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The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) Study: Design and Methods for Complex DNA Analysis

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Abstract

The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) study aims to delineate the independent and interaction effects of genomic (genetic and epigenetic) and psychological–environmental (maternally perceived racial discrimination, mental health, and parenting behavior) factors on blood pressure (BP) among African American mother–child dyads over time. The purpose of this article is to describe the two-step genetic and epigenetic approach that will be executed to explore Gene × Environment interactions on BP using a longitudinal cohort design. Procedure for the single collection of DNA at Time 1 includes the use of the Oragene 500-format saliva sample collection tube, which provides enough DNA for both the Illumina Multi-Ethnic Genotyping and 850K EPIC methylation analyses. BP readings, height, weight, percentage of body fat, and percentage of body water will be measured on all participants every 6 months for 2 years for a total of 4 time points. Genomic data analyses to be completed include multivariate modeling, assessment of population admixture and structure, and extended analyses including Bonferroni correction, false discovery rate methods, Monte Carlo approach, EIGENSTRAT methods, and so on, to determine relationships among both main and interaction effects of genetic, epigenetic, and psychological environmental factors on BP.

Keywords

African Americans, blood pressure, DNA methylation, Infinium MethylationEPIC (850K) BeadChip, gene–environment interaction, women, children

Previous studies have attempted to explain the phenomenon of high blood pressure (BP) among African Americans (AAs) by investigating the independent effects of biological, psychosocial, and other environmental determinants that may contribute to the development of hypertension (HTN). The risk factors these studies have explored include physical activity, salt sensitivity, body mass index (BMI), obesity, gene–environment (G×E) interactions, and psychological stress such as racism (Taylor, Maddox, & Wu, 2009; Taylor, Sun, Chu, Mosley, & Kardia, 2008; Taylor, Sun, Hunt, & Kardia, 2010). Although earlier studies (Boutain, 2001; Clark, Anderson, Clark, & Williams, 1999) independently showed that increases in BP are significantly associated with discrimination-related psychological stress and HTN-related genomic precursors, the interaction between these two variables has not been studied.

The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) study aims to investigate the strength of association of both main effects of genetic (G) and psychological environmental (E) variables and G×E interaction effects of these variables on predicting high BP in AA mothers and their children over time. This study adds to the body of knowledge of G×E interactions for HTN that could aid in reducing health disparities among AAs. We describe study design and analytic methods for the InterGEN study in two articles: In the present article, we explain our analyses of the genetic factors (Gs) related to candidate genes and DNA methylation analyses of AA women and their children's risk for high BP, while the second covers participant recruitment

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and analysis of psychological environmental factors (E) over time. We thus focus here on the selection of candidate genes, DNA collection, laboratory procedures, and genomic and epigenomic data analytic strategies.

Background

Genetics of HTN in AAs

Studies have shown that investigating traditional risk factors (e.g., obesity, gender, race, and other lifestyle habits) is not sufficient to identify causative agents that increase the likelihood of developing HTN (Hansen, Gunn, & Kaelber, 2007; Roger et al., 2011; Taylor et al., 2008; Taylor et al., 2010). The high prevalence of HTN among AAs has been associated with both genomic and environmental factors (Franceschini et al., 2013; Taylor et al., 2009). The heritability of HTN is well documented in AAs, although genetic differences only account for approximately 60% of the variance observed in BP (Fox et al., 2010; Levy et al., 2009; Snieder, Harshfield, & Treiber, 2003). These factors also have great potential to predict high BP in AA children prior to phenotypic expression of HTN (Taylor et al., 2009).

The genes and single nucleotide polymorphisms (SNPs) under investigation in the InterGEN study are positional candidate genes that researchers have most consistently found to be significantly associated with high BP in previous studies among AA adults and children (see references in Tables 1 and 2). Several studies have identified genetic variants associated with high BP in AAs (Table 1). For example, findings have linked the *SLC4A5* gene with HTN susceptibility in AAs in several studies (Barkley et al., 2004; Hunt et al., 2006; Li, Kraev, & Lytton, 2002; Taylor et al., 2009). Large cohort studies have revealed relationships between SNPs located on Chromosome 2 at or near the *SLC4A5* gene and high BP among people of African ancestry (Barkley et al., 2004; Cooper et al., 2002; Morrison et al., 2004). Due to functional similarities (Table 2) to the *SLC4A5* gene, we also selected the *SLC24A4* and *SLC25A42* genes to analyze as candidate genes for BP regulation in AA mothers and children. No prior studies have explored these genes as factors in BP. Research has also demonstrated an association between high BP in AA women and children and the SNP rs1879282 of the *CAPN13* gene, which is involved in apoptosis, cell division, modulation of integrin–cytoskeletal interactions, and synaptic plasticity (Dear & Boehm, 2001). A previous study using rat models determined that *CAPN13* is associated with essential HTN, but the association has not been evaluated in humans (Averna et al., 2001).

