

**The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure  
Study (InterGEN): Design and Methods for Recruitment and Psychological Measures**

Cindy A. Crusto,

PhD, is Associate Professor, Yale School of Medicine, New Haven, Connecticut, and Department of Psychology,  
University of Pretoria, South Africa.

Veronica Barcelona de Mendoza\*,

PhD, MSN, RN, APHN-BC, is Post-Doctoral Associate, Yale School of Nursing, Orange, Connecticut.

Christian M. Connell,

PhD, is Associate Professor, Yale School of Medicine, New Haven, Connecticut.

Yan V. Sun,

PhD, is Assistant Professor of Epidemiology, Emory University Rollins School of Public Health, Atlanta, Georgia.

Jacquelyn Y. Taylor,

PhD, PNP-BC, RN, FAHA, FAAN, is Associate Professor, Yale School of Nursing, Orange, Connecticut.

\*Corresponding author: Veronica Barcelona de Mendoza, PhD, MSN, RN, APHN-BC, Yale School of Nursing, 400  
West Campus Drive, Orange, CT 06477 (e-mail: [veronica.barcelona@yale.edu](mailto:veronica.barcelona@yale.edu)).

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**Abstract**

**Background:** Although studies show that genomics and environmental stressors affect blood pressure, few studies have examined their combined effects, especially in African Americans.

**Objective:** We present the recruitment methods and psychological measures of the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) study, which seeks to investigate the individual and combined effects of Genetic (G) and Environmental (E) (psychological) stressors on blood pressure in African American mother-child dyads. Genetic methods are presented elsewhere, but here we present the recruitment methods, psychological measures and analysis plan for these environmental stressors.

**Method:** This longitudinal study will enroll 250 mothers-child dyads (N=500). Study participation is restricted to women who: (1) are  $\geq 21$  years of age, (2) self-identify as African American or Black, (3) speak English, (4) do not have an identified mental illness or cognitive impairment, and (5) have a biological child between three to five years old. The primary environmental stressors assessed are parenting stress, perceived racism and discrimination, and maternal mental health. Covariates include age, cigarette smoking (for mothers) and gender (for children). The study outcome variables are systolic and diastolic blood pressure.

**Analysis:** Statistical modeling of environmental stressors on blood pressure will be done using descriptive statistics and generalized estimating equation (GEE) models. Genetic x Environment (G×E) interaction analyses will be described in a companion paper and will include laboratory procedures.

**Implications:** The methodology presented here may provide the foundation for other studies and development of interventions to reduce the risk for hypertension and to propose targeted health promotion programs for this high-risk population.

**MESH Key Words:** blood pressure, African Americans, Gene-Environment Interaction

Hypertension is the most common risk factor for cardiovascular disease in the US (Go et al., 2014) and African American adults bear a disproportionately high prevalence (42.1%) compared to Whites (28.0%), Hispanics (26.0%) and Asians (24.7%) (Nwankwo, Yoon, Burt, & Gu, 2013). Rates of hypertension have risen significantly among eight to 17 year olds in the US (Muntner, He, Cutler, Wildman, & Whelton, 2004), and signs of atherosclerosis can be identified as early as three years old, which if left untreated lead to irreversible coronary heart disease early in adulthood (Wung, Hickey, Taylor, & Gallek, 2013).

Previous studies have attempted to explain the phenomenon of hypertension among African Americans by investigating the independent effects of social determinants, including risk factors such as physical activity, salt sensitivity, body mass index, genetic predisposition and psychological stressors such as racism (Taylor, Sun, Chu, Mosley, & Kardia, 2008; Taylor, Maddox, & Wu, 2009), however these and other traditional risk factors are not sufficient to illuminate the complex etiology of hypertension in this population (Hansen, Gunn, & Kaelber, 2007; Roger et al., 2011; Taylor et al., 2008). Demographic variables such as socioeconomic status and gender may influence blood pressure (Cundiff, Uchino, Smith, & Birmingham, 2015), however, most Americans (86%) with uncontrolled high blood pressure have some type of health insurance and a regular provider (Centers for Disease Control and Prevention (CDC), 2011), demonstrating that improvements in adherence to treatment and an individualized approach may be necessary.

