HHS Public Access

Author manuscript

Biol Res Nurs. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Biol Res Nurs. 2018 March ; 20(2): 145–152. doi:10.1177/1099800417748759.

Perceived racial discrimination and DNA methylation among African American women in the InterGEN study

Veronica Barcelona de Mendoza, PhD, MSN, MPH, RN, PHNA-BC[a], **Yunfeng Huang, MPH**[b], **Cindy A. Crusto, PhD**[c], **Yan V. Sun, PhD**[b], and **Jacquelyn Y. Taylor, PhD, PNP-BC, RN, FAHA, FAAN**[a]

Veronica Barcelona de Mendoza: veronica.barcelona@yale.edu; Yunfeng Huang: yunfeng.huang@emory.edu; Cindy A. Crusto: cindy.crusto@yale.edu; Yan V. Sun: yan.v.sun@emory.edu

[a]Yale School of Nursing, 400 West Campus Drive, Orange, CT 06477

[b]Emory University Rollins School of Public Health, Department of Epidemiology, 201 Dowman Drive, Atlanta, Georgia 30322

[c]Yale School of Medicine, Department of Psychiatry, 300 George St, New Haven, CT 06511

Abstract

Introduction—Experiences of racial discrimination have been associated with poor health outcomes. Little is known, however, about how perceived racial discrimination influences DNA methylation (DNAm) among African Americans. Here, we examine the association of experiences of discrimination and DNAm among African American women in the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure Study (InterGEN).

Methods—The InterGEN study examines the effects of genetic and psychological factors on blood pressure among African American women and their children. Measures include the Major Life Discrimination (MLD) and the Race-Related Events (RES) scales. The 850K EPIC Illumina BeadChip was used for evaluating DNAm in the Epigenome-Wide Association Study (EWAS). In this analysis, we examined discrimination and DNAm at baseline in the InterGEN study.

Results—A total of 152 women contributed data for the RES-EWAS analysis and 147 for the MLD-EWAS analysis. Most were between 30-39 years old, non-smokers, had some college education, and had incomes <$15,000/year. After controlling for age, smoking and cell composition, MLD was significantly associated with DNAm at nine CpG sites (FDR corrected p<. 05). For the RES-EWAS analysis,no DNAm sites passed the epigenome-wide significance level after genomic control. Suggestive associations were observed between DNAm and RES at CpG sites after genomic control (raw $p<10^{-5}$).

Conclusion—We observed significant epigenetic associations between disease-associated genes (e.g. schizophrenia, bipolar disorder and asthma) and perceived discrimination as measured by the MLD scale. Future health disparities research should include epigenetics in high-risk populations to elucidate functional consequences induced by the psychosocial environment.

Corresponding author: Yale School of Nursing, 400 West Campus Drive, Orange, CT 06477, Phone: (203) 737-2364, Fax: (203) 785-6455, jacquelyn.taylor@yale.edu.

Declaration of Conflicting Interests: The Authors declare that there are no conflict of interests.

Keywords

DNA methylation; Epigenomics; African Americans; Women; Racism

Perceived experiences of discrimination have been associated with stress and poor physical and mental health outcomes for adults consistently in nursing and medical literature (Paradies et al., 2015; Williams & Mohammed, 2009), including increased risk of chronic illness such as hypertension (Orom, Sharma, Homish, Underwood, & Homish, 2016) and obesity (Cunningham et al., 2013), as well as preterm birth (Alhusen, Bower, Epstein, & Sharps, 2016) and depression (Molina & James, 2016). These experiences contribute to health disparities between racial/ethnic groups and adversely affect population health (Bailey et al., 2017). Experiences of perceived discrimination or racism have been defined as unfair treatment based on one's race or ethnicity (Clark, Anderson, Clark, & Williams, 1999). This social classification is based on phenotype (Jones, 2001). Studies have increasingly focused on how experiences of interpersonal racism affect biomarkers for disease, such as allostatic load and hormonal dysregulation (Paradies et al., 2015; Williams & Mohammed, 2009). The mechanisms by which these psychosocial experiences affect physical and mental health, however, are still unclear (Brondolo et al., 2011).

