DNA methylation and exposure to violence among African American young adult males

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ABSTRACT

Exposure to violence (ETV) has been linked to epigenomics mechanisms such as DNA methylation (DNAm). We used epigenetic profiling of blood collected from 32 African American young adult males who lived in Washington DC to determine if changes in DNAm at CpG sites affiliated with nervous and immune system were associated with exposure to violence. Pathway analysis of differentially methylated regions comparing high and low ETV groups revealed an enrichment of gene sets annotated to nervous system and immune ontologies. Many of these genes are known to interact with each other which suggests DNAm alters gene function in the nervous and immune system in response to ETV. Using data from a unique age group, young African American adult males, we provide evidence that lifetime ETV could impact DNA methylation in genes impacted at Central Nervous System and Immune Function sites.

Method:
Methylation analysis was performed on DNA collected from the blood of participants classified with either high or low lifetime ETV. Illumina® MethylationEPIC Beadchips (~850k CpG sites) were processed on the iScan System to examine whole-genome methylation differences. Differentially methylated CpG-sites between high (n = 19) and low (n = 13) groups were identified using linear regression with violence and substance abuse as model covariates. Gene ontology analysis was used to identify enrichment categories from probes annotated to the nearest gene.

Results:
A total of 595 probes (279 hypermethylated; 316 hypomethylated) annotated to 383 genes were considered differentially methylated in association with ETV. Males with high ETV showed elevated methylation in several signaling pathways but were most impacted at Central Nervous System and Immune Function affiliated sites. Eight candidate genes were identified that play important biological roles in stress response to violence with HDAC4 (10%), NR4A3 (11%), NR4A2 (12%), DSCAML1(12%), and ELAVL3 (13%) exhibiting higher levels in the low ETV group and DLGAP1 (10%), SHANK2 (10%), and NRGI(11%) having increased methylation in the high ETV group. These findings suggest that individuals subjected to high ETV may be at risk for poor health outcomes that have not been reported previously.

1. Introduction

For many African Americans (AA), experiencing violence, discrimination, and environmentally induced stressors, such as childhood neglect or abuse, are well documented (Jacobs et al., 2014; Pew Research Center, 2016). AAs carry a disproportionate burden of incidence, morbidity, and mortality from chronic diseases such as hypertension and obesity (Allport et al., 2019; Barengolts et al., 2019; Yang et al., 2019; Li et al., 2019; Faucher et al., 2019; Assari et al., 2019; Nagy et al., 2020; Goode et al., 2017; Go et al., 2014) in addition to exposure to violence (ETV) which has also been shown to have a negative impact on health (Griggs et al., 2019; Goldmann et al., 2011; Woodson et al., 2010; Paranjape and

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Therefore, it is worthwhile to examine if lifetime exposure to violence is a contributing factor in AA health disparity. Lifetime ETV is defined in this study as the cumulative effects of ETV during childhood, before age 18, and exposure to community violence after age 18. The key questions we would like to understand in this study is how lifetime exposure to violence, especially during the most vulnerable periods of childhood and adolescence, translates into changes at a molecular level. One possible mechanism is through methylation changes since ETV has been shown to affect the epigenome (Olofsson et al., 2012). Cytosine DNA methylation (DNAm) in humans occurs primarily at CG dinucleotides, which are also called CpG sites (Alberts, 2008). Over half of the promoters in human genes contain CpG islands which are CpG-rich regions that are binding sites for regulatory factors that modulate gene transcription. The most studied type of epigenetic regulation is by DNAm of the promoter regions (Mansell et al., 2019). DNAm generally acts as an On-Off switch that turns genes On when the DNA in not methylated and Off when the DNA is methylated (Alberts, 2008). Cytosine methylation occurs at the 5th position of the base which faces into the major groove of the DNA helix. Since transcription factors bind to the major groove of DNA, DNAm at CpG islands often blocks transcriptional activators from binding to the promoter regions (Alberts, 2008). That is one of the reasons why DNA methylation usually turns genes off. Another reason why DNAm turns genes off is that methyl-DNA-binding proteins, such as MeCP2, which is mutated in Rett Syndrome patients, binds to methylated cytosines and acts as a transcriptional repressor (Jyot et al., 2013). Blood is usually the surrogate tissue used in humans to DNAm changes caused by environmental or social stressors because other tissues are not generally available in humans (Ebrahimi et al., 2020). In mouse studies, epigenetic changes in the blood often correspond to epigenetic changes in the brain, thus justifying a surrogate tissue approach in humans (McKay et al., 2011). In recent years, contrasting results have been reported about the use of blood to study brain alterations. However, blood can be used to study peripheral rather than central biomarkers.