Experiences of racial discrimination also have been associated with increased blood pressure in adults (Roger et al., 2011) and poor health in children (Sanders-Phillips, Settles-Reaves, Walker, & Brownlow, 2009). Mothers who have vicariously witnessed racial discrimination against their children report this experience as being particularly distressing

(Nuru-Jeter, et al., 2009). Studies have varied in measurement of discrimination, and most have focused on adults (Dolezsar, McGrath, Herzig, & Miller, 2014; Taylor et al., 2012), signaling the need for more research on effects of discrimination on mothers and their children.

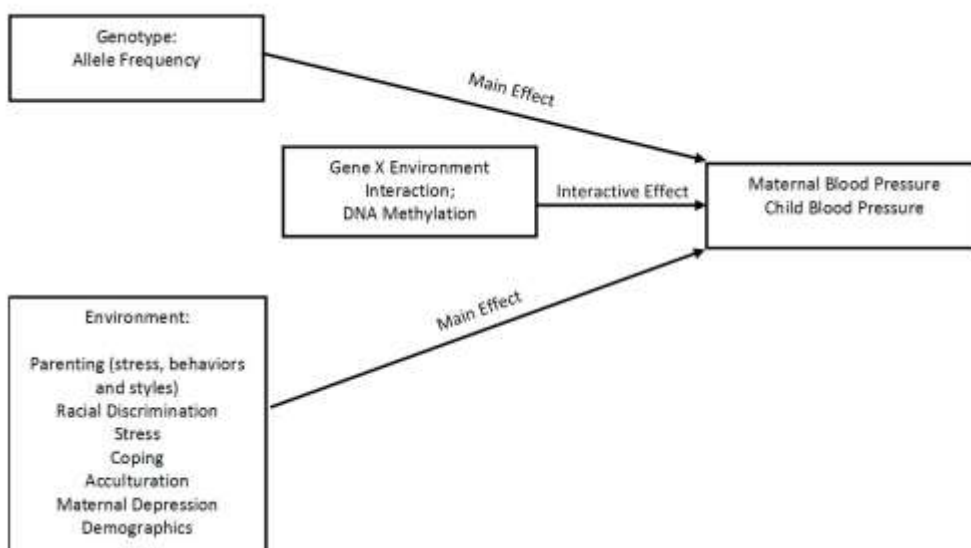
Maternal depression is a significant risk factor for poor outcomes, including blood pressure, in young children (Artinian, Washington, Flack, Hockman, & Jen, 2006), as it affects a mother's pattern of interaction with her children during early critical periods of development (Stein et al., 2014). This lack of social-emotional connection between a mother and her infant early in life can predict stress sensitivity in infants (Hane & Fox, 2006; Pederson, Gleason, Moran, & Bento, 1998) and may be associated with increases in blood pressure. Similarly, parenting stress has been significantly associated with high blood pressure in mothers (Taylor, Washington, Artinian, & Lichtenberg, 2007).

Genetic factors are also associated with blood pressure risk. Large cohort studies have identified single nucleotide polymorphisms (SNPs) located on chromosome 2 that are associated with hypertension susceptibility in African Americans (Barkley et al., 2004; Taylor et al., 2009), but additional genes are probably involved and have not yet been studied in African American children. Epigenetic factors (DNA modifications that turn genes "on" or "off" but do not change the DNA sequence (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009)) may also play a role, as environmental exposures cause DNA methylation and varying patterns of gene expression and disease (Fraga et al., 2005). Some epigenetic modifications can be inherited across generations (i.e. parent-child transmission), and early environmental exposures such as psychological stressors, abuse and socioeconomic status in pregnancy can change DNA methylation patterns in the fetus (Heijmans et al., 2008; Lam et al., 2012).

