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Associations between the neural-hematopoietic-inflammatory axis and DNA methylation of stress-related genes in human leukocytes: Data from the Washington, D.C. cardiovascular health and needs assessment

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ABSTRACT

Chronic stress is associated with cardiovascular disease (CVD) risk and elevated amygdala activity. Previous research suggests a plausible connection between amygdala activity, hematopoietic tissue activity, and cardiovascular events; however, the underlying biological mechanisms linking these relationships are incompletely understood. Chronic stress is thought to modulate epigenomic modifications. Our investigation focused on associations between amygdala activity (left (L), right (R), maximum (M), and average (Av) AmygA), and splenic (SpleenA), and bone marrow activity (BMA) as determined by ^{18}Fluorodeoxyglucose (FDG) on Positron Emission Tomography/Computed Tomography (PET/CT) scans. Subsequently, we assessed how these markers of chronic stress and hematopoietic activity might relate to the DNA methylation of stress-associated genes in a community-based cohort of African American individuals from Washington D.C. at risk for CVD. To assess the relationships between AmgyA, SpleenA, BMA, and DNA methylation, linear regression models were run and adjusted for body mass index and 10-year predicted atherosclerotic CVD risk. Among 60 participants (93.3% female, mean age 60.8), M-AmygA positively associated with SpleenA ($\beta = 0.29$; $p = 0.001$), but not BMA ($\beta = 0.01$; $p = 0.89$). M-AmygA ($\beta = 0.37$; $p = 0.01$ and $\beta = 0.31$; $p = 0.02$, respectively) and SpleenA ($\beta = 0.73$; $p < 0.01$ and $\beta = 0.59$; $p = 0.005$, respectively) were associated with both IL-1β and TNFα. Decreased M-AmygA, SpleenA, IL-1β, and TNFα were associated with methylation of *NFκB1* at cg07955720 and *STAT3* at cg19438966. Our findings suggest a potential association between AmygA, SpleenA, and pro-inflammatory cytokines in the setting of chronic stress, suggesting an adverse hematopoietic effect. Furthermore, findings reveal associations with epigenetic markers of *NFκB* and *JAK/STAT* pathways linked to chronic stress.

Abbreviations

^{18}F-FDG-PET/CT	^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography
AmygA	Amygdala activity
ASCVD	Atherosclerotic cardiovascular disease
BMA	Bone marrow activity
BMI	Body mass index
CVD	Cardiovascular disease

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DC-CHNA	DC Cardiovascular Health and Needs Assessment
DC CHOC	DC Cardiovascular Health, and Obesity Collaborative
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment-insulin resistance
HPA	Hypothalamic-pituitary-adrenal
hs-CRP	High sensitivity C-reactive protein
IFN	Interferon
IL	Interleukin
IRB	Institutional review board
LDL-C	Low-density lipoprotein cholesterol
JAK	Janus kinase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
ROI	Region of interest
SD	Standard deviation
SES	Socioeconomic status
SpleenA	Splenic activity
STAT	Signal transducer and activator of transcription
SUV	Standardized uptake value
TBR	Target-to-background ratio
TNF	Tumor necrosis factor

1. Introduction

Chronic psychosocial and environmental stress (PSES) is a significant public health concern that can have negative impacts on physical and mental health, including an increased risk for cardiovascular disease (CVD) (Pedersen et al., 2017). One potential neural mechanism by which chronic PSES may exert an influence on CVD risk is through increased activity in the amygdala (Powell-Wiley et al., 2021; Tawakol et al., 2017), a brain region involved in emotional processing and stress responses (Sergerie et al., 2008; Tawakol et al., 2019). Research into stress-associated neurobiological activity has shown that the amygdala region of the brain plays an important role in both acute and chronic responses to stressors (Muscatell et al., 2015; Tawakol et al., 2019). Furthermore, additional findings suggest chronic stress is associated with increased amygdala activity (AmygA) and altered functioning of the amygdala (Ortiz-Whittingham et al., 2024; Powell-Wiley et al., 2021; Zhang et al., 2018).

The investigation of the complex interplay between chronic stress and AmygA is particularly relevant to minoritized populations, such as individuals living in resource-limited neighborhoods (Hobson et al., 2022; McCutcheon et al., 2018). These populations are disproportionately exposed to social, environmental, and psychosocial factors that promote chronic stress (Powell-Wiley et al., 2022; Williams, 2018). Chronic stress, in turn, has been proposed to be an important driver of CVD disparities (Powell-Wiley et al., 2022).

The neural-hematopoietic-inflammatory axis is hypothesized to be an important pathway connecting stress-related neural activation (AmygA), the hemopoietic system (spleen, bone marrow), and subsequent immune cell dysfunction and inflammation, promoting poor CVD outcomes (Baumer et al., 2023; Stiekema et al., 2017). Prior studies have demonstrated a relationship between AmygA, splenic activity, bone marrow activity, arterial inflammation, and CVD events (Dar et al., 2019; Tawakol et al., 2017; van der Valk et al., 2017), with fewer studies investigating these relationships in the setting of chronic stress (Powell-Wiley et al., 2021; Tawakol et al., 2019). The spleen is a major immune cell reservoir, and splenic metabolic activity, measured by ^{18}FDG-PET/CT, has been connected to CVD events (Emami et al., 2015) and chronic stress (Gharios et al., 2024). Gharios et al. reported that when comparing the metabolic splenic activity of patients with post-traumatic stress disorder (PTSD) to those without PTSD, individuals with PTSD displayed higher splenic activity and increased inflammatory burden assessed by high-sensitivity C-reactive protein (hs-CRP) (Gharios et al., 2024). However, the role of transcriptomic or epigenomic changes in linking chronic stress-related neural activation with hematopoietic activity and CVD risk are not yet fully understood. A study by Cole et al. demonstrated a link between chronic loneliness as a chronic stress marker (Campagne, 2019) and transcriptional changes in immune cell gene expression related to inflammatory pathways (Cole et al., 2007). An important mechanism by which chronic stress might be associated with gene expression changes could involve epigenomic modification, such as deoxyribonucleic acid (DNA) methylation (Johnstone and Baylin, 2010). Associations between neurobiological measures of stress, like AmygA, and epigenomic modifications via DNA methylation remain largely unexplored.

Thus, the aim of the present exploratory pilot study was to investigate the potential relationship between AmygA as a marker of chronic stress and neurobiological activation, hematopoietic activity, and DNA methylation patterns of stress-related genes among a population at high risk for CVD, disproportionally exposed to chronic stress, and residing in resource-limited neighborhoods. Specifically, the hypothesis of this study is that greater AmygA would be associated with higher bone marrow and splenic activity and pro-inflammatory cytokine levels and altered DNA methylation of stress-related genes in a community-based participatory research study cohort of African American individuals in Washington, D.C. Furthermore, we evaluated whether DNA methylation might be associated with the presence of inflammatory cytokines (IL-1β, IFNγ, and TNFα), which have been associated with neighborhood-level socioeconomic deprivation in our prior studies (Farmer et al., 2021; Ortiz-Whittingham et al., 2023).

2. Materials and methods

2.1. Study population

The Washington, D.C. Cardiovascular Health and Needs Assessment (DC-CHNA) was an observational study that used community-based participatory research to evaluate CV health, psychosocial factors, cultural norms, and neighborhood environment characteristics in predominantly African American communities in Washington, D.C., with high rates of CVD. The research team partnered with a community advisory board, the DC Cardiovascular Health and Obesity Collaborative (DC CHOC), which included community and church leaders, as well as academic partners in research, to consult on the planning and implementation of the study (Ceasar et al., 2017).

2.2. Participant recruitment and consent

The DC-CHNA recruitment was accomplished through community partnerships, as previously described (Yingling et al., 2017). The National Heart, Lung, and Blood Institute (NHLBI) Institutional Review Board (IRB) approved both the DC-CHNA (NCT01927783) and the clinical protocol for cardiometabolic testing for those at risk for CVD (NCT01143454) in accordance with the principles of the Declaration of Helsinki. Additionally, all guidelines for good clinical practice and those set forth in the Belmont Report were followed. Informed written consent was obtained from all participants for both protocols. Participants who agreed to both protocols were enrolled at the National Institutes of Health (NIH) Clinical Center for anthropometric and clinical laboratory measurements, cardiometabolic phenotyping, and physical examinations. Participant samples were de-identified prior to distribution, and all participant samples intended for storage were de-identified, labeled, and catalogued in the Biospecimens Inventory System as recommended by NHLBI guidelines for human specimens.