One remarkable finding over the past couple of decades is that recent epigenetic studies, including those focused on DNAm, have found an association between adverse life experiences (such as exposure to community and family violence, discrimination, and trauma) and modulation of gene regulatory regions that can change behaviors, influence personality, and increase the risk for mental health disorders and psychosocial stressors (Vick and Burris, 2017; Barker et al., 2015; Jovanovic et al., 2017). Other studies have demonstrated the association between DNAm and perceived discrimination among African American women (Barcelona de Mendoza et al., 2018), with the highest rates of perceived discrimination (35%) by African American in comparison to other women (Jacobs et al., 2014). Results from another recent DNAm study in Brazil showed altered gene expression across the lifespan of those who experienced repeated community and domestic violence (Serpeioni et al., 2020). A meta-analysis conducted across five studies exploring the association between DNAm, disadvantaged neighborhoods, and cardiovascular disease risk indicated an association between DNAm changes in expression to the stress- and inflammation-related genes and disadvantaged neighborhoods, and risk of cardiovascular disease (Giurgescu et al., 2019). In a longitudinal study, childhood victimization predicted elevated levels of C-Reactive Protein (CRP) at age 18 with an association that was specific to women (Baldwin et al., 2018). Increased epigenetic aging and epigenetic age are positively correlated with experienced direct, but not witnessed, violence has also been shown (Jovanovic et al., 2017). Additionally, increased levels of DNAm at CpG sites across the genome have also been associated with socioeconomic status (SES) in a cohort of young adults (McDade et al., 2019). While several studies have investigated the relationship of epigenomic changes and stressful life events, such as exposure to violence, in disadvantaged neighborhoods, there is no study that investigate the genomewide changes among African American young adults. To address this issue, we explored the relationship between DNAm and exposure to different levels of violence, using genome-wide DNAm data extracted from whole blood. To our knowledge, there are no published studies that have examined how lifetime exposure to violence is associated with DNAm across the epigenome among AA young adult males. We hypothesized that higher levels of lifetime exposure to violence would result in DNAm changes involved in the immune response in African American young adult males.

2. Methods

2.1. Participants and procedure

This study selected 32 males who scored in the highest (n = 19) and the lowest (n = 13) 30% of 638 African American males and females (aged 18–25) from economically and socially disadvantaged neighborhoods of Washington DC on a self-reported scale measuring lifetime exposure to violence (Lifetime ETV) for epigenetic profiling. The Lifetime ETV scale combined 34 items measuring ETV during childhood (before age 18) and 35 items on exposure to community violence since adulthood. This study compares the high and low ETV male groups to determine if ETV resulted in changes to DNAm sites affiliated with immune function. This study controlled for drug use in the past 30 days. The IRB was approved by Howard University Office of Regulatory Research Compliance (IRB-13-PED-06).

To qualify for inclusion in the study, respondents had to be between the ages of 18 and 25 as of their most recent birthday, self-identify as African American or Black, screen as HIV negative (to exclude those with HIV compromised immune systems from the larger study on immune function), and currently live in one of the predominantly disadvantaged wards in Washington, DC. The full study entailed a comprehensive survey about participants’ ETV before and after the age of 18, adverse life experiences, discrimination, and childhood socioeconomic characteristics, current health problems and symptoms, current drug use, sleep quality measures, depressive symptom measures, and current HIV risk behaviors.

While mouse epigenetic studies of behavior can involve brain tissues, such tissues are impossible to collect in humans except from human brain banks. Instead, people who study epigenetic regulation of behavior in humans utilize a surrogate tissue such as blood or saliva which can be collected in non-invasive manners (Solomon et al., 2018; Murata et al., 2019). Other tissues have been collected in humans for epigenetic studies, such as fat or muscle biopsies (Taylor et al., 2019), but such collections are much more invasive than collecting blood or saliva and consequently more difficult to collect. Despite the limitation of surrogate tissues in humans, several studies have identified epigenetic biomarkers in genes that correlate with stressful conditions in humans (Sen et al., 2015a, 2015b; Intarasununont et al., 2012).

2.2. Survey of exposure to childhood and community violence

To measure exposure to childhood violence, exposure to community violence as adults, the survey included questions from previously developed and tested instruments. The childhood exposure to violence scale contained 34 questions that asked participants to respond to circumstances that might have happened during their childhood from birth through age 18. The response options were “1 time,” “2 times,” “3 times,” “4 times,” “5 times or more,” “no times,” and “prefer not to answer.” This scale has a test-retest reliability coefficient of 0.90 and Cronbach’s α = 0.85 (Finkelhor et al., 2010, 2015; Stith and Hamby, 2002; Little and Hamby, 2001). Community exposure to violence as adults was measured by 35 items. Participants were asked to describe the violence that they experienced, saw, or heard about since they turned 18. The responses were “never,” “once or twice,” “a few times,” “many times,” or “prefer not to answer.” This scale has an internal consistency of 0.85, test-retest reliability of 0.90, and Cronbach’s α = 0.61, 0.79, and 0.86, respectively for violence experienced, seen, and heard (Richters and Saltzman, 1990).
2.3. Genomic DNA extraction method