Several genes and SNPs on Chromosome 11 have been associated with HTN in AAs. SNP rs679620, located within the *MMP3* gene, has been significantly associated with HTN and obesity, particularly in AA women (Taylor et al., 2008). *MMP3* regulates arterial matrix composition, which is important for atherosclerotic progression and BP regulation (Lifton, Gharavi, & Geller, 2001; Sherva et al., 2011). Researchers have also posited that *MMP3* rs679620 is involved in the progression of atherosclerosis (Kingwell et al., 2001; Taylor et al., 2012). Research evidence identified a strong association between elevated BP and a 1.26-Mb region on Chromosome 11 in AAs (Fox et al., 2011). In the same meta-analysis, researchers identified a number of SNPs across this region of Chromosome 11 (i.e., SNPs in the genes *KCNQ1*, *P2RY2*, and *IPO7*) that were in linkage disequilibrium, with several missense variants from the Coronary Artery Risk Development in Young Adults (CARDIA) study reaching genome-wide significance and warranting further investigation (Fox et al., 2011). The CARDIA cohort, compared to populations from the Atherosclerosis Risk in Communities (ARIC) or Jackson Heart Study (JHS), was composed of much younger individuals, suggesting that associations found on Chromosome 11 are stronger in younger populations (Fox et al., 2011). Studies have also shown other genes on Chromosomes 2 (*PMS1*), 5 (*ARRDC3*/*ADGRV1* and *SUB1*/*NPR3*), 15 (*SV2B*), 16 (*CACNA1H*), and 21 (*C21orf91*) to be significantly associated with HTN (See Table 1 for references).

G×E Interaction and HTN Among AAs

A preliminary study of G×E interactions among AA mother and child dyads between genes and BMI identified an SNP (*CAPN13* rs1879282) with significant ($p < .01$) gene–BMI interaction on both systolic and diastolic BP among AA female offspring after accounting for multiple comparisons (Taylor et al., 2010). This preliminary study provided insight into the interaction of genomic and environmental effects and the ability of their relationship to predict increases in BP among AA mothers and offspring. J. Taylor and colleagues (2008) also found a significant protective effect with the G×E interaction of *MMP3* rs679620 ($p = .0009$) and BMI on diastolic BP among AA women in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. In a more recent study, Taylor, Maddox, and Wu (2009) found significant deleterious G×E interaction effects of low physical activity and rs1017783 (systolic BP: $p = .011$; diastolic BP: $p = .023$) and rs6731545 (systolic BP: $p = .016$; diastolic BP: $p = .049$), both located in *SLC4A5,* in a small sample of 108 AA mothers and daughters. Additional studies by Taylor, Sun, Chu, Mosley, and Kardia (2008), Taylor, Sun, Hunt, and Kardia (2010), Taylor et al. (2012) with samples of less than 200 AA and West African mothers and daughters yielded significant results for genetic precursors for HTN, including *SLC4A5, MMP3*, and *CAPN13*. In the study published in 2012, Taylor et al. also found marked associations between *SLC4A5* and perceptions of racism based on skin tone among 137 AA women and daughters. In a more recent study, Taylor and colleagues (2016) examined discovery and replicate samples of AAs and found that two SNPs located on Chromosomes 14 (*NEDD8* rs11158609: $p = 2.09 \times 10^{-7}$) and 17 (*TTYH2* rs8078051: $p = 9.65 \times 10^{-7}$) were associated with increases in SBP, including the main genetic effects and G×E interactions with cigarette smoking.

Table 1. Genes Associated With Hypertension in African Americans.