One potential mechanism for how negative social exposures (such as perceived discrimination) based on phenotype affect health is through DNAm across the epigenome. DNA methylation (DNAm), or the addition of methyl groups to promoter regions of genes, may effectively silence or activate gene expression. DNAm has been implicated in many disease processes, most notably in cancer (Liang & Weisenberger, 2017), but also in autoimmune diseases (Teruel & Sawalha, 2017), diabetes (Bansal & Pinney, 2017), schizophrenia (Shorter & Miller, 2015), and cardiovascular diseases (Muka et al., 2016). To our knowledge, there are no published studies thus far that have examined how perceived racism and discrimination as an environmental exposure is associated with DNAm across the epigenome among African American women. However, other studies have reported significant interactions between single nucleotide polymorphisms (SNPs) and experiences of discrimination associated with blood pressure among African Americans (Taylor et al., under review; Taylor et al., 2012).

African Americans commonly experience racism or discrimination (Stepanikova & Oates, 2017). A recent national survey by the Pew Research Center found that 71% of African Americans reported being treated unfairly because of their race, and 11% said this discrimination was a regular occurrence (Pew Research Center, 2016). One large, longitudinal study of multiethnic women reported that African Americans had the highest rates of perceived discrimination (35%), compared to Chinese (20%), Hispanic (12%) and Caucasian (3%) women (Jacobs et al., 2014). Considering that African Americans carry a disproportionate burden of incidence, morbidity and mortality from chronic diseases such as hypertension and obesity, (Centers for Disease Control and Prevention (CDC), 2005), it is worthwhile to examine how perceived racism and discrimination affect the epigenome. The purpose of the present study was to examine the influence of perceived racism and

discrimination on DNAm in a sample of African American mothers in the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure Study (InterGEN).

Materials and Methods

The InterGEN study is an ongoing, longitudinal cohort study in Connecticut that examines the effects of genetic, epigenetic, and psychological factors on blood pressure. Recruitment began in April 2015, and 159 mother/child dyads have been enrolled as of April 2017. African American women were recruited from early child-care and education (ECE) centers, primary care clinics, and community events (i.e., health fairs). Eligibility criteria are that women: (a) are ≥ 21 years old; (b) identify as African American or Black (via self-report); (c) speak English; (d) not have a psychiatric or cognitive disorder which may limit accuracy of reporting of study data; and (e) have a biological child three to five years old. Full study procedures have been described elsewhere (Crusto, Barcelona de Mendoza, Connell, Sun, & Taylor, 2016; Taylor, Wright, Crusto, & Sun, 2016).

Trained research assistants approached mothers for recruitment, conducted screening to verify eligibility, and obtained written, informed consent per established study protocols. InterGEN consists of four study visits, each approximately six months apart over the span of two years. During the baseline (Time 1) study visit, clinical measurements of blood pressure, height, weight, are taken, and saliva is collected for DNA analysis from both mother and child. Repeated clinical data and psychological measures are collected at the three follow-up visits. Demographic information, health history and psychological measures (including parenting, experiences of perceived racism and discrimination, and depression) are collected through mother's report using Audio Computer-Assisted Self-Interview (ACASI) software. Although an InterGEN research assistant is present for data collection, social desirability bias is minimized through use of computer-based data collection. For this analysis, we examined the association between discrimination (measured by Major Life Discrimination (MLD) and Race-Related Events (RES)) and DNA methylation (EWAS) at baseline (Time 1 in the InterGEN cohort study). Study procedures were reviewed and approved by Yale University's Institutional Review Board (approval #1311012986). Data are available via written request to the InterGEN study investigators.

Experiences of perceived racism and discrimination were measured using two scales. The 22-item Race-Related Events Scale (RES) assesses exposure to stressful and potentially traumatizing experiences of race-related stress in adults (Waelde et al., 2010). These events include being treated unfairly, harassed or hurt because of their race. Participants indicated if they had ever experienced each event (yes/no), and all 22 items were summed for a total RES score, ranging from 0-22. The RES has demonstrated good reliability (α = .78-.88) in a diverse sample of caregivers recruited from Head Start centers (Crusto, Dantzler, Roberts, & Hooper, 2015).