High molecular weight genomic DNA was extracted from 300 μl of whole blood from participants using the Qiagen DNAeasy DNA extraction kit for blood and tissue (Qiagen Sciences Inc.) according to the manufacturer’s protocol. The concentration and integrity of the DNAs were measured using a NanoDrop 2000c Microvolume Spectrophotometer. The DNA samples (200 ng aliquots) were used in genome-wide DNAm analysis by the Genome Sciences Core at Wayne State University.

2.4. Global methylation analysis

Methylation analysis was performed using Illumina®Methylatio-nePICO Bead chips prepared as described in the Illumina® Infinium® HD Assay Methylation Protocol Guide (15019519 v01) before processing on the Illumina iScan System. Input DNA (250 ng) was bisulfite treated using the Zymo EZ DNA Methylation Kit. Zymo’s Human Methylated and Non-methylated DNA controls are treated with samples. Controls are PCR amplified and run on a gel to confirm both methylated and unmethylated bands are present. After bisulfite conversion was confirmed, the bisulfite treated DNA was manually prepared for sequence-specific array-based hybridization using whole-genome amplification (WGA), enzymatic endpoint fragmentation and chemical precipitation. The WGA product was re-suspended and captured by array hybridization. Arrays were then mounted in the Tecan GenePaint automated slide processor on the Tecan Freedom Evo® robotic liquid handling system for primer extension and staining. The amount of fluorescence was measured and used to determine the methylation level of the CpG sites.

2.5. Differential methylation analysis

Raw data from the Infinium assays underwent quality control including staining, extension, hybridization, and bisulfite conversion checks (Aryee et al., 2014). After probe correction to remove probes with low intensity and normalization (Triche et al., 2013), differentially methylated CpG-sites (expressed as M values) between high (n = 19) and low (n = 13) groups were identified using linear regression with violence and substance abuse as model covariates (Ritchie et al., 2015). Substance abuse was calculated by averaging self-reported alcohol, marijuana, cocaine, glue, and heroin use during the thirty-day window prior to the survey. Average Delta β values indicating the differential methylation were calculated by subtracting the average β value of high violence from that of low violence groups. The differentially methylated probes with gene annotation (|Δβ| ≥ 0.1; p-value ≤ 0.05) were further analyzed for significant biological pathways using gene ontology analysis (Huang et al., 2009).

3. Results

3.1. Descriptive characteristic of participants

Descriptive characteristics and childhood SES variables of participants are summarized in Table 1. The mean age of the participants was 20.7 ± 2.4 and they were all unmarried (1 was engaged). 21.9 % did not finish high school and 62.5% reported completing high school or GED. 81.3 % made less than $15,000 a year and 68.8% grew up in families with incomes < $40,000 a year and 28.1% were unemployed. 72% were exposed to violence during childhood, two-thirds were exposed to community violence during their young adulthood and 78.1% were exposed to both childhood and community violence. 62.5% of participants had at least one drink during their lifetime, 53.1% had ever smoked a cigarette and 68.7% had ever used marijuana. About 47% had restless sleep, and 34.4% felt lonely and fearful in the past week.

The results of genome-wide DNAm analysis of 19 participants with high ETV showed significantly higher DNAm at multiple loci/genes in comparison with the 13 participants with low ETV. Of the 866091 probes on the EPIC Beadchips analyzed, 476292 passed QC across all 32 samples. Probes that had a greater than 10% change in β (p-value ≤ 0.05) between the groups of high and low Lifetime exposure to violence were classified as differentially methylated sites (DMS) (Fig. 1). In total, 595 probes annotated to 383 genes were considered DMS. (See Appendix A supplementary table showing all 383 genes).

Gene Ontology analysis of genes annotated to DMS (top 30 in Table 2), revealed 85 significant categories (FDR ≤ 0.05) with several related to the nervous system: nervous system development (GO:0007099), neuro-axis trans-synaptic signaling (GO:00098916), chemical synaptic transmission (GO:0007268), synaptic signaling (GO:009536), trans-synaptic signaling (GO:0099537), neurogenesis (GO:0022008) and central nervous system development (GO:0007417). Only one category, inflammatory response to antigenic stimulus (GO:0002437), was related to immune function.