2.3. Study outcomes

2.3.1. Amygdala activity

As previously described (Powell-Wiley et al., 2021), AmygA was quantified using ^{18}FDG-PET/CT images by dividing the maximum standardized uptake value (SUV) in the amygdala by the mean SUV in the ipsilateral temporal lobe to calculate the target-to-background ratio (TBR). These measurements utilized 0.5 cm^3 spherical regions of interest (ROIs). The measures of AmygA analyzed in the study were: 1) maximum AmygA or the highest TBR between the two AmygA (M-AmygA) (Powell-Wiley et al., 2021), 2) TBR of the left AmygA (L-AmygA), 3) TBR of the right AmygA (R-AmygA), and 4) average AmygA TBR (Av-AmygA).

2.3.2. Hematopoietic system activity measurement

As previously described (Patel et al., 2021), spherical ROIs were positioned within lumbar and thoracic vertebrae (T1 to L5) and the spleen on ^{18}FDG-PET/CT images to quantify bone marrow activity (BMA) and splenic activity (SpleenA), respectively. The average of the maximum SUVs within the twelve vertebral ROIs was used to measure BMA. One 8 cm^3 spherical ROI was used within the spleen to capture the maximum SUV; this value was used for SpleenA.

2.3.3. Biomarker measurements

Fasting serum and plasma samples were collected during the NIH Clinical Center visits and stored at −80 °C until analysis. EDTA plasma samples were used for cytokine (IL-1β, IL-6, TNFα) level detection via multiplexed ELISA (Meso Scale Diagnostics, Rockville MD, USA) as previously described (Andrews et al., 2021).

2.3.4. Epigenetic DNA methylation profiling

Buffy coats were obtained from EDTA purple top tubes by centrifugation at 1,200×*g* for 20 min at 4 °C. After removing the EDTA plasma layer, each buffy coat was collected and stored at −80 °C until further processing. The DNA methylation assay was performed using the methods previously described (Pang et al., 2022). Following the manufacturer's instructions, DNA was isolated using the AllPrep DNA/RNA Kit (80,284 Qiagen). The Infinium MethylationEPIC BeadChip Kit (WG-317-1003 Illumina, US) was used with 250 ng of DNA as the input, following the manufacturer's recommendations. A total of 5 genes selected for analysis of methylation sites were based on previous associations with loneliness and stress (Cole et al., 2007).

2.3.5. MethylationEPIC V1.0 DNA methylation pre-processing and analysis

500 ng of DNA was treated with bisulfite using the EZ DNA Methylation kit from Zymo Research, following the manufacturer's instructions. The bisulfite-treated DNA samples were randomly assigned to a well on the Infinium HumanMethylationEPICV1 BeadChip, which was then amplified, hybridized, stained, washed, and imaged with the Illumina iScan SQ instrument to obtain raw image intensities. To preprocess the DNA methylation array data, we used the *minfi* pipeline (Aryee et al., 2014), and low quality samples were identified using the *qcfilter* function from the ENmix package (Xu et al., 2016), using default parameters. All samples passed the QA/QC ($p < 0.05$) and were deemed high-quality.

2.3.6. Psychosocial measures

Social isolation was measured using a subscale of the Chronic Stress Scale with a higher score indicating increasing social isolation (Turner and Marino, 1994). Depressive symptoms were measured by the Center for Epidemiologic Studies Depression Scale — Revised (CESD-R), where a higher score indicates more depressive symptoms (Eaton et al., 2004). Socioeconomic status is the self-reported individual-level household income with a higher value indicating higher self-reported socioeconomic status.

2.4. Covariates

The NIH Clinical Center clinical team conducted physical examinations and documented demographic information, clinical histories, and anthropometric measurements upon enrollment of the DC-CHNA cohort. Body mass index (BMI) measurements were determined using height and weight measurements (Nuttall, 2015). The 10-year predicted atherosclerotic cardiovascular disease (ASCVD) risk score (Grundy et al., 2019), which considered factors such as age, race, sex, total cholesterol, high-density lipoprotein cholesterol (HDL-C), blood pressure, and medical history (including diabetes and smoking status), was also measured for each participant. This risk score estimates a patient's likelihood of experiencing a CVD event within the next ten years (Wong et al., 2022).

2.5. Statistical analysis

Descriptive data were generated for the study population, reporting continuous variables as means with standard deviations (SD) and categorical variables as frequencies and percentages. Nested adjusted linear regression modeling was utilized to test the hypothesis that M-AmygA, BMA, SpleenA, and pro-inflammatory cytokines were associated. We also examined associations between amygdala activity and splenic activity with DNA methylation of candidate genes related to psychosocial stress in prior studies (Cole et al., 2007). Finally, we examined associations between pro-inflammatory cytokines and DNA methylation of candidate genes associated with amygdala or splenic activity. The associations were tested based on biologically plausible pathways. In Fig. 1, models were unadjusted (model 1), adjusted for BMI (model 2), and adjusted for BMI and 10-year predicted ASCVD risk (model 3). ASCVD risk and BMI were selected as covariates due to their potential confounding role in the relationships of interest, considering that this protocol's inclusion criteria required at least one CVD risk factor. All analyses beyond Fig. 1 were shown for the fully adjusted model 3. All estimates were reported as standardized β, and p-values <0.05 were considered statistically significant. STATA (StataCorp. 12, College Station, TX, USA: StataCorp LLC) and R (RCoreTeam, 2023) were used for all analyses. Additionally, p-values were adjusted for multiple testing with a permutation-based method using R package multtest (Pollard et al., 2005). The correction is performed across all the loci across the five genes tested.

3. Results

3.1. Patient characteristics

The study population consisted of 60 African American participants from the DC-CHNA who were predominantly female (n = 56, 93.33%), with overweight or obesity on average (BMI of 33.00 ± 7.85 kg/m^2; range 19.9–51.5 kg/m^2) (Table 1). Most participants presented with hyperlipidemia (n = 33, 55%) and were at intermediate risk for an ASCVD event in 10 years (mean 10-year predicted ASCVD risk score of 10.75% ± 8.51).

Our analysis in this study is focused on amygdala activity as an established pathophysiological measure of chronic stress (Ferrara et al., 2020). In the first set of analyses, we aimed to determine if the measure of chronic stress might be associated with measures of ^{18}FDG-PET/CT-determined amygdala activity in our community-based cohort (Supplementary Table 1). In the BMI and ASCVD 10-year risk score-adjusted model, social isolation was positively associated with the Av-AmygA ($\beta = 0.31$, $p = 0.03$), while household-level socioeconomic status trended towards a negative association with L-AmygA ($p = 0.09$).