The 9-item Major Discrimination scale assesses experiences of unfair treatment in adults (Krieger, Smith, Naishadham, Hartman, & Barbeau, 2005; Williams, Yan, Jackson, & Anderson, 1997). Participants indicated if they had ever experienced each major discrimination event, such as being unfairly fired, denied a bank loan, or stopped and

searched by the police (yes/no). Each event was attributed to one main reason from 11 options, including gender, race, age, skin color, religion, weight or height, and others. The 9 items were summed (0 = No, 1 = Yes) for a total Major Lifetime Discrimination score (MLD), ranging from 0-9.

Saliva samples for DNA were collected from mothers and children using the Oragene (OG)-500 format tubes (Bahlo et al., 2010), which requires participants to spit until the contents reach the fill line (2 mL). Detailed DNA collection and analysis procedures have been described elsewhere (Taylor et al., 2016). Samples were transported from the field to the research laboratory where they were refrigerated at 4°C until DNA extraction and analysis were completed. Standard protocol for DNA extraction and purification was conducted as indicated in the standard operating procedures guidelines using ReliaPrep kits; more details on DNA and methylation processing can be found in Taylor, Wright, Crusto & Sun, 2016. All tubes and plates that contain an individual's DNA are labeled with a barcode to ensure precise sample tracking, and recorded in the laboratory's computerized freezer inventory via barcodes upon arrival to the laboratory. All DNA pipetting is performed with robotic workstations that incorporate barcode scanning to track the transfer of the biological material from tube to tube, tube to plate, and plate to plate. These customized barcode scanning programs are integrated with the robotics deck configuration and are networked to the laboratory. This concurrent electronic sample tracking ensures that an individual's DNA in each well of every plate generated can be identified for correct merging to genotype calls. These quality control assurance procedures are monitored frequently and updated as needed for continued improvement of sample management. The Illumina Infinium Methylation EPIC (850K) BeadChip was used for epigenome wide DNAm measurement. Quantile-normalization of beta values for autosomal CpG sites was performed. All individual samples passed laboratory based quality control procedures (missing rate <10% and no sex mismatch). CpG sites were excluded if they had detection p-value greater than 0.01 (N=71), had a missing rate greater than 10% (N=514), overlapped with single nucleotide polymorphisms (N=14,184), or were listed in the recent Illumina quality notice (Illumina, 2017) (N=977). A total of 831,658 autosomal and 19,007 X-chromosomal CpG sites were included in the association analyses.

Participants self-reported age in years, whether they smoked cigarettes (yes/no) and other demographic data at the initial interview. We controlled for age and maternal smoking, which are accepted confounders in epigenetic studies (Klebaner et al., 2016). We also adjusted for batch effects, and potential heterogeneity in cell proportions from saliva using the reference-free EWAS (Epigenome-Wide Association Studies) method (Houseman, Molitor, & Marsit, 2014).

All variables were examined using univariate analyses and descriptive statistics, including mean and standard deviation for continuous and frequencies for categorical variables. The correlation coefficient was calculated between the two discrimination variables. The following linear regression model used to study the influence of perceived discrimination on DNAm:

$$Y_i = D_i + \text{smoking status} + \text{age} + e_i,$$

where each discrimination variable (D) was modeled separately, controlling for maternal smoking and age confounding factors, to predict the dependent variable (Y), the beta value of DNAm. We applied the genomic control approach (Devlin & Roeder, 1999) to address residual unmeasured confounding and inflation of type I error. False discovery rate (FDR) was used to correct for multiple comparisons. All analyses were conducted in the R statistical computing environment (R Development Core Team, 2006), and a selection of packages from Bioconductor.

Results

Of the 159 InterGEN participants contributing data for this analysis, six had missing data for the RES discrimination variable, 11 were missing data for the MLD variable, and three were missing maternal smoking status. CpG sites with ≥ 50% missing rate were excluded, and all subjects with complete discrimination data were included in analyses, leaving N=152 for the RES-EWAS analysis and N=147 for the MLD-EWAS analysis. Most participants were between the ages of 30-39 years old, non-smokers, had achieved at least some college education or higher, and had an annual household income of less than $15,000/year. Other demographic information is presented in Table 1. The correlation coefficient was calculated between the two discrimination variables, and was 0.62, indicating moderate correlation between them.