Interaction analysis of the 90 genes categorized as related to the nervous system or immune response revealed interactions between 53

| Variables | Mean (SD) or percent |
|-----------|---------------------|
| Age 18-25 | 20.7 (2.4)          |
| Income    |                     |
| <$14,999  | 81.3%               |
| $15,000-$29,999 | 12.5%               |
| $30,000+$ | 6.2%                |
| Education |                     |
| Did not finish HS | 21.9%               |
| High school or GED | 62.5%               |
| Finished vocational or trade school | 3.1%               |
| Attended or graduated college | 12.5%               |
| Unemployment | 28.1%               |
| Source of childhood family income |     |
| People who worked | 71.9%               |
| Welfare or public assistance | 12.5%               |
| Worked and welfare | 15.5%               |
| Childhood Household Income |  |
| <$14,999 | 40.6%               |
| $15,000-$29,999 | 18.8%               |
| $30,000-$39,999 | 9.4%                |
| $40,000-$49,999 | 12.5%               |
| $50,000 and more | 18.8%               |
| Rentedy Residence (% yes) | 59.4%               |
| Lifetime childhood ETV | 72.0%               |
| Lifetime community ETV as adults | 75.0%               |
| Lifetime childhood and community ETV | 78.1%               |
| Ever drank alcohol (yes) | 62.5%               |
| Ever smoked cigarette (yes) | 53.1%               |
| Ever used marijuana (yes) | 68.7%               |
| Felt lonely past week | 34.4%               |
| Restless sleep past week | 46.9%               |
| Felt fearful past week | 34.4%               |

Fig. 1. Volcano plot of differentially methylated CpG sites. CpG sites with an absolute Delta β of 10% (p-value ≤ 0.05) are depicted in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
genes in 3 clusters* (Fig. 2). Our interaction network analysis identified several gene network pathways some that clustered in the central nervous system or immune response pathways. Neuregulin 1 (NRG1) which promotes excitatory neurons has been implicated in schizophrenia (Mei and Xiong, 2008) but also is involved in pro-regenerative immune response (Alizadeh et al., 2018). and dopaminergic neurons (Luo et al., 2008). The large cluster contains several genes involved in innate immunity including Doublecortin Like Kinase 1 (DCLK1) a regulator of IL17, tripartite motif-containing protein 3 (TRIM3), myelin basic protein (MBP), complement C3 (C3), NR4A3 (Nagaoaka et al., 2017; Odagiu et al., 2016; Boulet et al., 2019) and TNF (Zhang et al., 2018; Ozato et al., 2008; Nakagawa et al., 2003; Kerepesi et al., 2006; Francisco et al., 2015). Additional immune related genes include RELA Proto-Oncogene (RELA) a NF-κB subunit expressed in the macrophage (Pittet et al., 2011), HDAC11 which regulates interferon signaling (Cao et al., 2019) and HRAS a critical component of protective immunity (Iborra et al., 2011). Genes with neurological functions include controllers of neuronal apoptosis (APLP1 (Tang et al., 2007), NDRG4 (Wen et al., 2019)), neuronal differentiation (TMP-2 (Perez-Martinez and Jaworski, 2005)), excitatory neurons (FGFR2 (Stevens et al., 2010)), neuronal migration (DAB2IP (Lee et al., 2012)), hippocampal neurons (PRKCH (Buchser et al., 2010)), V2b neurons (FoxN4 (Li et al., 2005)) and axonal growth (RasGRF1). Several genes have ties to neurological disorders including autism (SMAD9 bmp regulator SKI/SMAD4 (Zhang et al., 2017), CACNB2 (Breitenkamp et al., 2014), STX1A (Durdjakova et al., 2014) (Nakamura et al., 2008), UNC13A (Ljupstein et al., 2017) RBFOX1 (Lee et al., 2016)), Alzheimer’s disease (Elavl3 (Ogawa et al., 2018) (Scheckel et al., 2016), DLGAP1 (Hadar et al., 2016), SHANK2 (Zhang et al., 2018; Ozato et al., 2008; Nakagawa et al., 2003; Kerepesi et al., 2006; Francisco et al., 2015). Additional immune related genes include RELA Proto-Oncogene (RELA), NR4A3 an immune category have known interactions. *Not all of the genes from the nervous system or immune response category have known interactions. For example, UNC5A and DSCAML1 are connected to each other but no other gene. It’s not currently known if these genes function in a coordinated way. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

![Fig. 2. Interaction network for genes annotated to differentially methylated sites related to nervous system and immune response. Genes with hyper-methylated sites are depicted in red.](image-url)

