, 6000 Medical Center East, North Tower, Nashville, TN 37232, USA

[e]Department of Animal Sciences, University of Illinois, Urbana-Champaign, 306 Animal Sciences Laboratory, 1207W. Gregory Dr, Urbana, IL 61801, USA

[f]Department of Entomology, Neuroscience Program and Carl R. Woese Institute for Genomic, Biology, University of Illinois, Urbana-Champaign, 1206W. Gregory Dr, Urbana, IL 61801, USA

[g]Department of Psychiatry & Biobehavioral Sciences and Medicine, UCLA School of Medicine, 11-934 Factor Building, Los Angeles, CA 90095, USA

[h]Department of African American Studies, Carle Illinois College of Medicine, 702S. Wright Ave, Urbana, IL 61822, USA

Abstract

Chronic stress threatens an individual's capacity to maintain psychological and physiological homeostasis, but the molecular processes underlying the biological embedding of these experiences are not well understood. This is particularly true for marginalized groups, presenting a fundamental challenge to decreasing racial, economic, and gender-based health disparities. Physical and social environments influence genome function, including the transcriptional activity of core stress responsive genes. We studied the relationship between social experiences that are associated with systemic inequality (e.g., racial segregation, poverty, and neighborhood violence) and blood cell (leukocytes) gene expression, focusing on the activation of transcription factors (TF) critical to stress response pathways. The study used data from 68 women collected from

*Corresponding author at: Department of Sociology, University of Illinois, Urbana-Champaign, 702 South Wright St, Champaign, IL 61820, USA. rubymen@illinois.edu (R. Mendenhall).

Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105174.

a convenience sample in 2013 from the Southside of Chicago. Comparing single, low-income Black mothers living in neighborhoods with high levels of violence (self-reported and assessed using administrative police records) to those with low levels of violence we found no significant differences in expression of 51 genes associated with the Conserved Transcriptional Response to Adversity (CTRA). Using TELiS analysis of promoter TF-binding motif prevalence we found that mothers who self-reported higher levels of neighborhood stress showed greater expression of genes regulated by the glucocorticoid receptor (GR). These findings may reflect increased cortisol output from the hypothalamic-pituitary-adrenal (HPA) axis, or increased GR transcriptional sensitivity. Transcript origin analyses identified monocytes and dendritic cells as the primary cellular sources of gene transcripts up-regulated in association with neighborhood stress. The prominence of GR-related transcripts and the absence of sympathetic nervous system-related CTRA transcripts suggest that a subjective perception of elevated chronic neighborhood stress may be associated with an HPA-related defeat-withdrawal phenotype rather than a fight-or-flight phenotype. The defeat-withdrawal phenotype has been previously observed in animal models of severe, overwhelming threat. These results demonstrate the importance of studying biological embedding in diverse environments and communities, specifically marginalized populations such as low-income Black women.

Keywords

Subjective neighborhood stress; Glucocorticoid receptors; Sociogenomics; Structural inequality; Neighborhood violence; Conserved transcriptional response to adversity; African American women; Conservation/withdrawal response

1. Introduction

Stress and trauma are risk factors for a wide variety of illnesses. For example, maternal stress, diet, and infection during pregnancy are associated with increased prevalence of neurodevelopmental and psychiatric disorders in children (Bale et al., 2010). Similarly, poverty and maltreatment during childhood increase risk of cardiovascular disease, autoimmune and metabolic disorders, and depression (Danese et al., 2009; Miller et al., 2011). Health effects of stress are also observed throughout adulthood; for example, adults experiencing stressful life events are more likely to express common cold symptoms (Cohen et al., 2012). Even subtle social conditions, like the absence of adequate support networks (Williams and Sternthal, 2010), can become stressors that impact health. Negative health outcomes are particularly prevalent when stressful social conditions are experienced in combination with other factors that arise from systemic inequality, e.g., limited access to health care, or experiences of racial discrimination (Cole, 2014; Cole et al., 2015b; Robinson and Whitfield, 2005; Thames et al., 2019). This pattern increases the vulnerability of marginalized groups to mental and physical illnesses (Williams and Sternthal, 2010).

Recent studies in humans and other primates have investigated the relationship between social inequality and the physiological pathways that link stress to disease (reviewed in Snyder-Mackler et al. (2020)). For example, in female rhesus macaques, low social status, a condition characterized by elevated harassment and a lack of social support,

is associated with a dysregulated stress response, including changes in immune cell gene expression and chromatin accessibility (Snyder-Mackler et al., 2019). Similarly, in humans, low socioeconomic status (SES) during childhood has lifelong impacts on the glucocorticoid-influenced anti-inflammatory actions of the immune system (Miller et al. 2009). Investigating how experiences related to social inequality impact immune system function can help identify the specific factors that underpin widespread racial, economic, and gender-based health disparities (Williams and Collins, 2001); this approach is an important step towards reducing these disparities (Crenshaw, 1991; American Psychological Association, 2017).