3.2. Associations between amygdala, splenic, and bone marrow activity

In the unadjusted model, every one-SD increase in M-AmygA was

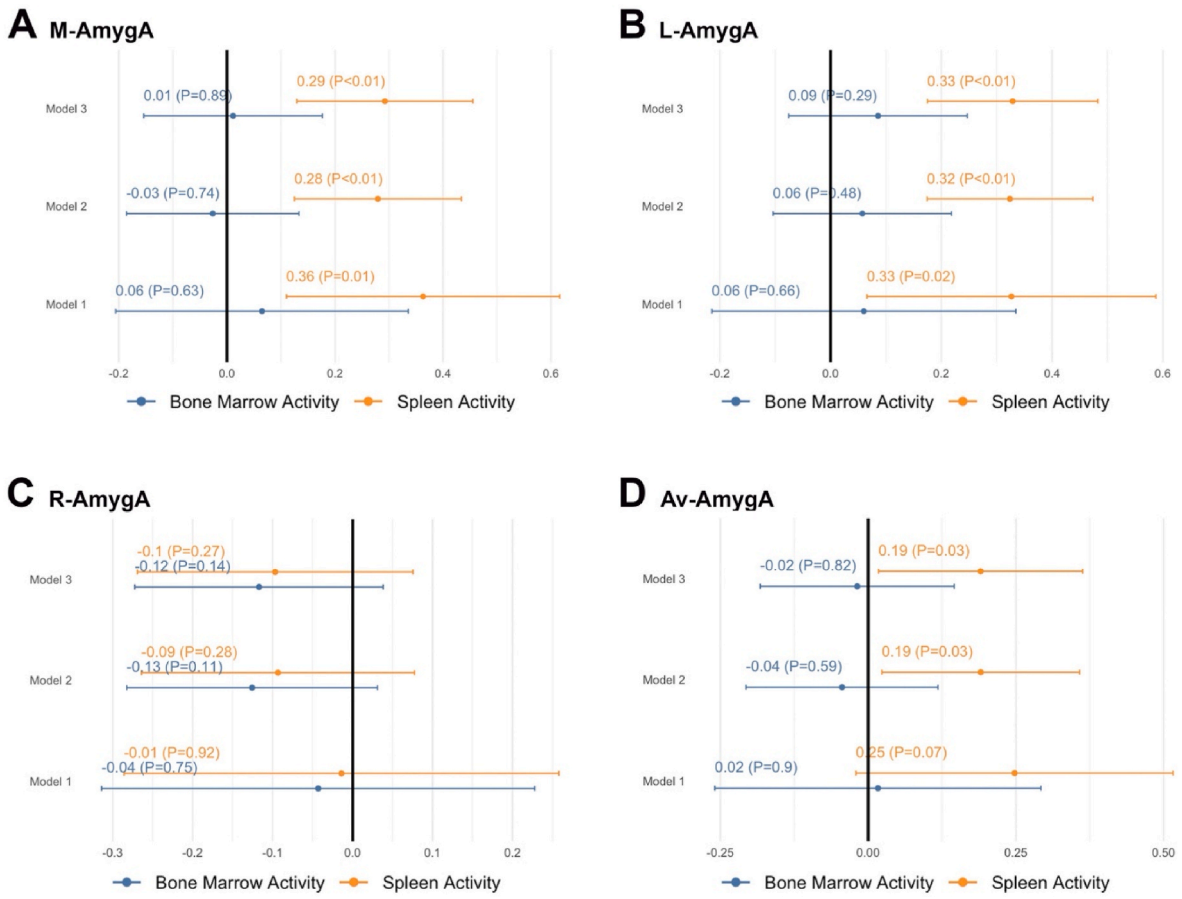

Fig. 1. Association between all AmygA and splenic and bone marrow activity in DC-CNHA cohort, 2014–2017. Model 1 was unadjusted, model 2 was adjusted for BMI, and model 3 was adjusted for both BMI and 10-year predicted ASCVD risk. Shown are the Forest plots of the associations of max (M-AmygA) (A), left (L-AmygA) (B), right (R-AmygA), and the average AmygA (Av-AmygA) with splenic or bone marrow activity.

associated with a 0.36-SD increase in SpleenA ($p = 0.01$). This remained significant when adjusted for BMI alone ($\beta = 0.28$; $p = 0.001$) and BMI and 10-year predicted ASCVD risk ($\beta = 0.29$; $p = 0.001$). Neither unadjusted nor adjusted models demonstrated an association between M-AmygA and bone marrow activity (Fig. 1A). The activity of L-AmygA and R-AmygA (Fig. 1 B/C) and the Av-AmygA activity was not associated with BMA (Fig. 1D). However, when focusing the analysis on L-AmygA or R-AmygA (Fig. 1 B/C), only the activity in the L-AmygA was significant associated with splenic activity. Av-AmygA to SpleenA associations were only significant after adjusting for BMI alone and BMI and ASCVD 10-year risk score (Fig. 1D). Therefore, all subsequent analyses focused on M-AmygA and L-AmygA.

3.3. Associations between amygdala and splenic activity and DNA methylation of candidate genes

Given the associations found with M-AmygA and SpleenA, we investigated the relationships between amygdala or splenic activity and DNA methylation of the 5 genes utilized in our candidate DNA methylation site analysis. Methylation sites significantly associated with AmygA and/or SpleenA are found in Table 2; all other regression estimates are in Supplementary Table 2.

The *NFκB1* gene had 51 methylation sites, from which three methylation sites displayed significant associations with M-AmygA and five methylation sites significantly associated with SpleenA after adjustment for BMI and ASCVD. Two methylation sites, in particular *NFκB1*-cg01983105 and *NFκB1*-cg07955720, displayed significant associations with M-AmygA ($\beta = -0.33$, $p = 0.01$ and $\beta = -0.31$, $p = 0.02$, respectively) and SpleenA ($\beta = -0.22$, $p = 0.01$ and $\beta = -0.26$, $p = 0.002$, respectively). For these associations, increasing M-AmygA or SpleenA metabolic activity was associated with decreasing methylation on each respective site within the *NFκB1 gene*. When investigating associations of M-AmygA or SpleenA and the 30 methylation sites on the *NFκB2* gene, we found three methylation sites significantly associated with either M-AmygA or SpleenA; however, no methylation site was associated with both.

Next, we determined potential associations with the 55 and 27 methylation sites for *JAK1* or *JAK2* genes, respectively. For the *JAK1* gene, seven significant associations with either M-AmygA or SpleenA were identified, with only one site, *JAK1*-cg25192081, significantly associated with both M-AmygA and SpleenA ($\beta = -0.38$, $p = 0.003$ and $\beta = -0.17$, $p = 0.045$, respectively). For JAK2, we found one significant association between SpleenA and *JAK2*-cg19920525. Although this association was not statistically significant, it did reveal a linear trend with M-AmygA ($p = 0.09$). Lastly, we analyzed 48 methylation sites of *STAT3,* from which M-AmygA or SpleenA associated with 6 methylation sites. *STAT3*-cg19438966 displayed significant associations with both M-AmygA and SpleenA ($\beta = -0.42$, $p = 0.001$ and $\beta = -0.23$, $p = 0.01$, respectively).

Interestingly, most associations were observed between M-AmygA and a gene methylation site, driven by the left amygdala activity (L-AmygA in Table 2). Most significant associations were lost after multiple testing correction. Only the SpleenA to *NFκB1*-cg07955720 ($\beta = -0.26$, $p = 0.002$ vs $p = 0.043$) and the M-AmygA to *STAT3*-cg19438966 ($\beta = -0.42$, $p = 0.001$ vs $p = 0.03$) associations remained significant after multiple testing correction. Therefore, further analyses within this manuscript focused on these two methylation sites within the *NFκB1 and STAT3* genes.

Table 1
Characteristics of participants from Washington, D.C. Cardiovascular Health and Needs Assessment who provided survey data and blood samples.

Sociodemographic	DC-CHNA cohort (n = 60)[a]
African American	60 (100%)
Sex, female	56 (93.33%)
Age (years)	60.83 ± 10.52
CVD Risk Factors[b]	
Type 2 diabetes mellitus, n (%)	13 (21.67%)
Hyperlipidemia, n (%)	33 (55.00%)
Hypertension, n (%)	38 (63.33%)
Smoking history, n (%)	7 (11.67%)
BMI (kg/m2)	33.00 ± 7.85
LDL-C (mg/dL)	105.5 ± 33.02
HDL-C (mg/dL)	66.57 ± 20.58
TG (mg/dL)	84.97 ± 26.43
TC (mg/dL)	188.98 ± 35.20
Fasting insulin (mU/mL)	16.76 ± 11.98
Fasting glucose (mg/dL)	104.73 ± 16.72
10-year predicted ASCVD risk score (%)[c]	10.75 ± 8.51
Stress and Inflammatory Markers[b]	
IL-1β (pg/mL)[d]	0.19 ± 0.24
IFNγ (pg/mL)	8.83 ± 9.56
TNFα (pg/mL)	1.61 ± 0.99
Imaging Measures	
M-Amygdala activity (TBR)[e]	1.13 ± 0.10
Splenic activity (SUV)[e]	4.17 ± 0.91
Bone marrow activity (SUV)[e]	4.21 ± 1.21
Psychosocial Measures	
Social Isolation[f]	0.42 ± 0.63
Depressive Symptoms[g]	4.44 ± 4.70
Household-levels Socioeconomic Status (SES), $in 10 K/annum[h]	54.45 ± 35.21

ASCVD = atherosclerotic cardiovascular disease; HDL-C = high-density lipoprotein cholesterol; IL = interleukin; LDL-C = low-density lipoprotein cholesterol; SUV = standardized unit uptake value; TC = total cholesterol; TG = triglyceride; TNF = tumor necrosis factor.

[a] Continuous variables expressed as mean ± standard deviation and categorical variables as total N (%).

[b] Clinical reference ranges: LDL-C optimal <100 md/dL; HDL-C low <40; TG normal <150 mg/dL; TC normal <200 mg/dL; insulin reference range 2.6–24.9 mU/mL; glucose 70–99 mg/dL; hs-CRP low-risk <1.0, average risk 1.0–3.0, high-risk >3.0 mg/L.