Gene Name	Gene Symbol	Function	References
Chromosome 2			
Solute carrier family 4 (sodium bicarbonate cotransporter), member 5	*SLC4A5*	Sodium bicarbonate cotransporter involved in intracellular pH regulation and electrogenic sodium bicarbonate transport	Barkley et al. (2004); Hunt et al. (2006); J. Y. Taylor et al. (2009)
Calpain 13	*CAPN13*	Nonlysosomal, intracellular calcium-activated neutral cysteine protease related to the apoptosis pathway	Averna et al. (2001); Dear and Boehm (2001)
Postmeiotic segregation increased 1	*PMS1*	Encodes a protein involved with DNA-mismatch repair	Adeyemo et al. (2009)
Chromosome 5			
Arrestin domain containing 3/Adhesion G protein-coupled receptor V1	*ARRDC3/ADGRV1*	Encodes protein associated with β-3 adrenergic receptor binding/calcium-binding protein expressed in the central nervous system	Adeyemo et al. (2009), Fox et al. (2011), Morrison et al. (2004)
SUB1 homolog/ natriuretic peptide receptor 3	*SUB1/NPR3*	Associated with transcription coactivator activity and single-stranded DNA binding/encodes a natriuretic receptor that clears circulating and extracellular natriuretic peptides via endocytosis	Adeyemo et al. (2009); Fox et al. (2011); Morrison et al. (2004)
Chromosome 11			
Metallopeptidase 3	*MMP3*	Encodes an enzyme that degrades fibronectin, laminin, cartilage proteoglycans, and some types of gelatin and collagen; activates procollagenase	Lifton, Gharavi, and Geller (2001); Sherva et al. (2011); J. Taylor et al. (2008); J. Y. Taylor et al. (2012)
Potassium channel, voltage gated KQT-like subfamily Q, member 1	*KCNQ1*	Encodes voltage-gated potassium channels required for cardiac repolarization	Fox et al. (2011)
Purinergic receptor P2Y, G-protein coupled, 2	*P2RY2*	Encodes a receptor activated by ATP that is associated with signaling pathways	Fox et al. (2011)
Importin 7	*IPO7*	Mediates nuclear import of proteins via a Ran-dependent transport cycle	Fox et al. (2011)
Chromosome 14			
Solute carrier family 24, member 4	*SLC24A4*	Potassium-dependent sodium/calcium exchanger found to transport intracellular calcium and potassium ions in exchange for extracellular sodium ions	Adeyemo et al. (2009); Li et al. (2002)
Chromosome 15			
Synaptic vesicle glycoprotein 2B	*SV2B*	Protein may play a role in regulation of secretion in neural and endocrine cells	Adeyemo et al. (2009)
Chromosome 16			
Calcium channel, voltage-dependent, T type, α 1H subunit	*CACNA1H*	Encodes a protein involved in voltage-dependent calcium channel activity	Adeyemo et al. (2009)
Chromosome 19			
Solute carrier family 25, member 42	*SLC25A42*	Exchanges coenzyme A for adenosine 3,5-diphosphate across inner mitochondrial membrane	Fiermonte, Paradies, Todisco, Marobbio, and Palmieri (2009); Haitina, Lindblom, Renström, and Fredriksson (2006)
Chromosome 21			
Chromosome 21 open reading frame 91	*C21orf91*	Unknown	Fox et al. (2011)

Note. ATP = adenosine triphosphate.

Epigenetics and HTN

Cells have unique epigenetic codes that add another layer of differential programming information with strong consequences for cellular activity and development (Jenuwein & Allis, 2001). The epigenomic signature of a cell type is composed of chemical modifications (e.g., DNA methylation) to the DNA that do not change the DNA sequence but may result in altered gene expression or contribute to phenotypic variation (Feinberg, 2007). Environmental factors, along with genetics and stochastic processes, are the primary sources of epigenetic variation (Bjornsson, Fallin, & Feinberg, 2004; Sun,

Table 2. African and Caucasian Ancestry Allele Frequencies (AF) in the United States for Single Nucleotide Polymorphisms (SNPs) Associated With Hypertension in African Americans.