Racialized residential segregation is one source of systemic inequality that contributes to chronic stress and health disparities in minority communities (Massey and Denton, 1988; Williams and Collins, 2001; South and Deane, 1993; Williams and Jackson, 2005). There are many complex factors that give rise to these outcomes, including elevated exposure to pollutants and limited access to resources including health care (Williams and Collins, 2001). However, one unique social stressor faced by individuals in some low-income and predominantly Black communities is a high rate of neighborhood violence often related to disinvestment in communities (Dahlberg and Mercy, 2009; D'Onofrio and Wall, 2020; Jenkins, 2002; Rodriguez et al., 2015). Violent social conditions directly elevate mortality rates (Rodriguez et al., 2015), but we hypothesize that they could also have indirect health effects, operating through stress response mechanisms that predispose vulnerable individuals to disease. Under this framework, individuals disproportionately exposed to violence and those more likely to perceive their neighborhood conditions as exceptionally stressful will be particularly at risk (Fowler et al., 2009; Kang, 2016; Meyer et al., 2014).

Individuals in social roles that are demanding (e.g. mothers) are especially vulnerable to conditions associated with systemic inequality including community violence (Ross, 2016). These individuals may be particularly prone to the negative health outcomes of stress (Miller et al. 2008; Miller et al. 2014). Women are often in high-demand social roles, and in low-income Black communities, they are uniquely burdened by providing financial resources to their families, helping other family members in their social networks, and providing care to an ill or disabled spouse (Mendenhall et al., 2013; Lee et al. 2003). The impacts of these stressors appear to be enhanced by women's reactions to and perceptions of their circumstances in these communities (e.g., feelings of discouragement and self-blame) (Williams et al., 2003;Legewie, 2019). In neighborhoods with high levels of violence, Black women face the additional high-level stressor of protecting their children from imminent harm (Mendenhall et al., 2013). This combination of factors could explain why Black women show marked health disparities compared to their White counterparts, including substantially higher cancer and maternal-infant mortality rates (Roberts, 2011).

In this study, we evaluated the relationship between neighborhood violence and stress physiology in Black women. We predicted that experiences of violence are associated with patterns of immune function that could give rise to poor health outcomes. We focused our study in the predominantly Black south side neighborhoods of Chicago, which have unusually high rates of gun violence (Luhby, 2016; Crenshaw, 1991; Roberts, 2011), and elevated prevalence of cancer and diabetes relative to the rest of the city

(Chicago Department of Public Health, 2013; Professional Research Consultants, 2012). To quantify experiences of violence and stress, we surveyed Black women to evaluate perceived (i.e., subjective) experiences. We also used crime reports and the prevalence of abandoned buildings and empty lots in women's neighborhoods as objective indicators of neighborhood violence and stress. To assess immune system function, we performed blood cell transcriptomic analyses to determine if violence is correlated with differential activation of biological pathways that may impact disease risk (Carrasco and Van de Kar, 2003). Specifically, we evaluated patterns of immune related gene expression in leukocytes, a metric that is increasingly associated with psychosocial and environmental stressors (Brown et al., 2019; Cole, 2010; Cole et al., 2009; Miller et al., 2008; Snyder-Mackler et al., 2019; Thames et al., 2019). In keeping with the similar transcriptomic studies referenced above, we used differentially expressed genes as inputs into higher-order set-based bioinformatics analyses designed to test a small number of specific a priori hypotheses. It is important to note that this study was neither designed nor sufficiently powered to detect statistically reliable associations between stress and the transcript abundance for specific individual genes.

Based on previous studies of human stress genomics, we used bio-informatics approaches to evaluate two major hypotheses (Cole, 2013). The first hypothesis was that neighborhood stress is correlated with variation in the expression of genes previously characterized as the Conserved Transcriptional Response to Adversity (CTRA). The second hypothesis was that neighborhood stress associates with differences in the activity of inflammation-related transcription factors (TFs) including the pro-inflammatory nuclear factor kappa beta and Rel (NF-kB/Rel) family of TFs and the anti-inflammatory glucocorticoid receptor (GR). We anticipated that there may be some differences in the transcriptional correlates of subjective and objective stress measurements in light of previous studies investigating stress as a function of social isolation and social status (Cole et al., 2015a). For example, it is possible that subjective perceptions operate through stress-induced neuroendocrine responses whereas objective conditions may involve imperceptible physicochemical or microbial mechanisms (Cole, 2013). We interpret the bioinformatics results in light of the potential psychological and physiological mechanisms that link the social environment and systemic inequality to racial, economic, and gender health disparities. This study contributes to a growing body of literature that integrates social science and stress biology with the goal of determining how human social and cultural conditions impact health.