In the EWAS analyses, DNAm at nine CpG sites were found to be associated with MLD after genomic control (FDR corrected $p<0.05$; which indicates that these associations should not be false positive assuming a 0.05 false discovery rate for the whole epigenome (Figures 1a & 1b). Furthermore, reduced DNAm (hypomethylation) was observed with increasing MLD at most of the significantly associated CpG sites (seven out of nine, 77.8%) (Table 2). However, for the RES-EWAS analysis, no DNAm site passed the epigenome-wide significance level after genomic control. Marginal negative and positive associations were observed between DNAm and RES at six and five CpG sites after genomic control ($p<10^{-5}$), respectively. No common DNA methylation site was identified in comparing the top 20 epigenetic associations of RES with the epigenome-wide significant associations of MLD.

Discussion

In this study, we found a significant inverse relationship between perceived racial discrimination measured by the MLD scale, and DNAm in a sample of African American women enrolled in the InterGEN study. Women who reported greater perceived discrimination as measured by the MLD scale had decreased DNAm on 9 genes/CpG sites. However, there were no significant associations found between the RES and DNAm.

The genes associated with increased reports of perceived racism and discrimination and hypomethylation were as follows: *WWOX, LOC101928443* (uncharacterized in HapMap), *ARHGAP15, FAT2, MAD1L1, LRRN3, and SORCS1.* The WW domain-containing

oxidoreductase (*WWOX*)is a protein-coding tumor suppressor gene that has been implicated in various cancers such as osteosarcoma (Wen et al., 2017), colorectal (Tian et al., 2017), and bone and breast cancer (Maroni, Matteucci, Bendinelli, & Desiderio, 2017), among others. Epigenetic mechanisms of *WWOX* have been implicated in influencing the phenotype of certain cancers (Maroni et al., 2017). The functional pathways for *WWOX* includes the transcriptional regulation by the AP-2 (TFAP2) family of transcription factors and gene expression (Gene Cards, 2017), and have also been associated with lung function (Burdett et al., 2017). *ARHGAP15* is also a protein-coding gene that has been associated with cancers and cognitive function in animal models (Sun et al., 2017; Zamboni et al., 2016). The *ARHGAP15* gene is associated with three pathways; GPCR downstream signaling, signaling by GPCR and signal transduction (Path Cards, 2017). FAT Atypical Cadherin 2 (*FAT2*) is another protein-coding tumor suppressor gene that has been linked with skin, spinal, and slower growth of other carcinomas (Cao et al., 2016). ARHGAP15 is also associated with asthma, and interestingly, with educational attainment (Burdett et al., 2017), suggesting education could be used as a proxy phenotype to study genetic influences on mental illness (Okbay et al., 2016). MAD1 Mitotic Arrest Deficient Like 1 (*MAD1L1*) is another tumor suppression protein-coding gene that has been associated with various cancers, but when hypomethylated, recurrence of tumors has followed (Cui et al., 2016). This locus is also associated with Schizophrenia and bipolar disease (Burdett et al., 2017). However, diverse DNAm profiles have been documented on *MAD1L1* across differing multi-ethnic populations residing in the same city (Giuliani et al., 2016). This diversity suggests that the DNAm profile of individuals is influenced by genetic ancestry as well as environmental and demographic stressors (Giuliani et al., 2016).

Leucine Rich Repeat Neuronal 3 (*LRRN3*) is a well-known protein-coding gene that has been significantly associated with inflammation, cigarette smoking and chronic obstructive pulmonary disease (Martin, Talikka, Hoeng, & Peitsch, 2015; Obeidat et al., 2016; Poussin et al., 2017). The *LRRN3* gene has also recently been linked with differential gene expression by age after traumatic brain injury (TBI) (Cho et al., 2016). Lastly, the Sortilin Related VPS10 Domain Containing Receptor 1 (*SORCS1*) is a protein-coding gene that is also associated with a brain disorder, however unlike *LRRN3* it is not TBI but narcolepsy and neurodegeneration in Alzheimer's (Knight et al., 2016; Printy, Verma, Cowperthwaite, Markey, & Alzheimer's Disease Neuroimaging Initiative, 2014; Scheinfeldt et al., 2015).