[c] 10-year predicted ASCVD risk score includes sex, age, race, total cholesterol, HDL-C, systolic blood pressure, personal history of diabetes, personal history of smoking, personal history of treatment for hypertension.

[d] IL-1β levels were undetectable in one individual (n = 59).

[e] For three study participants no imaging data were available (n = 57).

[f] Social isolation was measured using a subscale of the Chronic Stress Scale (Turner and Marino, 1994) (higher score = increasing social isolation) (n = 55).

[g] Depressive symptoms were measured by the Center for Epidemiologic Studies Depression Scale — Revised (CESD-R) (Eaton et al., 2004) (higher score = more depressive symptoms) (n-52).

[h] Socioeconomic status reflected self-reported individual-level household income (higher value = higher self-reported socioeconomic status) (n = 49).

3.4. Association between amygdala and splenic activity and plasma cytokines

Both M-AmygA and SpleenA significantly associated with IL-1β (β = 0.37, p = 0.01; β = 0.73, p = 0.0006, respectively) (Table 3). Furthermore, significant associations between both M-AmygA and SpleenA were also identified for TNFα (β = 0.32, p = 0.02; β = 0.59, p = 0.005, respectively). L-AmygA also associated with IL-1β (β = 0.29, p = 0.04) and TNFα (β = 0.31, p = 0.02). No significant associations were found for IFNγ. Additionally, the associations for M-AmygA and SpleenA remained significant after multiple testing corrections, while L-AmygA had associations that were trending toward statistical significance (Table 3).

3.5. Association between plasma level cytokines and DNA methylation of candidate genes

NFκB1-cg07955720 was inversely associated with IL-1β (β = −0.58; p < 0.001) and TNFα (β = −0.33; p = 0.001) (Table 4). Similarly, the *STAT3*-cg19438966 methylation site was also inversely associated with IL-1β (β = −0.41; p = 0.0009) and TNFα (β = −0.38; p = 0.01). All associations remained significant after multiple comparison adjustment (Table 4).

Given the complex relationships between the individual factors, we summarized our findings in Fig. 2.

4. Discussion

In this community-based pilot study of African American individuals from Washington, D.C., M-AmygA activity was positively associated with SpleenA, but not BMA. M-AmygA and SpleenA were also associated with decreased DNA methylation of genes in the *NFκB* and *JAK/STAT* pathways, which were related to higher IL-1β and TNFα levels. These findings suggest an association between markers of chronic stress-related neural-hematopoietic-inflammatory pathways and the epigenome. These results suggest a possible mechanistic link between chronic stress and CVD via immune function and epigenetic alterations (Powell-Wiley et al., 2021; Tawakol et al., 2017).

Chronic psychosocial and environmental stress, along with the cytokines TNFα or IL-1β have been linked to CVD (Amin et al., 2020; Ridker et al., 2017; Williams et al., 2019), with most of these cytokines being target genes of the glucocorticoid receptor. Hence, epigenetic regulation due to chronic stress might be the common factor linking chronic stress to inflammation, immune cell dysfunction, and increased CVD risk.

In response to chronic stress, the hypothalamic-pituitary-adrenocortical (HPA) axis is activated, resulting in the release of excess cortisol, a major stress hormone (Herman et al., 2016). Under normal circumstances, cortisol has an anti-inflammatory function via the glucocorticoid receptor; however, in the setting of chronic stress and excess cortisol, acquired glucocorticoid receptor resistance is observed (Wright, 2009), leading to decreased effects of cortisol on the receptor and enhanced expression of pro-inflammatory cytokines (Walsh et al., 2021). Glucocorticoids, like cortisol, get their anti-inflammatory function via suppression of the NFκB signaling pathway (Liden et al., 2000), leading to decreased expression of TNFα (in breast cancer cells (Fan et al., 2019)). Interestingly, a negative association between cortisol and NFκB in the setting of stress (Trier Social Stress Test) has been reported in humans (Wolf et al., 2009). Acute stress is associated with increased expression of *NFκB*, which stimulates the transcription of multiple target genes involved in inflammation (Bekhbat et al., 2017). As decreasing methylation may be associated with increasing gene expression, especially of promoter sites (Anastasiadi et al., 2018; Miranda and Jones, 2007), our data could suggest that increased AmygA may lead to increased NFκB1 expression via decreased methylation and subsequently increased TNFα expression. Similarly, IL-1β transcription has also been linked to NFκB-dependent gene expression (Liu et al., 2017).

In our study, numerous methylation sites were inversely associated with AmygA and SpleenA, coinciding with results from prior analyses of genes regulated by loneliness (Cole, 2008). Reduced M-AmygA and SpleenA was linked to methylation of *NFκB1*site cg007955720 and decreased levels of IL-1β and TNFα. This highlights hypomethylation of the *NFκB* gene as a potential mediating step between chronic stress and pro-inflammatory cytokine regulation, suggesting that lower methylation levels could lead to higher gene expression and contribute to the inflammatory response observed under chronic stress conditions.

Next, the JAK/STAT pathway is integral for the transcription of immune-related genes, such as IL-6 cytokine family members, implicated in cardiac hypertrophy (Wagner and Siddiqui, 2012) and other types of CVD (Kishore and Verma, 2012). Dysregulation of the

Table 2
Significant associations between amygdala and splenic activity and methylation sites in DC-CNHA cohort, 2014–2017. Shown are standardized beta values followed by the p-value. The multiple testing adjusted p-value is shown in the column to the right. Significance was assumed when the p-value reached <0.05 and is indicated by bold font. The Italic font indicates associations reaching significance.

Gene	Methylation Site	M-AmygA[a] (N = 57)		L-AmygA[a] (N = 57)		SpleenA[a] (N = 57)	
		β (p-value)	Adj. p-value	β (p-value)	Adj. p-value	β (p-value)	Adj p-value
NFκB1	cg01983105	−0.33 (0.01)	0.22	−0.37 (0.005)	0.12	−0.22 (0.0**1**)	0.17
	cg07955720	−0.31 (0.02)	0.30	−0.27 (0.046)	0.62	−0.26 (0.002)	0.043
	cg09931511	−0.21 (0.10)	0.86	−0.30 (0.02)	0.37	−0.19 (0.02)	0.31
	cg11140890	−0.24 (0.07)	0.72	−0.33 (0.01)	0.26	−0.22 (0.008)	0.15
	cg12615753	−0.30 (0.02)	0.31	−0.31 (0.02)	0.33	−0.17 (0.05)	0.57
	cg18284616	0.21 (0.10)	0.85	0.27 (0.04)	0.57	0.21 (0.01)	0.23
NFκB2	cg23738770[b]	0.27 (0.04)	0.44	0.32 (0.02)	0.26	0.01 (0.90)	1.00
	cg25811402[b]	0.26 (0.048)	0.54	0.38 (0.005)	*0.09*	0.02 (0.84)	1.00
	cg26571105	0.17 (0.18)	0.96	0.20 (0.13)	0.91	0.17 (0.047)	0.45
JAK1	cg10396322	−0.32 (0.01)	0.30	−0.08 (0.56)	1.00	−0.07 (0.44)	1.00
	cg10612943	−0.26 (0.04)	0.62	−0.38 (0.004)	0.12	−0.13 (0.14)	0.93
	cg11743000	−0.23 (0.08)	0.86	−0.23 (0.10)	0.88	−0.18 (0.04)	0.56
	cg12075498	0.31 (0.01)	0.35	0.23 (0.08)	0.84	−0.01 (0.95)	1.00
	cg12115700	0.11 (0.41)	1.00	0.19 (0.15)	0.97	0.18 (0.03)	0.48
	cg13420662	−0.06 (0.66)	1.00	−0.08 (0.58)	1.00	−0.23 (0.008)	0.16
	cg25192081	−0.38 (0.003)	0.10	−0.16 (0.24)	0.99	−0.17 (0.045)	0.61
JAK2	cg19920525	−0.22 (0.09)	0.74	−0.25 (0.07)	0.64	−0.18 (0.04)	0.36
STAT3	cg03647657	0.06 (0.63)	1.00	0.21 (0.11)	0.92	0.19 (0.02)	0.33
	cg06350351	0.21 (0.11)	0.92	0.14 (0.31)	1.00	0.19 (0.03)	0.42
	cg09804439[b]	0.33 (0.01)	0.22	0.15 (0.29)	0.99	0.06 (0.49)	1.00
	cg19438966	−0.42 (0.001)	0.03	−0.40 (0.003)	0.09	−0.23 (0.01)	0.19
	cg22128509	0.18 (0.17)	0.98	0.15 (0.26)	0.99	0.19 (0.03)	0.35
	cg25857307	−0.26 (0.046)	0.65	−0.29 (0.03)	0.47	−0.12 (0.17)	0.93

[a] Models are adjusted for BMI and 10-year predicted ASCVD risk.
[b] Methylation site in gene promoter; ASCVD = atherosclerotic cardiovascular disease; BMI = body mass index; JAK = Janus kinase; NF-κB = nuclear factor of κ light chain gene enhancer in B-cells; STAT = signal transducer and activator of transcription.