2. Materials and methods

2.1. Participants

The data used in this study are part of a larger study called The South Chicago Black Mothers' Resiliency Project. We recruited unmarried, low-income Black mothers (fluent in English) raising children under the age of 18 years in neighborhoods with high levels of violence (based on crime data) on the Southside of Chicago. There were several grandmothers who participated that do not currently have children under 18, but who are raising their grandchildren who are under 18. We included grandmothers because they often function as the primary caretakers. We refer to grandmothers as "mothers" throughout

this manuscript because they experience similar stress as the mothers when trying to keep children safe.

None of the women were currently lactating or were within eight weeks postpartum. Women who reported cardiac disease, cancer, respiratory conditions, autoimmune disorders, diabetes, or obesity were excluded from the study because these health conditions could possibly affect immune cell transcriptomes.

2.2. Recruitment

Due to an extensive history of exploitation and abuse, Black women are often reluctant to participate in research studies, especially those that require giving a biological sample like blood. To maximize recruitment, this study was conducted by Black researchers, and we paid $40 to all participants, as these two elements are known to improve study participation (Mendenhall et al., 2020).

2.3. Data collection

We recruited 93 total participants of variable age, education level, employment status, number of children, and pregnancy status. Informed consent was obtained from all participants. We limited our analyses to 68 women from whom we also obtained blood cell transcriptomic data (see Table 1 for Descriptive Statistics). Data were collected on Fridays (9 a.m. – 4 p.m.) and Saturdays (10 a.m. – 3:30 p.m.) over a three-month period in the fall of 2013. The mothers completed four psychosocial scales: the Patient Health Questionnaire, PHQ- 9 (Kroenke et al., 2002), Agricultural Coping Systems Inventory (Utsey et al., 2000), the Chronic Stress Scale (PhenxToolkit, 2011) and the PTSD Checklist (PCL-Civilian) (National Center for PTSD, 2015). They also completed a 15-page paper and pencil survey which included demographic and social support information, child and adult trauma, neighborhood safety, and stress and coping.

For objective measures of neighborhood conditions, we obtained crime data from publicly available reports published in the City of Chicago Public Safety Crime database (“City of Chicago | Data Portal,” 2016). We included the location and type of all property crimes and violent crimes responded to by the Chicago police throughout 2013. We used the Federal Bureau of Investigation (FBI) definition of violent crime, which includes five types of crime that are committed directly against a person: homicide (first- or second-degree murder), sexual assault, robbery, aggravated assault, and aggravated battery. The addresses in the dataset are shown at the block level to protect the privacy of the victims of these crimes. We used ArcGIS and software from the Environmental Systems Research Institute to map crimes within a four-block radius of the participant’s address. We used Google Street View photos, which were generally taken 12–15 months before the study, to quantify neighborhood conditions on the participants’ residential block.

2.4. Measurement tools

Responses from the PTSD Checklist (PCL-Civilian) (National Center for PTSD, 2015) as well as the Patient Health Questionnaire (PHQ- 9) (Kroenke et al., 2001) are included in the current study. The subjective stress measures were informed by a focus group study where

families discussed neighborhood violence, stress and coping. Neighborhood spatial analysis was conducted using geographical information system mapping (GIS).

2.4.1. Demographic variables—Nine demographic variables were included in the study (Table 1). Participants' were asked to provide their age, their years of education, and how many children under the age of 18 they currently have in their homes. Participants were also asked if they were employed at the time of the study or not (employment status), and if they were pregnant at the time of the study or not. Participants were asked about their current education status, referring to whether or not they were actively taking courses in pursuit of additional education opportunities. Participants were asked about their current financial stress, i.e., if they currently find it difficult to pay their bills every month. All participants reported their household income as below the federal poverty line. The responses to the PTSD checklist were used to determine whether or not the participants had a score that indicates that they have PTSD, which we incorporated as a final demographic variable.