SNPs	Position	Alleles	Ref. Allele	AF in African Ancestry	AF in European Ancestry
Chromosome 2					
SLC4A5					
rs10177833	74457718	A/C	A	0.52	.62
rs1667627	74429192	C/T	C	0.18	.47
rs7587117	74448655	C/T	C	0.17	.31
rs7571842	74460904	A/G	A	0.34	.57
rs2034454	74462641	G/T	T	0.10	.56
rs4853018	74466594	A/G	A	0.23	.40
rs7602215	74482892	C/T	C	0.23	.13
rs3771724	74485697	A/G	G	0.44	.86
rs1006502	74486357	A/G	A	0.56	.14
rs4853019	74488698	C/T	T	0.05	.16
CAPN13					
rs1879282	31029835	C/T	C	0.20	.24
PMS1					
rs5743185	190737838	A/G	G	0.96	.90
Chromosome 5					
ARRDC3/ADGRV1					
rs10474346	90564139	C/T	C	0.29	.12
SUB1/NPR3					
rs7726475	32575914	A/G	G	0.93	.65
Chromosome 11					
MMP3					
rs679620	102713620	C/T	T	0.30	.59
KCNQ1					
rs4930130	2619630	A/G	A	0.58	.89
P2RY2					
rs1791926	72944121	C/T	T	0.59	.98
IPO7					
rs12279202	9432090	C/T	C	1.00	.93
Chromosome 14					
SLC24A4					
rs11160059	92807330	C/T	T	0.10	0
Chromosome 15					
SV2B					
rs8039294	91743859	G/T	G	0.49	.93
Chromosome 16					
CACNA1H					
rs3751664	1254369	C/T	C	0.98	.90
Chromosome 19					
SLC25A42					
rs6511018	19186705	A/G	G	0.34	.17
rs12985799	19187575	C/T	C	0.33	.17
rs2012318	19208240	C/T	C	0.34	.17
Chromosome 21					
C21orf91					
rs2258119	19167479	C/T	T	0.66	.80

Note. Frequency data obtained from 1,000 Genomes Human build GRCh37 (http://browser.1000genomes.org/index.html). Populations used for AFs: African Ancestry in southwest US (ASW) and Utah residents with northern and western European Ancestry (CEU).

2014). These epigenetic modifications can explain why individuals with similar genome sequences, such as identical twins, can have different physical characteristics. Twins are epigenetically similar at birth, but as they age different environmental factors cause their epigenomic signatures to differentiate, which may result in varying patterns of gene expression and disease (Fraga et al., 2005). These changes in signature can occur over the life course, and some epigenetic marks can be transferred across generations (i.e., parent–child transmission). For example, early environmental exposure such as maternal diet, mood during pregnancy, early-life socioeconomic status, abuse, and psychological stressors experienced by pregnant mothers can change DNA methylation patterns in the fetus, which may lead to various diseases for these

children later in life (Champagne, 2012; Heijmans et al., 2008; Lam et al., 2012).

Epigenome-wide association studies in AAs have identified DNA methylation differences associated with age (Smith et al., 2014), sex (Sun et al., 2010), BMI (Demerath et al., 2015), cigarette smoking (Sun, Smith, et al., 2013), and inflammation (Sun, Lazarus, et al., 2013). However, few studies have evaluated the relationship between DNA methylation and BP in AAs. Of the studies that have been completed, the results are conflicting related to the direction of methylation change (gain, Wang et al., 2013; or loss, Smolarek et al., 2010), and authors acknowledge that age may explain variability (Wang et al., 2013). Significant G×E interactions related to high BP have also varied by the age of participants (Fox et al., 2011), and we suspect changes in methylation over time could explain some of this variance. To our knowledge, no studies have evaluated DNA methylation related to HTN in AA women and/or their children prior to phenotypic expression as young as 3 years of age.

Genomic Method

Procedures

After acquiring informed consent for genotyping of their DNA for studies of the effects of G×E interaction on BP outcomes, we enroll 250 AA mothers and their biological children aged 3–5 years in the InterGEN study. We provide detailed description of participant recruitment and environmental and interview data collected for the study in a companion article (Crusto, Barcelona de Mendoza, Connell, Sun, & Taylor, In press). Briefly, we enroll English-speaking mothers, 21 years of age or older, who self-identify as AA or Black and have no active psychological or cognitive impairment, with one of their children aged 3–5 years. We conduct face-to-face interviews using audio self-assisted interviewing software to obtain demographic and environmental influence data (e.g., living arrangement, experience of racial discrimination, etc.). Members of the university-based research team have been trained in the collection of DNA samples, BP readings, height and weight measurements for mothers and children, and psychological measures from mothers. Saliva samples will be collected for candidate gene genotyping and DNA methylation analyses at baseline concurrently with BP readings, height, weight, and all psychological measurements. All data and specimens from each participant will be identified through a unique seven-digit number, which we will use for all communications among the investigators to identify participants, their samples, and their data. The Yale University Institutional Review board approved these procedures.