Table 3
Association between amygdala and splenic activity and cytokines in DC-CNHA cohort, 2014–2017. Shown are standardized beta values followed by two p-values (unadjusted p-value, multiple testing correction adjusted p-value). Significance was assumed when the p-value reached <0.05 and is indicated by bold font. The Italic font indicates associations reaching significance.

Biomarker	M-AmygA[a]		L-AmygA[a]		SpleenA[a]	
	β (p-value)	Adj. p-value	β (p-value)	Adj. p-value	β (p-value)	Adj. p-value
IL-1β	0.37 (0.01)	0.03	0.29 (0.04)	*0.08*	0.73 (0.0006)	0.002
IFNγ	0.19 (0.18)	0.18	0.15 (0.28)	0.28	0.32 (0.12)	0.12
TNFα	0.32 (0.02)	0.048	0.31 (0.02)	*0.07*	0.59 (0.005)	0.01

[a] Models are adjusted for BMI and 10-year predicted ASCVD risk; ASCVD = atherosclerotic cardiovascular disease; BMI = body mass index; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor.

Table 4
Associations between methylation sites and the cytokines IL-1β and TNFα in DC-CNHA cohort, 2014–2017. Shown are standardized beta values followed by two p-values (unadjusted p-value, multiple testing correction adjusted p-value). Significance was assumed when the p-value reached <0.05 and is indicated by bold font.

Biomarker	*NFκB1*-cg07955720[a]			*STAT3*-cg19438966[a]		
	Standardized β coefficient	p-value	Adj. p-value	Standardized β coefficient	p-value	Adj. p-value
IL-1β	**−0.58**	**0.000006**	**0.00005**	**−0.41**	**0.0009**	**0.005**
TNFα	**−0.33**	**0.002**	**0.004**	**−0.38**	**0.01**	**0.02**

[a] Models are adjusted for BMI and 10-year predicted ASCVD risk; ASCVD = atherosclerotic cardiovascular disease; BMI = body mass index; IL = interleukin; TNF = tumor necrosis factor.

JAK/STAT pathway has been linked to accelerated inflammation and immune cell dysfunction and has been used as a therapeutical target in inflammatory and autoimmune diseases (Banerjee et al., 2017; Luo et al., 2021). We would postulate that the *JAK/STAT* pathway might play a role in connecting chronic psychosocial and environmental stress, immune cell dysfunction, and inflammation. Overall, lower AmygA and/or SpleenA tended to be associated with *JAK1/2* and *STAT3* methylation, further supporting this hypothesis. Interestingly, the *STAT3*-cg19438966 methylation site displayed the same patterns as the identified *NFκB1*site; with greater AmygA and SpleenA associated with hypomethylation, and pro-inflammatory cytokines IL-1β and TNFα, again highly suggestive of epigenetic changes as a possible mediator between chronic stress and immune system activation. Mechanistically, *STAT3* and the glucocorticoid receptor are intertwined and potentially act in a co-activator complex (Zhang et al., 1997), synergizing gene expression as shown in breast cancer (Conway et al., 2020). Stat3 hyperactivation has been linked to various cancers and CVD (Carpenter and Lo, 2014; Kishore and Verma, 2012) and has been connected to increased inflammation; however, its role in chronic stress-related inflammation is largely unexplored, with one mouse study substantiating the potential importance of Stat3 in chronic stress-related immune dysfunction (Hu et al., 2014).

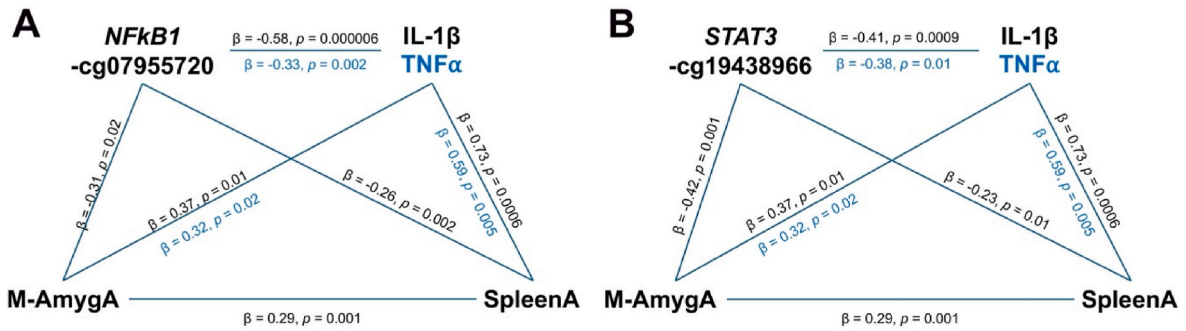

Fig. 2. Summary figure of relationships between M-AmygA, SpleenA, methylation sites, and cytokines adjusted for ASCVD 10-year risk and BMI in the DC-CHNA cohort, 2015–2019. [Note: no mediation analysis was performed, and no directionality was implied].

Associations with stress activity were present at other gene DNA methylation sites, signifying the complex interconnectedness between epigenomic regulation of stress-induced biological pathways. Interestingly, our data indicate the left amygdala may play a major role in relaying the effects between the brain and the immune system. Our findings are consistent with a study we published recently where we found lesser perceived neighborhood safety to be associated with enhanced left amygdala activity and functional connectivity, as determined by magnetic resonance imaging (MRI) (Ortiz-Whittingham et al., 2024). More research should be done exploring the potential importance of similar findings on subsequent biological sequelae, the immune system, and future CVD.

Additionally, while we did find AmygA and SpleenA to associate with one another, we could not detect a significant relationship between AmygA and BMA, as other studies have reported previously. While this might be a consequence of a smaller sample size in this study, differences in our study population, or due to not adjusting BMA measures for blood activity, we believe that our findings are of importance, as besides BMA, SpleenA, in particular, has been suggested to relate more closely to a pro-inflammatory state of circulating immune cells (Emami et al., 2015). We believe that our data, while hypothesis-generating from a smaller cohort, shed light on potential pathways involving DNA methylation which could be of importance and targetable in the future.

Chronic stress, especially in vulnerable groups akin to this study population, is closely intertwined with SDoH factors that are often structural in nature (Powell-Wiley et al., 2022). Targeting therapies to mitigate these systemic factors may prove difficult. Therefore, identifying multi-level interventions focused on healthy behavior, patient education, and care coordination and management that ameliorate the deleterious effects of chronic stress on CVD outcomes is imperative (Powell-Wiley et al., 2022). For example, a 3-month physical activity intervention for 43 participants with obesity decreased AmygA, SpleenA, and BMA (Pahk et al., 2022). While AmygA is a potential therapeutic target, DNA methylation is also modifiable with both pharmacotherapy and behavioral interventions. DNA methyltransferase inhibitors are used therapeutically to silence DNA methylation and prevent tumor progression; whereas physical activity and diet interventions modulate epigenomic methylation patterns (Hu et al., 2021; Maugeri, 2020; Rönn et al., 2013). A recent study in Native Hawaiian adults with diabetes reported that a social support intervention shifted the epigenomic landscape of monocytes towards a less inflammatory phenotype (Dye et al., 2022). Whereas the feasibility of a population health approach using stress-induced DNA methylation-targeted pharmacotherapy is likely of little yield, identifying culturally appropriate, multi-level, behavioral interventions that alter the stress-epigenomic pathways could address the modifiable disparities in stress-related adverse effects.