There were 2 genes in which the significant association between MLD and DNAm was not inversely correlated: one uncharacterized gene and the ZXD Family Zinc Finger C *(ZXDC).* The *ZXDC* gene is a protein-coding gene that has been linked with cardiovascular related diseases such as cerebral arterial disease (Shoemaker et al., 2015). Though the relationship between these genes and cardiovascular diseases requires further examination and study, these genes hold promise to improve understanding of the complex and multigenic response to environmental stimuli and how these responses may affect health.

Other studies have examined how perceived racism and discrimination relate to genetics and the development of chronic diseases on the population level, with much of the focus on hypertension and other common, chronic diseases. One such study investigated how SNPs on genes associated with hypertension interacted with experiences of discrimination to

influence blood pressure among African Americans enrolled in the Jackson Heart Study (N=2937) (Taylor et al., under review). In that study, two SNPs were identified that interacted significantly with MLD on both systolic and diastolic blood pressure in African Americans (Taylor et al., under review). These SNPs are located near the sodium bicarbonate co-transporter gene (*SLC4A5*). Others have examined the relationship between genetic polymorphisms and self-reported skin color (as a proxy for racial discrimination) on high blood pressure in a sample of 137 African American women from Detroit (Taylor et al., 2012). They found that one SNP-skin color interaction was significant, also on the *SLC4A5* gene (Taylor et al., 2012).

Researchers have also examined how family environment influences DNAm in African American populations. Brody and colleagues studied family environments (including parent-child conflict, parental emotional support and chaotic home environment) and epigenetic aging in adolescents from Georgia using two replication cohorts. They found that supportive family environments buffered epigenetic aging and DNAm among African American adolescents exposed to high levels of discrimination (Brody, Miller, Yu, Beach, & Chen, 2016). They also observed that adolescents in less supportive family environments had faster epigenetic aging in immune system cells in peripheral blood (Brody et al., 2016). Wright and colleagues also observed that higher parenting stress was associated with differential DNAm among a sample of African American women (n=74) also enrolled in the InterGEN study, especially in pathways associated with stress signaling (Wright et al., under review).

In the present study, discrimination was measured two ways, and interestingly, statistically significant results were only observed with the MLD variable. The MLD measures all experiences of discrimination over the lifespan, while the RES only captures those related to race. It may be that the cumulative effects of discrimination measured by the MLD resulted in longer and larger effects on the epigenome, which made it easier to detect epigenetic modifications. A large study of African American and Latino adults (N=617) from New York reported interaction between age and lifetime experiences of discrimination on ambulatory blood pressure, noting that effects of discrimination were not observed in younger participants (Beatty Moody et al., 2016). These findings support the idea that the accumulation of experiences of discrimination may be more important than the type of discrimination, when predicting long-term health effects. The measurement of discrimination can also include influences of structural racism as well as other, less studied indices, so that health disparities research can produce meaningful interventions (Riley, 2017). Further research on specific health outcomes accounting for perceived racism and discrimination and DNAm effects in this population are needed to truly understand and address health disparities.

This study had both strengths and limitations. Strengths included measures of discrimination as an environmental factor that has not traditionally been studied in the context of epigenetics, and in an all African American sample. The choice of saliva for EWAS analysis is also a strength as it is an accessible and acceptable tissue type, and a potential limitation, as its generalizability to other tissues may be limited due to tissue/cell type specificity. Some limitations are that our sample was relatively small, and this may have resulted in inadequate power to fully examine the effects of the RES on DNAm. It is encouraging that several

significant CpG sites were identified after stringent controls for multiple testing, confounding, and genomic inflation for MLD. Future replication studies with similar samples are needed to further validate the findings.

In conclusion, this study adds to the sparse literature examining the influence of perceived racial discrimination on DNAm in African Americans. This research is important for nursing practice as a better understanding of social determinants of health and their interplay with epigenetics could lead to improved, individualized treatments for chronic illness, as well as a better delineation of disease risk. Future health disparities research should include how environmental factors contribute to epigenetic changes in this high-risk population. Nursing research can and should incorporate both genome wide and epigenome wide methodologies to explore –omic interactions with perceived racism and discrimination on health outcomes among African Americans.