### Table 2

| Term                      | Description                             | Fold Enrichment | FDR    |
|---------------------------|-----------------------------------------|-----------------|--------|
| GO:0022610                | biological adhesion                      | 2.219861        | 3.03E-09|
| GO:0007155                | cell adhesion                            | 2.196325        | 6.84E-09|
| GO:0098742                | cell-cell adhesion via plasma membrane adhesion molecules | 4.655715 | 1.21E-06|
| GO:0007275                | multicellular organism development       | 1.486545        | 1.44E-06|
| GO:0044707                | single-multicellular organism process    | 1.40827         | 2.12E-06|
| GO:0032501                | multicellular organismal process         | 1.350336        | 2.27E-06|
| GO:0048731                | system development                       | 1.51563         | 4.27E-06|
| GO:0007156                | homophilic cell adhesion via plasma membrane adhesion molecules | 5.364321 | 5.86E-06|
| GO:0044700                | single organism signaling                | 1.356961        | 2.52E-05|
| GO:0048856                | anatomical structure development         | 1.392844        | 3.19E-05|
| GO:0023052                | Signaling                               | 1.345472        | 4.49E-05|
| GO:0007399                | nervous system development               | 1.744177        | 4.96E-05|
| GO:0044767                | single-organism developmental process    | 1.382599        | 5.69E-05|
| GO:0032502                | developmental process                     | 1.372452        | 6.16E-05|
| GO:0009887                | organ morphogenesis                      | 2.203251        | 7E-05   |
| GO:0098609                | cell-cell adhesion                       | 2.06874         | 8.000116|
| GO:0007154                | cell communication                       | 1.32265         | 8.000152|
| GO:0007267                | cell-cell signaling                      | 1.82729         | 1.543705|
| GO:0030198                | extracellular matrix organization        | 3.032487        | 1.382599|
| GO:0043062                | extracellular structure organization      | 3.023435        | 1.382599|
| GO:0040011                | locomotion                              | 1.770931        | 1.382599|
| GO:0051239                | regulation of multicellular organismal process | 1.543705 | 1.382599|
| GO:0048513                | animal organ development                 | 1.482196        | 8.000152|
| GO:0001501                | skeletal system development              | 2.502209        | 8.000194|
| GO:0051674                | localization of cell                    | 1.800652        | 8.002756|
| GO:0007165                | signal transduction                     | 1.800652        | 8.002756|
| GO:0098916                | anterograde trans-synaptic signaling     | 2.294712        | 2.294712|
| GO:0007268                | chemical synaptic transmission           | 2.294712        | 2.294712|
| GO:0099536                | chemical synaptic transmission           | 2.294712        | 2.294712|

*Not all of the genes from the nervous system or immune response category have known interactions. For example, UNC5A and DSCAML1 are connected to each other but no other gene. It’s not currently known if these genes function in a coordinated way. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Several studies suggest that epigenetic mechanisms such as DNA methylation (DNAm) networks involved in the central nervous system and the immune system. For example, McDade et al. (2019) found that physical maltreatment showed the strongest associations with high poverty children living with their families, particularly in genes related to immune regulation and cellular signaling. More recently, Naumova et al. (2012), found that children raised in an institution since birth showed greater epigenome-wide DNAm compared with high-poverty children living with their families, particularly in genes related to immune regulation and cellular signaling. More recently, based on a cross-sectional sample of high-risk youth, Cecil, et al. (Cecil et al., 2016), sought to characterize the DNAm ‘signatures’ of different forms of maltreatment, using an epigenome-wide approach. They found that physical maltreatment showed the strongest associations with DNAm, implicating multiple genes previously associated with psychiatric and physical disorders (e.g., GABBR1, GRIN2D, CACNA2D4, PSEN2).

We observed variable methylation levels within and across the low and high violence groups. Overall, we observed that for NR4A3, NR4A2, DSCAML1, HDAC4, and ELAV3 there was relatively higher methylation in the low violence group compared to high violence group. In contrast, for DLGAP1, NRG1, and SHANK2, there was relatively lower methylation in the low violence when compared to the higher violence exposure.

4. Discussion

There are several environmental determinants associated with health disparities in the USA including exposure to violence. Exposure to violence can directly influence health through biological mechanisms and can be measured as indicator of stressors. The well characterized biological pathway whereby environmental exposure to violence are transmitted through the body to elicit physiological response is via the so-called hypothalamic-pituitary-adrenal axis (HPA axis) system. Signals from the central nervous system in the form of chemical signals or electrical potential are sent to the HPA axis signifying the release of corticotrophin releasing hormone (CRH). The CRH in turn stimulates biosynthesis and release of adrenocorticotropic hormone that triggers the production of glucocorticoids (cortisol) which are the stress response markers (Welberg et al., 2001).