This study is one of the first to assess associations connecting the neural-hematopoietic-inflammatory axis with epigenomic factors. Additionally, it explores the role of biological stress in an African American population, a community exposed to both acute and chronic stress in a variety of social, environmental, and psychosocial forms. However, this study has several limitations. First, causality cannot be established, given the cross-sectional design, and there is risk for reverse causation, even though the published literature may support directionality. Next, the study included a relatively small sample size (n = 60). As such, the results should be interpreted as hypothesis-generating performed in a pilot study population, and mediation analyses to further assess potential mechanistic pathways were not feasible. We believe that the smaller sample size limits the statistical power to observe associations between measures of chronic stress, like depressive symptoms, and amygdala activity. Additionally, the smaller sample size did not allow for examining the cumulative effects of chronic stressors, which ultimately might be associated with AmygA. Future studies should examine how cumulative chronic stress scores might impact the observed associations. The small sample size also does not allow for further adjustments typically seen in DNA methylation analyses, like white blood cell count, food intake, cancer history, socioeconomic status, or environmental exposures. We had no access to the ancestry information of our study participants. While focusing on one specific racial or ethnic group might be argued to impact the generalizability of our findings, the basic findings of this study are in accordance with other studies (Tawakol et al., 2019). Our work aims to fill the gaps as minoritized populations are still underrepresented in clinical research trials. Moreover, the pathways identified based on DNA methylation data (*NFκB* and *STAT3*) are very similar to a previous study focused on RNA sequencing (Cole et al., 2007). Certainly, future studies should be conducted in larger, diverse, population-based cohorts. Also, analyses in large community-based intervention studies could examine how these associations are impacted by multi-level behavioral interventions focused on diet, physical activity, and stress management.

5. Conclusions

Thus, in a community-based cohort of African American individuals at risk for CVD, the results demonstrate a potential association between AmygA and SpleenA in chronic stress, implicating an adverse hematopoietic effect from chronic stress. Furthermore, epigenetic mechanisms of chronic stress and subsequent inflammation may be related to an inverse relationship between AmygA and the methylation of *NFκB* and *STAT3* genes. This study supports the need for a greater understanding of the complex relationships between chronic stress, proinflammatory genes and processes, and epigenomic modifications, particularly related to the NF-κB and Jak/Stat pathways. Further understanding of these stress-related pathways in population-based cohorts may identify novel targets for interventions that can mitigate the negative effects of chronic stress on cardiovascular health.

CRediT authorship contribution statement

Manuel A. Cintron: Writing – review & editing, Writing – original draft, Data curation. **Yvonne Baumer:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alina P.S. Pang:** Writing – review & editing, Formal analysis, Data curation. **Elizabeth M. Aquino Peterson:** Formal analysis, Writing – review & editing. **Lola R. Ortiz-Whittingham:** Writing – review & editing, Formal analysis, Data curation. **Joshua A. Jacobs:** Writing – review & editing, Writing – original draft, Formal analysis. **Sonal Sharda:** Writing – review & editing, Investigation, Formal analysis. **Kameswari A. Potharaju:** Writing – review & editing, Writing – original draft, Formal analysis. **Andrew S. Baez:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Cristhian A. Gutierrez-Huerta:** Writing – review & editing, Formal analysis, Data curation. **Erika N. Ortiz-Chaparro:** Writing – review & editing, Formal analysis, Data curation. **Billy S. Collins:** Writing – review & editing, Data curation. **Valerie M. Mitchell:** Writing – review & editing, Data curation. **Abhinav Saurabh:** Writing – review & editing, Formal analysis, Data curation. **Laurel G. Mendelsohn:** Writing – review & editing, Formal analysis, Data curation. **Neelam R. Redekar:** Writing – review & editing, Methodology, Formal analysis. **Subrata Paul:** Writing – review & editing, Methodology, Formal analysis. **Michael J. Corley:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Tiffany M. Powell-Wiley:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2025.100976.

Data availability

Data will be made available on request.

References

Amin, M.N., Siddiqui, S.A., Ibrahim, M., Hakim, M.L., Ahammed, M.S., Kabir, A., Sultana, F., 2020. Inflammatory cytokines in the pathogenesis of cardiovascular disease and cancer. SAGE Open Med 8, 2050312120965752. https://10.1177/2050312120965752.

Anastasiadi, D., Esteve-Codina, A., Piferrer, F., 2018. Consistent inverse correlation between DNA methylation of the first intron and gene expression across tissues and species. Epigenetics Chromatin 11, 37. https://10.1186/s13072-018-0205-1.

Andrews, M.R., Ceasar, J., Tamura, K., Langerman, S.D., Mitchell, V.M., Collins, B.S., Baumer, Y., Gutierrez Huerta, C.A., Dey, A.K., Playford, M.P., Mehta, N.N., Powell-Wiley, T.M., 2021. Neighborhood environment perceptions associate with depression levels and cardiovascular risk among middle-aged and older adults: data from the Washington, DC cardiovascular health and needs assessment. Aging Ment. Health 25, 2078–2089. https://10.1080/13607863.2020.1793898.

Aryee, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., Irizarry, R.A., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369. https://10.1093/bioinformatics/btu049.

Banerjee, S., Biehl, A., Gadina, M., Hasni, S., Schwartz, D.M., 2017. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 77, 521–546. https://10.1007/s40265-017-0701-9.

Baumer, Y., Pita, M.A., Baez, A.S., Ortiz-Whittingham, L.R., Cintron, M.A., Rose, R.R., Gray, V.C., Osei Baah, F., Powell-Wiley, T.M., 2023. By what molecular mechanisms do social determinants impact cardiometabolic risk? Clin. Sci. (Lond.) 137, 469–494. https://10.1042/cs20220304.

Bekhbat, M., Rowson, S.A., Neigh, G.N., 2017. Checks and balances: the glucocorticoid receptor and NFκB in good times and bad. Front. Neuroendocrinol. 46, 15–31. https://10.1016/j.yfrne.2017.05.001.

Campagne, D.M., 2019. Stress and perceived social isolation (loneliness). Arch. Gerontol. Geriatr. 82, 192–199. https://10.1016/j.archger.2019.02.007.

Carpenter, R.L., Lo, H.W., 2014. STAT3 target genes relevant to human cancers. Cancers 6, 897–925. https://10.3390/cancers6020897.

Ceasar, J., Peters-Lawrence, M.H., Mitchell, V., Powell-Wiley, T.M., 2017. The communication, awareness, relationships and empowerment (C.A.R.E.) model: an effective tool for engaging urban communities in community-based participatory research. Int. J. Environ. Res. Publ. Health 14, 1422.

Cole, S.W., 2008. Social regulation of leukocyte homeostasis: the role of glucocorticoid sensitivity. Brain Behav. Immun. 22, 1049–1055. https://10.1016/j.bbi.2008.02.006.

Cole, S.W., Hawkley, L.C., Arevalo, J.M., Sung, C.Y., Rose, R.M., Cacioppo, J.T., 2007. Social regulation of gene expression in human leukocytes. Genome Biol. 8, R189. https://10.1186/gb-2007-8-9-r189.

Conway, M.E., McDaniel, J.M., Graham, J.M., Guillen, K.P., Oliver, P.G., Parker, S.L., Yue, P., Turkson, J., Buchsbaum, D.J., Welm, B.E., Myers, R.M., Varley, K.E., 2020. STAT3 and GR cooperate to drive gene expression and growth of basal-like triple-negative breast cancer. Cancer Res. 80, 4355–4370. https://10.1158/0008-5472.Can-20-1379.

Dar, T., Radfar, A., Abohashem, S., Pitman, R.K., Tawakol, A., Osborne, M.T., 2019. Psychosocial stress and cardiovascular disease. Curr. Treat. Options Cardiovasc. Med. 21, 23. https://10.1007/s11936-019-0724-5.

Dye, C.K., Corley, M.J., Ing, C., Lum-Jones, A., Li, D., Mau, M., Maunakea, A.K., 2022. Shifts in the immunoepigenomic landscape of monocytes in response to a diabetes-specific social support intervention: a pilot study among Native Hawaiian adults with diabetes. Clin. Epigenet. 14, 91. https://10.1186/s13148-022-01307-6.

Eaton, W.W., Smith, C., Ybarra, M., Muntaner, C., Tien, A., 2004. Center for epidemiologic studies depression Scale: review and revision (CESD and CESD-R). In: Maruish, M.E. (Ed.), The Use of Psychological Testing for Treatment Planning and Outcomes Assessment: Instruments for Adults. Lawrence Erlbaum Associates Publishers, pp. 363–377.

Emami, H., Singh, P., MacNabb, M., Vucic, E., Lavender, Z., Rudd, J.H., Fayad, Z.A., Lehrer-Graiwer, J., Korsgren, M., Figueroa, A.L., Fredrickson, J., Rubin, B., Hoffmann, U., Truong, Q.A., Min, J.K., Baruch, A., Nasir, K., Nahrendorf, M., Tawakol, A., 2015. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardiosplenic axis in humans. JACC Cardiovasc Imaging 8, 121–130. https://10.1016/j.jcmg.2014.10.009.