2.5. Metrics of stress

Because biological impacts of stress are correlated with individual stress perception (Cole et al., 2014), we devised two ways to describe social violence in neighborhoods: a subjective measure of participants' perceived experiences (hereafter "subjective stress"), and an objective measure of neighborhood factors (hereafter "objective stress"). For both measures, higher scores indicate greater stress.

2.5.1. Subjective stress—Based on conversations with community members and focus groups (Following Minkler et al. (2003) and Israel et al. (1998), we created a measure of subjective stress based on two scores derived from the above surveys (Table 2). The first score reflects a participant's perception of neighborhood violence ("Perceived Neighborhood Violence Score", PNVS) and the second reflects their self-reported stress response to environmental conditions (the "Environmental Conditions Stress Score", ECSS). For the PNVS, we summed a response for each question on a 1–4 scale for each participant. We took a similar approach for the ECSS; participant answers could range from 1 to 4. The Z-scores for the PNVS and ECSS were then summed to form the subjective stress score for each participant. We used the Z-score because it reflects complex and multidimensional nature of the neighborhood perception (Caldwell et al., 2019).

2.5.2. Objective stress—The objective stress score also combined two measures, the "Neighborhood Crime Score" (NCS) and the "Block Deprivation Score" (BDS). The latter was included because when discussing violent crime in their neighborhoods, participants associate violence with empty abandoned space, like abandoned homes or lots. Using publicly available crime reports (see Section 2.3 for description of data collection), we tallied the number of crimes (all crimes were given equal weight) to create the neighborhood crime score (NCS). For the BDS, we quantified neighborhood conditions on the participants' residential block (see Section 2.3). The BDS ranged from zero (no abandoned buildings and no empty lots) to nine. The participants discussed the NCS and BDS as similar threats therefore we combined the five variables from the NCS to the BDS to generate one stress

measure. After standardization, the Z-scores for the NCS and the BDS were summed to create the objective stress score.

2.6. Blood sampling and gene expression microarrays

We collected blood from the participants for microarray transcriptomic measurements using PAXgene tubes. All of the 93 women interviewed agreed to give blood samples, samples were obtained from 73 (77%), and we obtained usable data from 68 (see below). Blood samples were stored in a biosafety level 2 lab freezer (−20 °C) and total RNA was extracted from leukocytes using the PAXgene RNA blood kit at the University of Illinois at Urbana-Champaign. The samples were tested for suitable mass (PicoGreen assay) and quality (Agilent TapeStation). 69 samples yielded RNA of adequate mass (> 200 ng; achieved range 324–4914 ng), purity (A260/A280 > 1.8; range 1.9–2.7), and integrity (RNA integrity number > 7; range 7.5–9.2) for downstream analyses. Those samples were shipped overnight to the University of California, Los Angeles (UCLA) Social Genomics Core Laboratory. Samples were analyzed by microarray assays conducted in the UCLA Neuroscience Genomics Core Laboratory following previously published protocols (Fredrickson et al., 2013). Briefly, RNA was converted to fluorescently labeled cRNA (Ambion TotalPrep) and hybridized to Illumina HT-12 v4 bead arrays following the manufacturer's standard protocol. Fluorescence images were scanned with an Illumina iScan instrument and subsequently converted to estimates of relative RNA abundance using Illumina GenomeStudio software with quantile normalization. One sample yielded sub-optimal hybridization intensity and was removed from all subsequent analyses, leaving 68 transcriptome profiles available for analysis.

2.7. Statistical analysis

Gene expression data were $\log_2$-transformed for analysis using linear models (with standard ordinary least squares linear statistical model analyses implemented using the Java JAMA matrix algebra package). These models assessed the relationship between transcript abundance and the summed Z-score measures of subjective and objective stress. Both stress measures were incorporated as covariates in a single model that also controlled for the covariates of age, number of children, pregnancy status, years of education, current employment status, current education status, and current financial stress. Results with a p-value less than 0.05 were considered statistically significant. Post hoc tests examined each stress variable in isolation, adjusting for covariates.

Two primary hypotheses were tested. The first was that neighborhood stress associates with variation in the expression of genes previously characterized as the Conserved Transcriptional Response to Adversity (CTRA). The CTRA is characterized by increased expression of pro-inflammatory genes and decreased expression of antiviral response genes and genes associated with IgG antibody synthesis in blood cells. Previous studies have associated CTRA expression profiles with a variety of contexts for psychosocial and environmental stressors in human and animal models, including variation in socioeconomic status, social threat, and low social status (Cole, 2014). Following previous studies (Cole et al., 2015), we tested an association between the expression levels of a pre-specified set of 53

CTRA indicator genes and stress measures using a maximum likelihood approach (Cole et al., 2015b; Fredrickson et al., 2013).