Measures and DNA Sample Collection

BP. Our procedure for preparing participants for BP measurement is in accordance with JNC-7 (The Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) recommendations (Chobanian et al., 2003). We categorize repeated BP readings for children as *low, normal, preHTN,* or *HTN* based on percentile rankings of age, gender, and height, as outlined by the American Academy of Pediatrics (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Adult participants with BP readings of 160/100 or higher will be immediately referred to a hospital emergency department for treatment, which is standard of care.

Height and weight. BMI, which represents the relationship between weight and height (kg/m^2), is associated with body fat and health risk. High BMI (i.e., obesity) is a major risk factor for HTN (Chobanian et al., 2003). We measure weight, percentage of body fat, and percent of body water with a Tanita high-capacity electronic scale (BF-684W/Tanita, Tokyo, Japan) and the Tanita children's scale (BF-689/Tanita Tokyo, Japan). We measure height to the nearest 10th of an inch in barefoot participants. For the purposes of this study, we categorize mothers with BMIs from 25 to 29 kg/m^2 as overweight and those with BMIs of 30 kg/m^2 or more as obese (Chobanian et al., 2003). When assessing risk factors such as BMI in children, the calculation is the same but the cut-off points differ. We thus calculate BMI for children using the standard formula but, for categorization, use BMI-for-age charts for girls and for boys, with percentile rankings in accordance with the Centers for Disease Control and Prevention (2015).

DNA collection. We selected saliva for collection and extraction of DNA due to the noninvasive nature of the collection procedure and its portability. Additionally, we have successfully used saliva in preliminary studies to examine genomic variation in AA women with HTN. We collect saliva samples into Oragene (OG)-500 Format tubes (Bahlo et al., 2010) at baseline for mothers and children. This collection method provides a sufficient amount of DNA for the genome-wide SNP genotyping and DNA methylation analyses for this study. For example, the Ilumina Multi-Ethnic Genotyping Array (MEGA) Beadchip (candidate gene analysis; Illumina, 2015a) and the Infinium MethylationEPIC Beadchip for DNA methylation analysis (Illumina, 2015b; Moran, Arribas, & Esteller, 2016) require <500 ng of DNA for adequate analyses. The OG-500 collection tube allows for the extraction of approximately 110 µg of DNA.

We will ask participants to refrain from eating, drinking, smoking, or chewing gum for 30 min before collecting the saliva sample. Participants will spit into the collection tube several times until the liquid reaches the fill line (2 ml). If participants are unable to produce enough saliva to reach the fill line, we give them a clear, artificially sweetened lollipop that helps them to produce more saliva.

Once samples have been collected, we label all tubes with barcodes to ensure precise sample tracking and enter these barcodes into the computerized freezer inventory when we first receive the samples in the laboratory. Saliva samples will be refrigerated at 4°C until DNA extraction and analysis is completed.

Candidate gene and DNA methylation analysis. We use the Illumina MEGA BeadChip to assess the 14 candidate genes we described above as well as the ancestry informative markers (AIMs) for AAs. We selected the MEGA chip because it has expanded coverage of SNPs for multiethnic populations (Illumina, 2015a). We use the Illumina Infinium Methylation EPIC (850K) BeadChip to analyze epigenome-wide DNA methylation. This BeadChip directly quantifies DNA methylation at 853,307 CpG dinucleotides, giving near complete coverage of known genes. We perform hybridization on a per-sample basis. The Infinium arrays are well annotated for CpG dinucleotides in CpG island and non-CpG island promoters, shore regions, coding regions, repetitive elements, miRNA promoter regions, FANTOM5 enhancers, ENCODE open chromatin and enhancers, and DNase hypersensitivity sites and include 91.1% of the loci from the HumanMethylation450 BeadChip. DNA methylation is determined at each of the CpG sites on the 850K array by measuring the fluorescent signals from the M (methlyated) and U (unmethylated) probes specific for each site included in the array, covering approximately 99% of all RefSeq genes and 96% of CpG islands (Bibikova et al., 2011; Moran et al., 2016). We confirm DNA methylation by methylation-specific polymerase chain reaction (Herman, Graff, Myohanen, Nelkin, & Baylin, 1996) and by bisulfite sequencing (Zinn, Pruitt, Eguchi, Baylin, & Herman, 2007).