However, the underlying biological mechanism whereby exposure to violence may alter stressor in the body is unknown. In this study we carried out genome-wide DNAm analysis and found that individuals with high levels of self-reported violence victimization had significant hypermethylation of key CpG sites located in the genes influencing nervous system development, cell adhesion, and cellular signaling as compared to those similarly situated individuals without high violence exposure. Previous studies have shown that experiencing community and domestic violence was associated with gene methylation involved in the neural development in adolescents (Serpeloni et al., 2019), and epigenomic mechanisms possibly associated with risk for health problems later in life in maltreated children. Additionally, increased levels of DNAm at CpG sites across the genome were found to be associated with low socioeconomic status (SES) in a cohort of young adults (McDade et al., 2019).

In our current studies we have observed that high exposure to violence is significantly associated with increased DNAm of genetic networks involved in the central nervous system and the immune system. Several studies suggest that epigenetic mechanisms such as DNA methylation changes play important biological roles in stress response to violence (Fig. 3). We observed variable methylation levels within and across the low and high violence groups. Overall, we observed that for NR4A3, NR4A2, DSCAML1, HDAC4, and ELAV3 there was relatively higher methylation in the low violence group compared to high violence group. In contrast, for DLGAP1, NRG1, and SHANK2, there was relatively lower methylation in the low violence when compared to the higher violence exposure.

Fig. 3. Heatmap for selected genes showing methylation levels (hypomethylation - blue; hypermethylation - orange) across individual subjects classified into the low (green) and high (red) ETV categories. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Of particular interest is the identification of genes including Neuroregulin 1 (NRG1) and nuclear receptor subfamily 4 A member 2 (NR4A2) from our GO analysis to be involved in both the nervous system and immune signal pathways. In support of our observation, a recent study by Uddin et al. (2018), identified methylation at 2 CpG sites in an epigenome-wide association studies to be associated with PTSD. One study also found methylation of NRG1 to be associated with inflammation (Song et al., 2016). Taken together, these observations suggest that aberrant methylation of key regulatory genes in the central nervous system and immune signal pathways may be a potential mechanism for inducing chronic inflammatory changes in various psychiatry disorders including depression, anxiety, and PTSD. Based on their study, Safe et al. (2016), suggest that NR4A2 is important for regulating both inflammation and resolution of inflammatory signaling in activated immune cells and glial cells.

Furthermore, our results showed a gene interaction analysis of the 90 significant genes categorized as related to the nervous system or immune response revealed interactions in 3 clusters. The findings of this study suggest that young African American men who are exposed to high levels of lifetime violence maybe at risk for many health risks and diseases that have not been reported in literature related to this age group. However, we note that no clinical data are available about the participants’ psychiatric symptoms. This will be pursued in a future study.

4.1. Study limitations and strengths

The results of this study should be considered in light of several limitations. First, the sample was only from the baseline phase of a longitudinal study and we cannot infer causal associations between exposure to violence and DNAm. Second, this study was based on 32 DNA samples of young African American, living in Washington DC, who were exposed to low and high exposure to violence and might not have enough power analysis. However, given the lack of study on DNAm among young African American, and the high cost associated with the DNAm testing, this study analyses lay groundwork for building a portrait of the potential contribution of lifetime violence maybe at risk for many health risks and diseases that have not been reported in literature related to this age group. However, we note that no clinical data are available about the participants’ psychiatric symptoms. This will be pursued in a future study.

5. Conclusion

While replication is required, this study suggests that overall genes that are differentially methylated are involved in pathways including neurological apoptosis, differentiation and migration, axonal growth, and neurological disorders including autism, Alzheimer’s, bipolar disorder, and Parkinson’s disease. Future studies will investigate how methylation of these gene network drives health disparities. These analyses lay groundwork for building a portrait of the potential contribution of violence exposure on methylation processes in young African American men.

Declaration of competing interest

The authors have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Appendix A. Supplementary data

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References

Alberts, B., 2008. Molecular Biology of the Cell, fifth ed. Garland Science, New York. Epub 2018/02/23 Alizada, A., Santosh, K.T., Katara, H., Goumi, A.S., Karimi-Abdolrezae, S., 2018. Neuroregulin-1 elicits a regulatory immune response following traumatic spinal cord injury. J. Neuroinflammation 15 (1), 53. https://doi.org/10.1186/s12974-018-1093-y. [pii]. PubMed PMID: 29467061; PubMed Central PMCID: PMC5828667.

Epub 2019/09/08 Barengolts, E., Green, S.J., Chlipala, G.E., Layden, B.T., Eisenberg, Y., Epub 2017/09/05 Baldwin, J.R., Arseneault, L., Caspi, A., Fisher, H.L., Mof. 2014/04/21 Ayere, M.J., Jaffe, A.E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A.P., Hansen, K.D., et al., 2014. Minfin: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30 (10), 1356–1369. https://doi.org/10.1093/bioinformatics/btu496.[pii]. PubMed PMID: 24478389; PubMed Central PMCID: PMC4016708.