The second primary hypothesis involved the possibility that neighborhood stress might associate with differences in the activity of inflammation-related transcription factors (TFs) including the pro-inflammatory nuclear factor kappa beta and Rel (NF-kB/Rel) family of TFs and the anti-inflammatory glucocorticoid receptor (GR). For analyses of TF activity, we applied TELiS analysis of promoter TF-binding motif prevalence (Cole et al., 2005) to consensus human genome core promoter sequences for all genes with a predicted difference of > 1.25 fold in average expression over a 4-SD range of variation in stress. The fold difference was estimated from the covariate-adjusted linear statistical model analysis model described in the first paragraph of Section 2.5 (i.e., covariate-adjusted fold-difference). It was not estimated from a *t*-test (i.e., covariate unadjusted). These analyses compared prevalence of TF-binding motifs (TRANSFAC position-specific weight matrices V $NFKAPPAB65_01 and V$GR_Q6) in promoter sequences of the genes showing expression variation (as defined above). Because our gene expression analyses evaluate pre-specified hypotheses and do not reflect statistical testing of individual genes, no multiple testing corrections are included in the results presentation (Cao and Zhang, 2014).

Human blood contains diverse cell types with different immune functional implications. In a secondary analysis, we evaluated the cell population origins of the transcriptional changes associated with variation in stress using a Transcript Origin Analyses (TOA) (Cole et al., 2011). This analysis tests whether the transcripts up-regulated in association with neighborhood stress derive predominately from monocytes in general (and more specifically from CD16-vs. CD16- sub-populations), and whether the down-regulated transcripts derive predominately from B lymphocytes. These populations have been associated with the CTRA in previous studies (Fredrickson et al., 2013). In TELiS and TOA analyses, statistical testing was based on standard errors derived from 200 cycles of bootstrap sampling of correlated residual vectors (i.e., controlling for any potential correlation among genes; Efron and Tibshirani, 1994).

3. Results

3.1. Subjective and objective stress scores

All of the Black mothers participating in this study were living below the poverty line in the generally adverse social environment of South-side Chicago, but they varied markedly in their subjective and objective stress scores. Subjective and objective stress were uncorrelated ($r = -0.12$, $p = .345$) which underscores the contribution of individual experiences and perceptual processes to the personal experience of adverse socio-environmental conditions. Objective stress showed modest positive correlations with years of education and current educational status, but was unrelated to current financial stress, employment status, or other covariates. Subjective stress was not notably correlated with any of the demographic or socio- economic covariates selected a priori for control in statistical models (all $r < 0.20$).

3.2. Conserved transcriptional response to adversity gene expression

To determine whether subjective or objective neighborhood stress conditions might potentially trigger the CTRA gene expression profile previously observed in blood cell (leukocyte) samples from other studies of adverse social conditions, we tested the association of stress measures with an a priori specified 53-gene CTRA indicator contrast involving 19 pro-inflammatory gene transcripts (positively weighted) and 34 interferon- and antibody-related gene transcripts (negatively weighted to reflect their inverse contribution to the CTRA profile), while controlling for potentially confounding effects of age, number of children, current pregnancy, years of education, current employment status and current education status. Results showed that CTRA gene expression was not significantly associated with objective stress scores (mean −0.011 ± SE 0.035 $\log_2$ RNA units / SD of objective neighborhood stress, p = .754) or subjective stress scores (−0.010 ± 0.020, p = .632).

3.3. Transcription factor activity

To determine whether subjective or objective neighborhood stress conditions modulate pro-inflammatory (NF-kB) or anti-inflammatory (GR) signaling pathways, we conducted TELiS promoter-based bioinformatics analyses of all genes showing ≥ 1.25-fold differential expression per 4-SD range of neighborhood stress scores (a range of continuous Z-score values covering the broad range of variation in scores from low = −2 SD relative to the mean to high = +2 SD). In an analysis including both subjective and objective stress scores as covariates, we identified 138 differentially expressed transcripts as a function of subjective stress (44 up-regulated and 94 down-regulated), while identifying 314 differently expressed transcripts as a function of objective stress (190 up-regulated and 124 down-regulated). Results identified no significant asymmetry of TF-binding sites for the pro-inflammatory TF, NF-κB, as a function of objective stress (1.49-fold ± 0.40, p = .236) or as a function of subjective stress (0.58-fold ± 0.38, p = .095). However, findings indicated a significant up-regulation of GR activity with greater subjective stress (1.67-fold ± 0.23, p = .016) but not with greater objective stress (0.80-fold ± 0.17, p = .153). Similar results emerged when subjective stress was analyzed without objective stress (but controlling for all other covariates; NF-κB: 0.62-fold ± 0.35, p = .107; GR: 2.10-fold ± 0.23, p = .001). When objective stress was analyzed without subjective stress, the results continued to show no significant difference in TF activity (NF-κB: 1.55-fold ± 0.44, p = .232; GR:.87-fold ± 0.19, p = .418). Similar results also emerged in analyses that did not adjust for any covariates (Supporting Information).