Data Analysis

We apply novel statistical approaches to assess the effects of both common and rare variants on quantitative traits in mother–child dyads using potentially functional variants from the Illumina MEGA Beadchip (candidate gene) array. We conduct additional secondary analyses to examine potentially functional putative variants that will be available due to the use of the Beadchip. Genomic data analyses to be completed include multivariate modeling, assessment of population admixture and structure, and extended analyses to determine relationships among epigenomic markers. The first phase of the analysis will focus on identification of large-scale, epigenomic changes in the 14 candidate genes listed in Table 1 and SNPs regulating BP in these regions shown in Table 2.

Power analysis. The statistical analyses will be completed for an estimated sample size of 200 mother–child dyads, which allows for a 20% attrition rate in our proposed sample of 250 families. The outcomes, systolic and diastolic BP, will be examined as continuous variables. Predictors will include both categorical (e.g., gender and genomic polymorphisms) and continuous variables (e.g., age, BMI, and psychological environmental factors). Assuming continuous outcome and additive genomic effects, the estimated sample size will have 80% power to detect about 5% R^2 in 200 unrelated AA subjects using the conservative Bonferroni correction.

Multiple comparisons and Type I error. We will be investigating a number of SNPs as effect modifiers of environmental risk factors with respect to BP measurements and for their main effects on BPs. We will focus on the aforementioned 14 candidate genes for our primary hypotheses, driven by biological plausibility and prior knowledge. However, even when focusing on these a priori hypothesized genes, we will be conducting a large number of statistical tests. We account for this multiple testing by considering the number of tests performed in assessing overall significance. Because the separate hypotheses may not be independent, applying Bonferroni procedure for correction will result in tests that are too conservative. Thus, we will apply false discovery rate (FDR) methods to control for multiple comparisons (Benjamini & Hochberg, 1995; Pritchard, Stephens, & Donnelly, 2000; Pritchard, Stephens, Rosenberg, & Donnelly, 2000; Reich & Lander, 2001; Yekutieli & Benjamini, 1999). The basic FDR adjustment involves ordering the nominal p values obtained for each hypothesis from the least to the greatest, with p_i being the p value corresponding to the ith hypothesis (h_i, $i = 1 \ \ldots \ m$). All h_i, $i = 1, 2, \ \ldots, k$, hypotheses are rejected, where k is the largest i for which $p_i \leq i^*\text{FDR}/m$, where the FDR is defined as

$$\text{FDR} = E\left[\frac{V}{R} R > 0\right] Pr(R > 0),$$

where V is equal to the number of false positive results and R is equal to the number of results found significant by the statistical test (Benjamini & Hochberg, 1995; Stütz et al., 2009). Using an FDR at the 10% level as our threshold for significance will result in 1 out of every 10 significant results, on average, being a false positive. In addition, as a complement to this method, we use a Monte Carlo approach to create an empirical null distribution of the test statistics by permuting outcomes among individuals.

G×E interaction associated with BP measurements. We also evaluate interaction effects associated with BP measurements between SNPs in candidate genes and environmental factors in AA mothers and their children. However, the proportion of African ancestry is a strong predictor of high BP among AAs (Table 2 delineates the variation of allele frequencies [AFs] of the candidate genes and SNPs within African populations; Barkley et al., 2004; Champagne, 2012; Hansen et al., 2007; Harding et al., 2010; Jenuwein & Allis, 2001; Taylor et al., 2009; Taylor et al., 2012). Because of the differences in AFs and their potential influences on BP, we must account for population admixture/structure (confounder of the outcome) in the analysis. For the pooled analysis of all mothers and children, we use a kinship coefficient to model the relatedness in a linear mixed model, which improves power across unrelated individuals (Hu, Hui, & Sun, 2014). To further address this issue, we estimate admixture using AIM sets for African/European ancestry for the 28 SNPs on the 14 candidate genes in all 500 subjects (mothers and children). Based on the results from the STRUCTURE program (Pritchard, Stephens, & Donnelly, 2000) and principal component analysis, we carefully examine and appropriately adjust for population variation in ancestry in