Epub 2016/06/23 Asari, S., Wisse, C., Bazargan, M., 2016. Obesity and polypharmacy among African American older adults: gender as the moderator and multimorbidity as the mediator. Int. J. Environ. Res. Publ. Health 16 (12). https://doi.org/10.3390/ijerph16122181. PubMed PMID: 21322752; PubMed Central PMCID: PMCPMC617277.

Epub 2017/09/09 Baldwin, J.R., Arseneault, L., Caspi, A., Fisher, H.L., Moffitt, T.E., Odgers, C.L., et al., 2018. Childhood victimization and inflammation in young adulthood: a genetically sensitive cohort study. Brain Behav. Immun. 67, 211–217. https://doi.org/10.1016/j.bbi.2017.08.025. PubMed PMID: 28867281; PubMed Central PMCID: PMC5951095.

Epub 2011/05/19 Bali, P., Im, H.L., Kenny, P.J., 2011. Methylation, memory and addiction. Epigenetics 6 (6), 671–674. https://doi.org/10.4161/epi.6.6.15905. PubMed PMID: 21586900; PubMed Central PMCID: PMCPMC3428666.

Epub 2017/12/21 Barcelona de Mendoza, V., Huang, Y., Crusto, C.A., Sun, Y.Y., Taylor, J.Y., 2018. Perceived racial discrimination and DNA methylation among African American women in the InterGEN study. Biol. Res. Nurs. 20 (2), 145–152. https://doi.org/10.1177/1099800417748789. PubMed PMID: 29258399; PubMed Central PMCID: PMCPMC5741522.

Epub 2019/09/08 Barengolts, E., Green, S.J., Chipala, G.E., Layden, B.T., Eisenberg, Y., Priyadarshini, M., et al., 2019. Predictors of obesity among gut microbiota biomarkers in African American men with and without diabetes. Microorganisms 7 (9). https://doi.org/10.3390/microorganisms7090220. PubMed PMID: 31491976; PubMed Central PMCID: PMCPMC780321.

Epub 2017/07/25 Barker, E.D., Walton, E., Cecil, C.A.M., 2018. Annual Research Review: DNA methylation as a mediator in the association between risk exposure and child and adolescent psychopathology. JCPP (J. Child Psychol. Psychiatry) 59 (6), 303–322. https://doi.org/10.1111/jcpp.12782. PubMed PMID: 28736860.

Epub 2019/07/10 Boulet, S., Daudelin, J.F., Oudagu, I., Pelletier, A.N., Yun, T.J., Lesage, S., et al., 2019. The orphan nuclear receptor NRA3 controls the differentiation of monocyte-derived dendritic cells following microbial stimulation. Proc. Natl. Acad. Sci. U. S. A. 116 (30), 15150–15159. https://doi.org/10.1073/ pnas.18212961161821296116. PubMed PMID: 31285338.

Epub 2014/04/23 Breitenkamp, A.F., Matthes, J., Nass, R.D., Sinzig, J., Lemhmuhl, K., Nürnberg, P., et al., 2014. Rare mutations of CACNB2 found in autism spectrum disease-affected families alter calcium channel function. PLoS One 9 (4), e95579. https://doi.org/10.1371/journal.pone.0095579PONE-D-13-44841[pii]. PubMed PMID: 24752249; PubMed Central PMCID: PMCPMC404086.

Epub 2010/09/27 Buchover, W.J., Slepak, T.L., Gutierrez-Arenas, O., Bixby, J.L., Lemmon, V.P., 2010. Kinase/phosphatase overexpression reveals pathways regulating hippocampal neuron morphology. Mol. Syst. Biol. 6, 391. https://doi.org/10.1038/msb.2010.52; PubMed PMID: 20664657; PubMed Central PMCID: PMCPMC2925531.

Epub 2015/08/02 Bunin, A., Sisirak, V., Ghosh, H.S., Gajkowska, L.T., Hou, Z.E., Miron, M., et al., 2015. Protein tyrosine phosphatase PTPRS is an inhibitory receptor on human and murine plasmacytoid dendritic cells. Immunity 43 (2), 277–288. https://doi.org/10.1016/j.immuni.2015.07.009S1074-7613(15)00276-9[pii]. PubMed PMID: 26231120; PubMed Central PMCID: PMCPMC4579994.

Caillet, S.J., Briggs, G., Cree, B.A.C., Baranzini, S.E., Fernandez-Vina, M., Ramsey, P.P., et al., 2008. Uncoupling the roles of HLA-DRB1 and HLA-DRB5 genes in multiple sclerosis. J. Immunol. 181 (8), 5473–5480. https://doi.org/10.4049/jimmunol.181.8.5473. PubMed PMID: IS10030286025300039.