3.4. Cellular Origins

To identify the cellular sources of the gene transcripts found to be differentially expressed as a function of stress, we conducted Transcript Origin Analyses as previously described (Cole et al., 2011) using reference transcriptome data from isolated subsets of circulating leukocytes (i.e., CD4± and CD8± T cells, B cells, NK cells, monocytes, and plasmacytoid dendritic cells. Genes down-regulated in association with objective stress derived disproportionately from plasmacytoid dendritic cells (mean cell type diagnosticity z-score =0.28 ± SE.10, p = .003) and B cells (0.52 ± 0.26, p = .023). No specific cell subset

was significantly linked to the set of genes up-regulated in association with objective stress, although a marginal trend emerged for a contribution from monocytes (0.77 ± 0.48, p = .055). By contrast, genes up-regulated in association with subjective neighborhood stress showed clear derivation from monocytes (1.03 ± 0.41, p = .007) and plasmacytoid dendritic cells (0.40 ± 0.20, p = .024). Gene transcripts down-regulated in association with subjective stress derived disproportionately from B cells (1.03 ± 0.34, p = .002). Similar results emerged for subjective stress in the absence of control for objective stress (up-regulated: monocyte, 0.96 ± 0.40, p = .010; dendritic cell, 0.46 ± 0.19, p = .009; down-regulated: B cell, 1.11 ± 0.29, p < .001) and for objective stress in the absence of subjective stress (up-regulated: monocyte 0.71 ± 0.45, p = .066; down-regulated: dendritic cell, 0.27 ± 0.10, p = .004; B cell, 0.47 ± 0.26, p = .033).

To determine whether the monocyte involvement in stress-related transcriptome correlates might reflect previously observed stress-related hematopoietic up-regulation of immature CD16- monocytes (Cole et al., 2015a; Powell et al., 2013), we conducted additional Transcript Origin Analyses testing for differential contributions from CD16- vs. CD16± monocyte subsets (using reference transcriptomes from (Ingersoll et al., 2010). Results showed no significant indication that distinct monocyte subsets contributed to the differential gene expression associated with objective neighborhood stress (up-regulated CD16- cell bias: 0.33 ± 0.23, p = .083; down-regulated CD16- cell bias: 0.19 ± 0.19, p = .158). Analysis of transcripts associated with subjective stress also found no CD16- monocyte-related transcriptome bias for up-regulated genes (CD16- cell bias: 0.11 ± 0.25, p = .330) but found a significant contribution of CD16- monocytes to the set of down-regulated transcripts (CD16- cell bias: 0.51 ± 0.29, p = .040; i.e., opposite what would be expected if CD16- monocytes were up- regulated in association with subjective stress).

4. Discussion

Low-income single Black mothers living in racially segregated neighborhoods with high levels of violence on the Southside of Chicago showed individual variation in both objective and subjective stress measures. Notably, these two values were uncorrelated and appear to be associated with distinct patterns of immune system function, as assessed by evaluating transcriptome profiles within the circulating leukocyte pool. Specifically, we found that subjective stress was a stronger predictor of differential gene expression than objective stress, that genes responsive to subjective but not objective stress were significantly associated with activation of anti-inflammatory pathways, and that subjective and objective stress were correlated with changes in gene transcription in distinct blood cell populations.

The more pronounced relationship between the blood transcriptome and subjective stress could reflect the broader and more even distribution of values for subjective versus objective stress in our surveyed population. Despite living in very similar neighborhoods, individuals have highly variable emotional responses to threats and symbols of violence, e.g., memorials to murder victims; by contrast, variation in objective stress (i.e., violent crimes within a four-block radius) is less pronounced. Our findings of a more substantial role for subjective versus objective stress is in agreement with a growing body of literature suggesting that perceived experience can be a better predictor of mental and physical health outcomes

than objective measures of experience (Cole et al., 2015a). Notably, variation in subjective stress could reflect individual differences in social experiences at earlier time points, e.g., during childhood or adult life; such experiences can have persistent effects on mental and physical health (Hertzman and Boyce, 2010), particularly for groups of color who face disproportionately high stress irrespective of socioeconomic achievement (Gaydosh et al., 2018; James et al., 1987, 1983; Miller et al., 2015). Alternatively, other unmeasured factors, like degree of social isolation and social status (beyond SES) could give rise to variation in subjective stress in our focal population (Holt-Lunstad et al., 2010; House et al., 1988; Snyder-Mackler et al., 2020).