Acknowledgments

Funding: This work was supported by the National Institutes of Health, National Institute of Nursing Research [R01NR013520].

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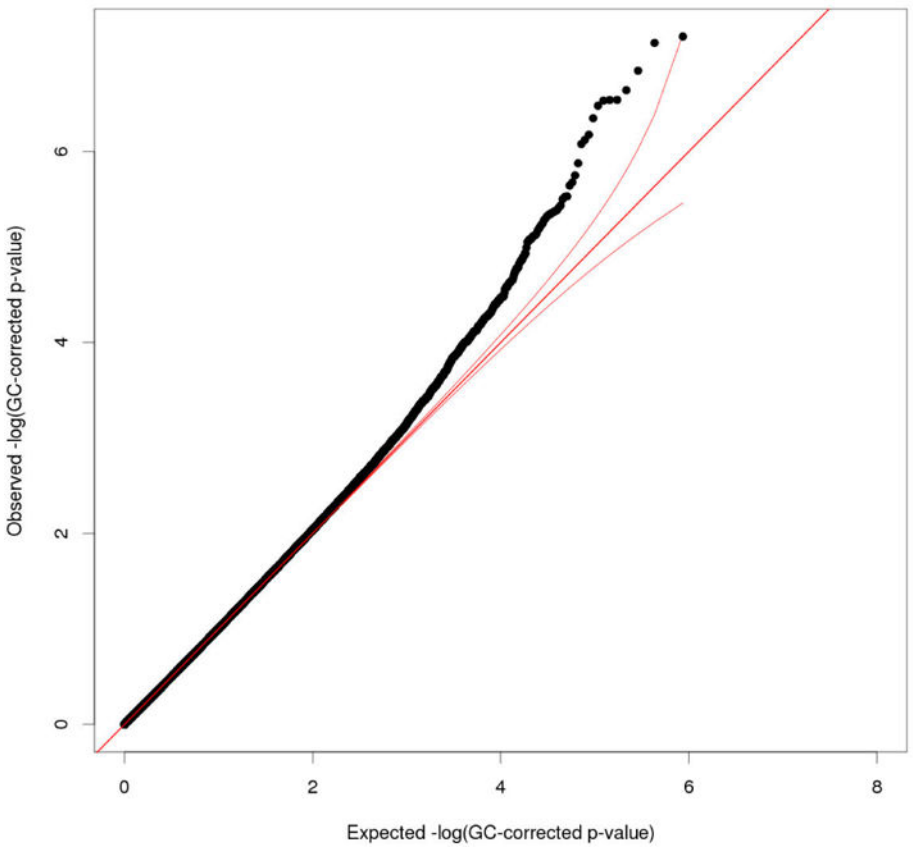
A



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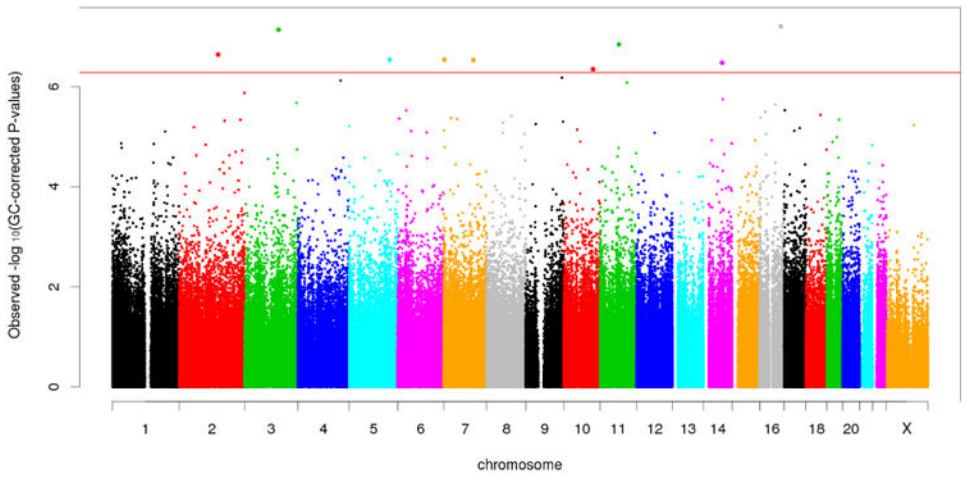

Figure 1.