Fan, P., Siwak, D.R., Abderrahman, B., Agboke, F.A., Yerrum, S., Jordan, V.C., 2019. Suppression of nuclear factor-κb by glucocorticoid receptor blocks estrogen-induced apoptosis in estrogen-deprived breast cancer cells. Mol. Cancer Therapeut. 18, 1684–1695. https://10.1158/1535-7163.Mct-18-1363.

Farmer, N., Gutierrez-Huerta, C.A., Turner, B.S., Mitchell, V.M., Collins, B.S., Baumer, Y., Wallen, G.R., Powell-Wiley, T.M., 2021. Neighborhood environment associates with trimethylamine-N-oxide (TMAO) as a cardiovascular risk marker. Int. J. Environ. Res. Publ. Health 18. https://10.3390/ijerph18084296.

Ferrara, N.C., Vantrease, J.E., Loh, M.K., Rosenkranz, J.A., Rosenkranz, J.A., 2020. Chapter 11 - protect and harm: effects of stress on the amygdala. In: Urban, J.H., Rosenkranz, J.A. (Eds.), Handbook of Behavioral Neuroscience. Elsevier, pp. 241–274. https://doi.org/10.1016/B978-0-12-815134-1.00011-8.

Gharios, C., van Leent, M.M.T., Chang, H.L., Abohashem, S., O'Connor, D., Osborne, M. T., Tang, C.Y., Kaufman, A.E., Robson, P.M., Ramachandran, S., Calcagno, C., Mani, V., Trivieri, M.G., Seligowski, A.V., Dekel, S., Mulder, W.J.M., Murrough, J. W., Shin, L.M., Tawakol, A., Fayad, Z.A., 2024. Cortico-limbic interactions and carotid atherosclerotic burden during chronic stress exposure. Eur. Heart J. 45, 1753–1764. https://10.1093/eurheartj/ehae149.

Grundy, S.M., Stone, N.J., Bailey, A.L., Beam, C., Birtcher, K.K., Blumenthal, R.S., Braun, L.T., de Ferranti, S., Faiella-Tommasino, J., Forman, D.E., Goldberg, R., Heidenreich, P.A., Hlatky, M.A., Jones, D.W., Lloyd-Jones, D., Lopez-Pajares, N., Ndumele, C.E., Orringer, C.E., Peralta, C.A., Saseen, J.J., Smith Jr., S.C., Sperling, L., Virani, S.S., Yeboah, J., 2019. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a Report of the American College of cardiology/American Heart association task force on clinical practice guidelines. Circulation 139, e1082–e1143. https://10.1161/cir.0000000000000625.

Herman, J.P., McKlveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., Myers, B., 2016. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Compr. Physiol. 6, 603–621. https://10.1002/cphy.c150015.

Hobson, J.M., Moody, M.D., Sorge, R.E., Goodin, B.R., 2022. The neurobiology of social stress resulting from Racism: implications for pain disparities among racialized minorities. Neurobiol Pain 12, 100101. https://10.1016/j.ynpai.2022.100101.

Hu, C., Liu, X., Zeng, Y., Liu, J., Wu, F., 2021. DNA methyltransferase inhibitors combination therapy for the treatment of solid tumor: mechanism and clinical application. Clin. Epigenet. 13, 166. https://10.1186/s13148-021-01154-x.

Hu, D., Wan, L., Chen, M., Caudle, Y., LeSage, G., Li, Q., Yin, D., 2014. Essential role of IL-10/STAT3 in chronic stress-induced immune suppression. Brain Behav. Immun. 36, 118–127. https://10.1016/j.bbi.2013.10.016.

Johnstone, S.E., Baylin, S.B., 2010. Stress and the epigenetic landscape: a link to the pathobiology of human diseases? Nat. Rev. Genet. 11, 806–812. https://10.1038/nrg2881.

Kishore, R., Verma, S.K., 2012. Roles of STATs signaling in cardiovascular diseases. JAK-STAT 1, 118–124. https://10.4161/jkst.20115.

Liden, J., Rafter, I., Truss, M., Gustafsson, J.-Å., Okret, S., 2000. Glucocorticoid effects on NF-κB binding in the transcription of the ICAM-1 gene. Biochem. Biophys. Res. Commun. 273, 1008–1014. https://doi.org/10.1006/bbrc.2000.3079.

Liu, T., Zhang, L., Joo, D., Sun, S.-C., 2017. NF-κB signaling in inflammation. Signal Transduct. Targeted Ther. 2, 17023. https://10.1038/sigtrans.2017.23.

Luo, Y., Alexander, M., Gadina, M., O'Shea, J.J., Meylan, F., Schwartz, D.M., 2021. JAK-STAT signaling in human disease: from genetic syndromes to clinical inhibition. J. Allergy Clin. Immunol. 148, 911–925. https://10.1016/j.jaci.2021.08.004.

Maugeri, A., 2020. The effects of dietary interventions on DNA methylation: implications for obesity management. Int. J. Mol. Sci. 21. https://10.3390/ijms21228670.

McCutcheon, R., Bloomfield, M.A.P., Dahoun, T., Quinlan, M., Terbeck, S., Mehta, M., Howes, O., 2018. Amygdala reactivity in ethnic minorities and its relationship to the social environment: an fMRI study. Psychol. Med. 48, 1985–1992. https://10.1017/s0033291717003506.

Miranda, T.B., Jones, P.A., 2007. DNA methylation: the nuts and bolts of repression. J. Cell. Physiol. 213, 384–390. https://10.1002/jcp.21224.

Muscatell, K.A., Dedovic, K., Slavich, G.M., Jarcho, M.R., Breen, E.C., Bower, J.E., Irwin, M.R., Eisenberger, N.I., 2015. Greater amygdala activity and dorsomedial prefrontal-amygdala coupling are associated with enhanced inflammatory responses to stress. Brain Behav. Immun. 43, 46–53. https://10.1016/j.bbi.2014.06.201.

Nuttall, F.Q., 2015. Body mass index: obesity, BMI, and health: a critical review. Nutr. Today 50, 117–128. https://10.1097/nt.0000000000000092.

Ortiz-Whittingham, L.R., Baumer, Y., Pang, A.P.S., Sampson, M., Baez, A.S., Rose, R.R., Noonan, S.H., Mendez-Silva, J., Collins, B.S., Mitchell, V.M., Cintron, M.A., Farmer, N., Remaley, A.T., Corley, M.J., Powell-Wiley, T.M., 2023. Associations between neighborhood socioeconomic deprivation, IFNγ, and high-density lipoprotein particle size: data from the Washington, D.C. cardiovascular health and needs assessment. Psychoneuroendocrinology 157, 106346. https://10.1016/j.psyneuen.2023.106346.

Ortiz-Whittingham, L.R., Zhan, L., Ortiz-Chaparro, E.N., Baumer, Y., Zenk, S., Lamar, M., Powell-Wiley, T.M., 2024. Neighborhood perceptions are associated with intrinsic amygdala activity and resting-state connectivity with salience network nodes among older adults. Psychosom. Med. 86, 116–123. https://10.1097/psy.0000000000001272.

Pahk, K., Joung, C., Kwon, H.W., Kim, S., 2022. Chronic physical exercise alleviates stress-associated amygdala metabolic activity in obese women: a prospective serial (18)F-FDG PET/CT study. Front. Endocrinol. 13, 1046838. https://10.3389/fendo.2022.1046838.

Pang, A.P.S., Higgins-Chen, A.T., Comite, F., Raica, I., Arboleda, C., Went, H., Mendez, T., Schotsaert, M., Dwaraka, V., Smith, R., Levine, M.E., Ndhlovu, L.C., Corley, M.J., 2022. Longitudinal study of DNA methylation and epigenetic clocks prior to and following test-confirmed COVID-19 and mRNA vaccination. Front. Genet. 13, 819749. https://10.3389/fgene.2022.819749.

Patel, N.H., Osborne, M.T., Teague, H., Parel, P., Svirydava, M., Sorokin, A.V., Teklu, M., Manyak, G., Zhou, W., Pantoja, C., Scott, C., Playford, M.P., Kapoor, P., Rodante, J. A., Keel, A., Chen, M., Tawakol, A., Mehta, N.N., 2021. Heightened splenic and bone marrow uptake of 18F-FDG PET/CT is associated with systemic inflammation and subclinical atherosclerosis by CCTA in psoriasis: an observational study. Atherosclerosis 339, 20–26. https://doi.org/10.1016/j.atherosclerosis.2021.11.008.