In contrast to previous studies examining adverse social environments experienced by other socio-demographic groups (Cole et al., 2015; Fredrickson et al., 2013), our results did not show stress-associated CTRA gene expression activation, nor activation of any cellular and signaling pathways previously associated with that transcriptomic state (i.e., no indication of CD16- monocyte up-regulation and no indication of NF-kB activation or GR down-regulation). Promoter-based bioinformatics analyses showed a relative up-regulation of GR activity (in correlation with subjective stress only) but no significant differences in NF-kB activity.

This combination of results is consistent with increased output from the HPA axis and/or increased sensitivity of the GR, but without the typical concomitant changes in NF-kB activity or its regulatory targets (Snyder-Mackler et al. 2020). Although the mechanistic implications of this distinct transcriptomic profile remain to be defined in future studies, this profile could indicate decreased sensitivity to glucocorticoid signaling in individuals with high subjective stress, a change in homeostasis that has implications for immune function at the level of gene regulation (Cohen et al., 2012). Elevated GR activity also occurs with normal aging (van Lieshout et al., 2020), suggesting our results support the “weathering hypothesis”, wherein human populations or groups chronically exposed to social and economic disadvantage display accelerated aging and thus increased disease risk at a relatively young age (Forde et al., 2019; Geronimus et al., 2006). One implication of our study is that, at least in our target population, elevated subjective stress is a risk factor for future disease.

We also detected down regulation of gene transcripts preferentially expressed by CD16- classical monocytes, which suggests this phenotype occurred without chronic SNS (sympathetic nervous system) related alterations in myelopoiesis and CD16- monocyte up regulation. The absence of characteristic SNS-related transcriptomic patterns and the presence of increased GR/HPA axis-related transcriptome profiles suggests that these Black mothers’ experience of subjective stress may invoke biological responses that are more characteristic of a “hunkered down” conservation/withdrawal response (HPA) than of a classical fight-or-flight response (SNS) (Henry and Stephens, 1977; Koolhaas, 2008; Koolhaas et al., 2010). It is important to note that the “hunkered down” conservation/withdrawal response is also associated with considering new actions after a period of lying low, which can be viewed as a renewed sense of agency. However, the lack of SNS activity is also consistent with prolonged exposure to chronic stress, which leads to accumulated tissue damage known as allostatic load (reviewed in Suvarna et al. (2020)). This damage manifests

in many organs beyond blood cells, including cardiac and neural tissues. Our results indicate that, although the women in our study population were healthy at the time of assessment, they were showing effects of chronic stress that put them at future risk of cardiovascular, metabolic, and cognitive disease (reviewed in Russell and Lightman (2019)).

The transcriptomic profiles displayed by women in our study are often triggered by threats to survival (Henry and Stephens, 1977; Koolhaas, 2008; Koolhaas et al., 2010; Schmale and Engel, 1975; Thierry et al., 1984). Such a phenotype is consistent with the psychological characteristics of neighborhood stress, which include high levels of violence. Approximately 51% of our sample report experiencing symptoms of post-traumatic stress disorder (PTSD). Future research will examine how PTSD affects the genome and lives of the participants. The authors will also explore how different types of coping strategies may be used as protective factors for these participants living in areas with high levels of violence. Overall, the results presented here suggest that when mothers believe that their neighborhoods are unsafe and stressful places to live due to drugs, gangs, and violence that may affect them or their children, these social threats are evident “under their skin”, affecting gene regulatory processes in the immune system that reflect chronic stress and increased disease risk.