A. QQ plot - Epigenome-wide Association Analysis with MLD score among n=147 AA mothers. (Genomic control approach was applied on raw p-values. Inflation Factor = 1.00)

B. Manhattan plot - Epigenome-wide Association Analysis with MLD score among 147 AA mothers. (Genomic control approach was applied on raw p-values; Red line: FDR adjusted p-value = 0.05)

Table 1

Baseline characteristics of study population, Intergenerational Blood Pressure Study, N=159.*

	n	%
Primary covariates		
Age		
20-29	64	40.2
30-39	77	48.4
40-49	18	11.3
Maternal Smoker		
No	124	78.0
Yes	32	20.1
Education		
< High School	8	5.1
High School graduate	57	36.3
Some college/graduate	92	58.6
Annual household income		
≤$15,000	71	47.6
>$15,000-$34,999	53	35.5
>$35,000	25	16.7
Health insurance type		
Private	19	12.1
Medicaid	96	61.5
Government/ACA	23	14.7
None	12	7.6
Ever received diagnosis of high blood pressure		
No	131	83.9
Yes	25	16.0
Current high blood pressure medication use		
No	148	94.2
Yes	9	5.7
BMI		
Underweight (<18.5)	9	5.6
Normal weight (18.5-24.9)	40	25.1
Overweight (25-29.9)	44	27.6
Obesity (≥30)	66	41.5

*Numbers may not sum to 159 due to rounding

Table 2

Epigenome-wide Significant Associations with MLD and RES score among AA mothers (n=147).

CpG Sites	Chr	bp	Gene	Exposure	Beta	SE	T-stat	P-value after GC[a]	FDR adjusted p-value[b]
cg09437479	16	78539954	*WWOX*	RES	-1.73E-03	7.47E-04	-2.32	3.56E-02	0.994
				MLD	-1.13E-02	1.88E-03	-6.03	6.25E-08	0.031
cg16143945	3	126168382	*ZXDC*	RES	5.96E-04	1.36E-04	4.38	1.02E-04	0.766
				MLD	2.27E-03	3.79E-04	6.00	7.30E-08	0.031
cg03317714	11	69912795	*LOC101928443*	RES	-1.85E-03	5.65E-04	-3.28	3.16E-03	0.941
				MLD	-8.69E-03	1.49E-03	-5.85	1.43E-07	0.035
cg19958721	2	144455439	*ARHGAP15*	RES	-1.45E-03	5.67E-04	-2.56	2.07E-02	0.981
				MLD	-8.23E-03	1.43E-03	-5.74	2.28E-07	0.035
cg05711042	5	150913871	*FAT2*	RES	-2.59E-03	6.59E-04	-3.93	4.50E-04	0.823
				MLD	-1.14E-02	2.00E-03	-5.69	2.88E-07	0.035
cg02578462	7	2028754	*MAD1L1*	RES	-1.59E-03	4.42E-04	-3.60	1.24E-03	0.888
				MLD	-6.49E-03	1.14E-03	-5.69	2.89E-07	0.035
cg07726178	7	110748868	*LRRN3; IMMP2L*	RES	-1.62E-03	4.10E-04	-3.95	4.27E-04	0.808
				MLD	-6.58E-03	1.16E-03	-5.68	2.94E-07	0.035
cg15209133	14	70020151		RES	2.21E-03	7.21E-04	3.06	5.76E-03	0.957
				MLD	1.13E-02	1.99E-03	5.66	3.32E-07	0.035
cg04928761	10	108756503	*SORCS1*	RES	-3.16E-03	1.01E-03	-3.13	4.87E-03	0.954
				MLD	-1.46E-02	2.62E-03	-5.59	4.49E-07	0.042

[a] GC: genomic control;

[b] FDR adjustment applied after GC approach