Pedersen, S.S., von Kanel, R., Tully, P.J., Denollet, J., 2017. Psychosocial perspectives in cardiovascular disease. Eur J Prev Cardiol 24, 108–115. https://10.1177/2047487317703827.

Pollard, K.S., Dudoit, S., van der Laan, M.J., 2005. Multiple testing procedures: the multtest package and applications to genomics. In: Gentleman, R., Carey, V.J., Huber, W., Irizarry, R.A., Dudoit, S. (Eds.), Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Springer, New York, New York, NY, pp. 249–271. https://10.1007/0-387-29362-0_15.

Powell-Wiley, T.M., Baumer, Y., Baah, F.O., Baez, A.S., Farmer, N., Mahlobo, C.T., Pita, M.A., Potharaju, K.A., Tamura, K., Wallen, G.R., 2022. Social determinants of cardiovascular disease. Circ. Res. 130, 782–799. https://10.1161/CIRCRESAHA.121.319811.

Powell-Wiley, T.M., Dey, A.K., Rivers, J.P., Chaturvedi, A., Andrews, M.R., Ceasar, J.N., Claudel, S.E., Mitchell, V.M., Ayers, C., Tamura, K., Gutierrez-Huerta, C.A., Teague, H.L., Oeser, S.G., Goyal, A., Joshi, A.A., Collins, B.S., Baumer, Y., Chung, S. T., Sumner, A.E., Playford, M.P., Tawakol, A., Mehta, N.N., 2021. Chronic stress-related neural activity associates with subclinical cardiovascular disease in a community-based cohort: data from the Washington, D.C. Cardiovascular health and needs assessment. Front. Cardiovasc. Med. 8, 599341. https://10.3389/fcvm.2021.599341.

RCoreTeam, 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Ridker, P.M., Everett, B.M., Thuren, T., MacFadyen, J.G., Chang, W.H., Ballantyne, C., Fonseca, F., Nicolau, J., Koenig, W., Anker, S.D., Kastelein, J.J.P., Cornel, J.H., Pais, P., Pella, D., Genest, J., Cifkova, R., Lorenzatti, A., Forster, T., Kobalava, Z., Vida-Simiti, L., Flather, M., Shimokawa, H., Ogawa, H., Dellborg, M., Rossi, P.R.F., Troquay, R.P.T., Libby, P., Glynn, R.J., 2017. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N. Engl. J. Med. 377, 1119–1131. https://10.1056/NEJMoa1707914.

Rönn, T., Volkov, P., Davegårdh, C., Dayeh, T., Hall, E., Olsson, A.H., Nilsson, E., Tornberg, A., Dekker Nitert, M., Eriksson, K.F., Jones, H.A., Groop, L., Ling, C., 2013. A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. PLoS Genet. 9, e1003572. https://10.1371/journal.pgen.1003572.

Sergerie, K., Chochol, C., Armony, J.L., 2008. The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. Neurosci. Biobehav. Rev. 32, 811–830. https://10.1016/j.neubiorev.2007.12.002.

Stiekema, L.C.A., Schnitzler, J.G., Nahrendorf, M., Stroes, E.S.G., 2017. The maturation of a 'neural-hematopoietic' inflammatory axis in cardiovascular disease. Curr. Opin. Lipidol. 28, 507–512. https://10.1097/MOL.0000000000000457.

Tawakol, A., Ishai, A., Takx, R.A., Figueroa, A.L., Ali, A., Kaiser, Y., Truong, Q.A., Solomon, C.J., Calcagno, C., Mani, V., Tang, C.Y., Mulder, W.J., Murrough, J.W., Hoffmann, U., Nahrendorf, M., Shin, L.M., Fayad, Z.A., Pitman, R.K., 2017. Relation between resting amygdalar activity and cardiovascular events: a longitudinal and cohort study. Lancet 389, 834–845. https://10.1016/S0140-6736(16)31714-7.

Tawakol, A., Osborne Michael, T., Wang, Y., Hammed, B., Tung, B., Patrich, T., Oberfeld, B., Ishai, A., Shin Lisa, M., Nahrendorf, M., Warner Erica, T., Wasfy, J., Fayad Zahi, A., Koenen, K., Ridker Paul, M., Pitman Roger, K., Armstrong Katrina, A., 2019. Stress-associated neurobiological pathway linking socioeconomic disparities to cardiovascular disease. J. Am. Coll. Cardiol. 73, 3243–3255. https://doi.org/10.1016/j.jacc.2019.04.042.

Turner, R.J., Marino, F., 1994. Social support and social structure: a descriptive epidemiology. J. Health Soc. Behav. 35, 193–212. https://10.2307/2137276.

van der Valk, F.M., Kuijk, C., Verweij, S.L., Stiekema, L.C.A., Kaiser, Y., Zeerleder, S., Nahrendorf, M., Voermans, C., Stroes, E.S.G., 2017. Increased haematopoietic activity in patients with atherosclerosis. Eur. Heart J. 38, 425–432. https://10.1093/eurheartj/ehw246.

Wagner, M.A., Siddiqui, M.A., 2012. The JAK-STAT pathway in hypertrophic stress signaling and genomic stress response. JAK-STAT 1, 131–141. https://10.4161/jkst.20702.

Walsh, C.P., Bovbjerg, D.H., Marsland, A.L., 2021. Glucocorticoid resistance and β2-adrenergic receptor signaling pathways promote peripheral pro-inflammatory conditions associated with chronic psychological stress: a systematic review across species. Neurosci. Biobehav. Rev. 128, 117–135. https://doi.org/10.1016/j.neubiorev.2021.06.013.

Williams, D.R., 2018. Stress and the mental health of populations of color: advancing our understanding of race-related stressors. J. Health Soc. Behav. 59, 466–485. https://10.1177/0022146518814251.

Williams, J.W., Huang, L.H., Randolph, G.J., 2019. Cytokine circuits in cardiovascular disease. Immunity 50, 941–954. https://10.1016/j.immuni.2019.03.007.

Wolf, J.M., Rohleder, N., Bierhaus, A., Nawroth, P.P., Kirschbaum, C., 2009. Determinants of the NF-κB response to acute psychosocial stress in humans. Brain Behav. Immun. 23, 742–749. https://doi.org/10.1016/j.bbi.2008.09.009.

Wong, N.D., Budoff, M.J., Ferdinand, K., Graham, I.M., Michos, E.D., Reddy, T., Shapiro, M.D., Toth, P.P., 2022. Atherosclerotic cardiovascular disease risk assessment: an American Society for Preventive Cardiology clinical practice statement. Am J Prev Cardiol 10, 100335. https://10.1016/j.ajpc.2022.100335.

Wright, R.J., 2009. Stress and acquired glucocorticoid resistance: a relationship hanging in the balance. J. Allergy Clin. Immunol. 123, 831–832. https://10.1016/j.jaci.2009.02.017.

Xu, Z., Niu, L., Li, L., Taylor, J.A., 2016. ENmix: a novel background correction method for Illumina HumanMethylation 450 BeadChip. Nucleic Acids Res. 44, e20. https://10.1093/nar/gkv907.

Yingling, L.R., Mitchell, V., Ayers, C.R., Peters-Lawrence, M., Wallen, G.R., Brooks, A.T., Troendle, J.F., Adu-Brimpong, J., Thomas, S., Henry, J., Saygbe, J.N., Sampson, D. M., Johnson, A.A., Graham, A.P., Graham, L.A., Wiley Jr., K.L., Powell-Wiley, T., 2017. Adherence with physical activity monitoring wearable devices in a community-based population: observations from the Washington, D.C.,

Cardiovascular Health and Needs Assessment. Transl Behav Med 7, 719–730. https://10.1007/s13142-016-0454-0.

Zhang, X., Ge, T.T., Yin, G., Cui, R., Zhao, G., Yang, W., 2018. Stress-induced functional alterations in amygdala: implications for neuropsychiatric diseases. Front. Neurosci. 12, 367. https://10.3389/fnins.2018.00367.

Zhang, Z., Jones, S., Hagood, J.S., Fuentes, N.L., Fuller, G.M., 1997. STAT3 acts as a Co-activator of glucocorticoid receptor signaling*. J. Biol. Chem. 272, 30607–30610. https://doi.org/10.1074/jbc.272.49.30607.