4.1. Limitations

We conducted an observational study, and the associations here may stem in part from other mechanisms in addition to any causal effects of subjective stress on gene expression. Although this study controlled for several potential confounding factors (including objective stress, demographic variables, and SES), other variables may affect both neighborhood stress perceptions and gene expression profiles. This study was neither designed nor sufficiently powered to detect statistically reliable associations between either stress score and specific individual gene transcripts. Gene-specific correlates of neighborhood stress scores may well exist, but future research using larger sample sizes would be required to detect such effects. The differentially expressed genes identified here should not be interpreted as statistically reliable on an individual basis and serve only as input into higher-order set-based bioinformatics analyses that test a small number of specific a priori hypotheses derived from previous research in human social genomics. These hypotheses are centered on variation in CTRA activity, bioinformatics predictions of NF-kB and GR transcription factor activity, and transcript cellular origins. Other sets of genes besides those tested here may also associate with neighborhood stress and remain to be resolved in future research using larger participant sample sizes. No direct measures of leukocyte subset prevalence were available in this study, so future research using direct enumeration of leukocyte subsets (e.g., by flow cytometry) will be required to confirm the bioinformatic inferences of cellular contribution reported here. Finally, given the limited sample size in this study, it is possible that some effects that only approached statistical significance might emerge as more significant findings in a larger sample with greater statistical power. This includes trends toward reduced NF-κB activity with high subjective stress, which would be consistent with the anti-inflammatory effects of HPA-axis activity, or trends toward increased classical monocyte-related gene expression with high objective neighborhood stress, which would be more typical of the CTRA.

5. Conclusions

Prior to this research, it was largely unknown how neighborhood violence affects immune system gene regulation. Our results suggest that an individual's perception of stress in their neighborhood (subjective stress) is associated with a profile of leukocyte gene regulation that is distinct from the CTRA pattern observed in many other types of social adversity. Individuals with high subjective stress scores showed the hallmarks of increased GR- influenced gene regulation characteristic of chronic stress, increased allostatic load, accelerated aging, and increased disease risk. These novel observations allow us to begin to understand the pathways that may contribute to differential disease risk and mortality confronting low-income African American mothers. These results also identify specific biological hypotheses that should be tested in future studies using more direct assays of HPA axis activity (e.g., cortisol measures like diurnal cortisol slopes or total daily cortisol levels), GR functional activity (e.g., *ex vivo* glucocorticoid sensitivity assays), chromatin dynamics associated with the transcription factors studied here (e.g., chromatin immunoprecipitation and Assay for Transposase-Accessible Chromatin), conducted on specific cellular populations (e.g., obtained via flow cytometry). Future research should tease out how those hormones and immune cells contribute to differential disease risk that might confront these women. This study moves away from individualized notions of health and illness. Instead, we consider how social inequality linked to race, class, and gender may operate through individual perception and immune function to contribute to health disparities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive Statistics of Women with profiled blood transcriptome (N = 68).

Demographic variables	Mean or%	Min	Max
Age	33.58	18	62
Years of Education	12.24	10	19
Number of children under 18 in household	1.92	0	7
Employment status (Employed)	24.6%	0	1
Pregnant at time of Study	9.1%	0	1
Current Education Status (Actively taking classes)	20.3%	0	1
Current Financial Stress (Difficulty paying bills)	46.1%	0	1
Household Income Below Federal Poverty Level (Yes)	100%	0	1
PTSD (Yes)	50.1%	0	1

Table 2

Questions used to derive subjective and objective measures of social environmental stress.

Subjective Stress	
Perceived Neighborhood Violence Score (PNVS), Alpha Score.89	**Range (7–20)**
Do you feel that it is stressful living in this neighborhood? How concerned are you with drugs and gangs in this neighborhood? How concerned are you with your children being shot in this neighborhood? How concerned are you with your children witnessing someone being shot in this neighborhood?	Mean 15.69 SD (3.27) Possible Responses for all variables: 4 = EXtremely 3 = Very 2 = Somewhat, and 1 = Not at all
Environmental Conditions Stress Score (ECSS), Alpha Score.73	**Range (6–24)**
How does seeing memorials to children killed in your neighborhood make you feel? How does seeing memorials to adults killed in your neighborhood make you feel? How does seeing drug houses in your neighborhood make you feel? How does seeing gang signs in your neighborhood make you feel? How does living on a low floor (with increased exposure to burglary and bullets) make you feel? How do busy streets (higher traffic, could mean greater exposure to crime) make you feel?	Mean 13.79 SD (6.63) Possible Responses for all variables: 1 = Very Good 2 = Somewhat Good 3 = Somewhat Stressed 4 = Very Stressed
Objective Stress	
Neighborhood Crime Score (NCS)	**Range (64–3497)**
Number of violent crimes (homicide, criminal sexual assault, robbery, aggravated assault, aggravated battery) committed within a 4-block radius of the participant's residence	Mean 255.56 SD (451.56)
Block Deprivation Score (BDS)	**Range (0–9)**
Number of abandoned buildings and lots within the participant's block	Mean 2.27 SD (2.19)