Epub 2020/01/23 Calle-Fabregat, C., Morante-Palacios, O., Ballerston, E., 2020. Understanding the relevance of DNA methylation changes in immune differentiation
Epub 2018/11/21 Uddin, M., Ratanatharathorn, A., Armstrong, D., Kuan, P.F., Aiello, A.E., Bromet, E.J., et al., 2018. Epigenetic meta-analysis across three civilian cohorts identifies NRG1 and HGS as blood-based biomarkers for post-traumatic stress disorder. Epigenomics 10 (12), 1585–1601. https://doi.org/10.2217/epi-2018-0049. PubMed PMID: 30456986; PubMed Central PMCID: PMCPMC6331697.

Epub 2018/12/26 Umeda, K., Negishi, M., Katoh, H., 2019. RasGRF1 mediates brain-derived neurotrophic factor-induced axonal growth in primary cultured cortical neurons. Biochem. Biophys. Rep. 17, 56–64. https://doi.org/10.1016/j.bbrep.2018.11.011. S2405-5808(18)30275-9 [pii]. PubMed PMID: 30582008; PubMed Central PMCID: PMC6295856.

Epub 2017/03/04 Vick, A.D., Burris, H.H., 2017. Epigenetics and health disparities. Curr. Epidemiol. Rep. 4 (1), 31–37. https://doi.org/10.1007/s40471-017-0096-x. PubMed PMID: 28255530; PubMed Central PMCID: PMCPMC5327425.

Epub 2001/04/20 Welberg, L.A., Seckl, J.R., Holmes, M.C., 2001. Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotropin-releasing hormone: possible implications for behaviour. Neuroscience 104 (1), 71–79. https://doi.org/10.1016/s0306-4522(01)00065-3. PubMed PMID: 11311532.

Epub 2018/12/30 Wen, L., Liu, L., Li, J., Tong, L., Zhang, K., Zhang, Q., et al., 2019. NDRG4 protects against cerebral ischemia injury by inhibiting p53-mediated apoptosis. Brain Res. Bull. 146, 104–111. S0361-9230(18)30292-2 [pii].10.1016/j.brainresbull.2018.12.010. PubMed PMID: 30593880.

Epub 2011/03/05 Woodson, K.M., Havix, C., Sanders-Phillips, K., 2010. Violence exposure and health related risk among African American adolescent female detainees: a strategy for reducing recidivism. J. Offender Rehabil. 49 (8), 571–584. https://doi.org/10.1080/10509674.2010.519669. PubMed PMID: 21373205; PubMed Central PMCID: PMCPMC3045759.

Epub 2016/10/28 Wu, Q., Yang, X., Zhang, I., Zhang, Y., Feng, L., 2017. Nuclear accumulation of histone deacetylase 4 (HDAC4) exerts neurotoxicity in models of Parkinson’s disease. Mol. Neurobiol. 54 (9), 6970–6983. https://doi.org/10.1007/s12051-016-0199-210.1007/s12051-016-0199-2 [pii]. PubMed PMID: 27785754.

Epub 2019/09/07 Yang, Y., Bai, Q., Zheng, W., Steinwandel, M., Blot, W.J., Shu, X.O., et al., 2019. Oral microbiome and obesity in a large study of low-income and African-American populations. J. Oral Microbiol. 11 (1), 1650597. https://doi.org/10.1080/20002297.2019.1650597. PubMed PMID: 31489128; PubMed Central PMCID: PMCPMC6713186.

Zhang, S., Takaku, M., Zou, L.Y., Gu, A.D., Chou, W.C., Zhang, G., et al., 2017. Reversing SKI-SMAD4-mediated suppression is essential for TH17 cell differentiation. Nature 551 (7678), 105. https://doi.org/10.1038/nature24282. PubMed PMID: 28641229; PubMed Central PMCID: PMCPMC5512294.

Epub 2018/04/01 Zhang, Y., Zoltan, M., Riquelme, E., Xu, H., Sahin, I., Castro-Pando, S., et al., 2018. Immune cell production of interleukin 17 induces stem cell features of pancreatic intraepithelial neoplasia cells. Gastroenterology 155 (1), 216–223. S0016-5085(18)30348-2 [pii].10.1053/j.gastro.2018.03.041. PubMed PMID: 29604293; PubMed Central PMCID: PMCPMC6050795.

Epub 2003/02/18 Zhong, Z., Wheeler, M.D., Li, X., Froh, M., Schemmer, P., Yin, M., et al., 2003. L-Glycerol: a novel anti-inflamatory, immunomodulatory, and cytoprotective agent. Curr. Opin. Clin. Nutr. Metab. Care 6 (2), 229–240. https://doi.org/10.1097/01.mco.0000058609.19236.a4. PubMed PMID: 12589194.