our SNP association analysis and G×E interaction analysis using the methods outlined in EIGENSTRAT (Price et al., 2006). SNPs, measures of environmental factors, and their interactions will then be modeled as predictors of BP phenotypes. Each phenotype and genomic marker will be modeled separately. The general model to be fitted will be as follows:

$$Y_i = \beta_0 + \beta_1 Z_i + \beta_2 E_i + \beta_3 G_{1i} + \beta_4 G_{2i} + \beta_5 G_{1i} \times E_i + \beta_6 G_{2i} \times E_i + e_i,$$

where Y_i is the BP outcome (e.g., systolic and diastolic BP) for person i, Z_i is a vector of adjustment variables, E_i is the relevant psychological environmental measures identified by methods described in the companion paper (Crusto et al., In press), and G_{1i} and G_{2i} are dummy variables to capture variation in SNP genotypes. SNPs with minor AFs of <5% will be flagged and related results interpreted with caution due to suboptimal power. Coefficients β_5 and β_6 will be used to test for G×E interactions. In all genomic analyses, we use appropriate methods to account for multiple testing as outlined above.

DNA methylation analysis. We convert the raw fluorescence data from the 850K array to β values for statistical analyses. Figure 1 outlines the 850K data normalization and quality control preprocessing steps that we use to convert the data to β values. The β values are continuous variables ranging from 0 to 1 and represent the ratio of fluorescence intensity at the methylated probe type compared to the combined fluorescence intensity of the CpG site (methylated + unmethylated probe types). Specifically, we use the following formula to calculate the ratio of fluorescent signals from the two alleles: $\beta = \max(M, 0)/|U| + |M| + 100$. We use an absolute value in the denominator of the formula as a compensation for any "negative signals" that may arise from global background subtraction. We exclude from the analysis DNA methylation sites for which the missing genotype call rate is greater than 10% as well as samples from individuals, in which the total call rate of the methylation data is less than 90%. Further, we exclude methylation sites from the analyses if they overlap with SNPs, are not uniquely mapped to the reference genome, or do not vary among individuals (monomorphic).

We evaluate DNA methylation markers in two forms. First, we analyze DNA methylation at a particular genomic location as a quantitative variable using the estimated β value (ranging between 0 and 1). Second, we use the differential methylation region method to investigate the regional epigenomic profiles as a whole. Specifically, we use a linear mixed modeling approach to allow integration of all available data into a single modeling framework. To evaluate the cross-sectional impact of epigenetic variation, after accounting for the genomic effects detected, we use the following linear mixed effects model considering age and BMI as covariates:

$$Y_{jk} = \beta_0 + \beta_1 \text{ Age}_{jk} + \beta_2 \text{ BMI}_{jk} + \beta_3 G_{jk} + \beta_4 E_{jk} + W_{0k} + \varepsilon_{jk},$$

where for a given measure of BP trait Y, Y_{jk} is the BP trait for participant j in mother–child dyad k, G_{jk} is the genomic predictors for participant j in mother–child dyad k, and E_{jk} is the epigenetic predictor or profile for participant j in dyad k. W_{0k} is the random effect for each dyad that accounts for the relatedness within the dyad. We also consider other covariates including, but not limited to, cigarette smoking (for mothers) and gender (for children) and evaluate the data to determine whether principal components estimated in the entire set of SNPs to adjust for population genomic substructure should be added to the model. In addition, we use the linear mixed model to evaluate the impact of psychosocial measures and epigenetic combinatorial effects on BP. For these analyses, we target differential DNA methylation of genes known to be associated with HTN (Wang et al., 2013).

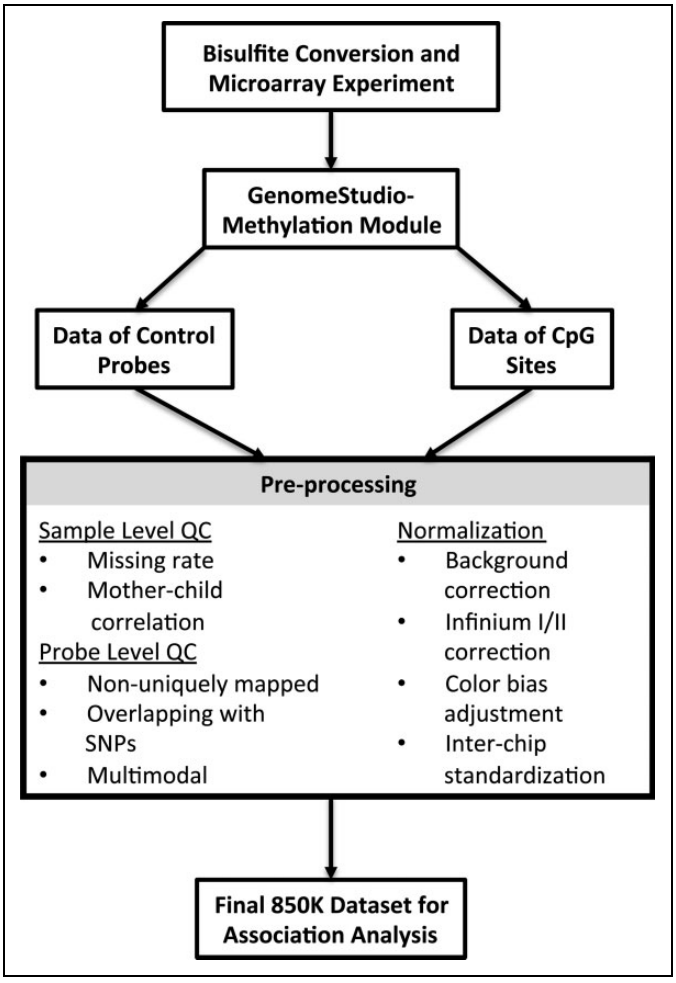

Figure 1. Data processing and analysis pipeline for illumina 850K BeadChip. QC = quality control; SNPs = single nucleotide polymorphisms.

Limitations and Future Directions

We need to interpret the results from our study cautiously due to several limitations, which also serve to outline anticipated future studies by our team. First, we will not have repeated DNA methylation measures, making it impossible to make any

causal inference related to BP over time. However, the snapshot that our results give us will provide both valuable information that we can use in a follow-up prospective longitudinal study and justification for conducting a similar study in other at-risk populations (e.g., Hispanic). The analysis methods outlined here are agnostic of clinical outcome or population and therefore have utility for future studies evaluating genomic, epigenetic, and environmental factors. Another limitation of the study is that we will not evaluate any downstream protein products that may result from variants identified as significant. Future studies we have planned include metabolomics and nutritional data to obtain a more complete picture of the participants' environment and allow us to determine how variants may influence participant physiology.

Summary

The results from this study, using an approach incorporating candidate genes, epigenetics, and environmental factors (e.g., discrimination, depression, and parenting behaviors), contribute to a more complete picture of the genomic (G) and psychological–environmental (E) architecture of complex traits, such as G×E interactions and epigenetic influences for high BP among AA mothers and their young children. In designing this study, we have utilized theoretical and research methodologies from the fields of genomics, nursing, and psychology. These methodologies inform the various approaches we use in this and future studies to increase our understanding of the increased risk for high BP among AA mothers and their young children and determine how best to identify risks and prevent and treat HTN in this population. Results from this investigation provide the foundation for the critically important development of theoretically and empirically informed strategies to reduce this health disparity.

This study is innovative for several reasons. First, it adds new interdisciplinary methodological approaches to the field by examining genomic and psychological variables concurrently for the first time to determine both main effects and interaction effects on BP in this at-risk population. Second, the use of multiple sources of information including individual-level biological and environmental data is an innovative approach to obtaining a robust understanding of the multitude of factors that impact mother and children's health. Third, our results may allow us to identify intermediate biological pathways influenced by HTN-related genes that could help explain the high prevalence of HTN in AAs. Finally, the findings may identify potential therapeutic targets for future interventional and translational studies for clinical prevention and treatment of HTN secondary to the effects of environmental and genetic factors.

Author Contribution

J. Taylor contributed to conception, design, data analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. M. Wright contributed to data interpretation, drafted and critically revised the manuscript, gave final approval, and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. C. Crusto contributed to conception and design, critically revised the manuscript, gave final approval, and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy. Y. Sun contributed to conception, design, data analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agrees to be held accountable for all aspects of work, ensuring integrity and accuracy.

Declaration of Conflicting Interests

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