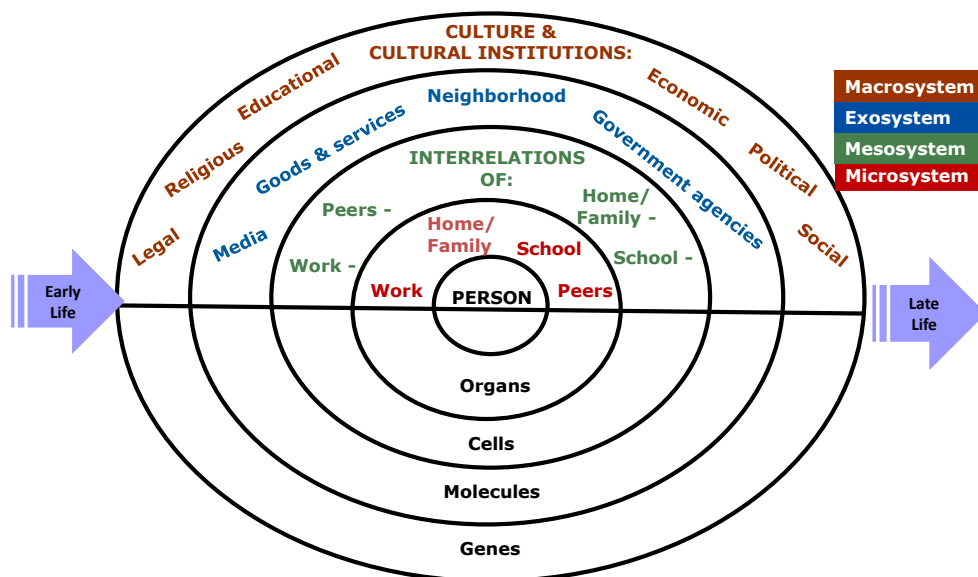
Although the individual effects of both genomic and environmental stressors on the development of hypertension have been studied, few have focused on mothers and their children, and there is a paucity of research on the interaction (non-additive synergistic effects) of genetic and environmental variables on increases in blood pressure. Further, to fully understand and interpret interaction effects, it is important to have a clear understanding of the variance contributed by main effects (in this case, environmental exposures). The use of multiple sources of information including individual-level biological data (e.g., genetic and epigenetic factors, blood pressure), as well as demographic and psychological data gathered via interview is an innovative approach to assessing the multitude of factors that impact maternal and child health. Results from this investigation will provide the platform for the critically important development of theoretical and empirically informed strategies to reduce health disparities in hypertension among African American mothers and their children.

The theoretical framework for this study (Figure 1) is informed by Bronfenbrenner's bioecological theory of human development, which states that child development results from an interaction between the individual and his or her environment or context (Bronfenbrenner, 2005). Bronfenbrenner's model incorporates transactions between systems (micro-, meso-, exo- and macro-systems) including proximal processes such as parenting and environmental stressors, distal processes such as economic systems, and changes in conditions and the individual's life over time (chronosystem). An adaptation of this model (Figure 2) has been developed which incorporates biological changes to the individual over time, and includes genes (Glass & McAtee, 2006; Tebes, 2016, in press). This expanded model provides a framework to examine proximal processes such as environmental stressors which co-occur with changes to genes. These stressors may directly influence infant outcomes as a result of insults to the epigenome. In

**Figure 1.** Theoretical framework for the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure Study (InterGEN).



**Figure 2.** An Embodied Social Ecological Model for Understanding Contexts for Human Behavior (Tebes, 2016, in press).



Tebes (2016). Adapted from: Glass, T. A., & McAtee, M. J. (2006) & Bronfenbrenner, U. (1977).

Tebes, J. K. (2016). Community Psychology for This Century: Perspectives on the Future of the Field from the Generations after Swampscott. *American Journal of Community Psychology*. Society for Community Research and Action (SCRA) published by Wiley and reprinted with permission.

addition, we apply the concept of resilience to the examination of cardiovascular outcomes. Resilience is a phenomenon often studied in child development and psychology in which children succeed despite adversity, such as poverty, discrimination and living with a mother experiencing depression. We contribute to the fourth wave of resilience research (Wright, Masten, & Narayan, 2013) by extending Bronfenbrenner's theory to propose a model where genetic and environmental factors at multiple levels of analysis (i.e. parental coping and parenting styles) interact to shape cardiovascular outcomes. Our study is especially unique as it allows for longitudinal study of psychological stressors and therefore addresses the chronosystem that is often understudied in theoretical contexts using this model (Tudge, Mokrova, Hatfield, & Karnik, 2009). This gene-environment model is a novel approach that advances both nursing science and psychology.

Therefore, in this paper we present the sub-aims [psychological environmental factors (E)] of the larger G×E study, as the methods for the genetic (G) portion of the study are described elsewhere (Taylor, Wright, Crusto, & Sun, 2016, in press). The sub-aims focusing on the psychological environmental factors that are explained here are as follows: (1) Examine the effects of mother's perceived discrimination on their and their child's BP over a 2-year period. (2) Examine the effects of mother's mental health (depressive symptoms) on their and their child's BP over a 2-year period. (3) Examine the effects of mother's parenting behavior (stress and behavior) on their and their child's BP over a 2-year period. We hypothesize that exposure to psychological environmental risks is associated with increased blood pressure readings among both African American mothers and children over time. This research is innovative as we are including children as young as three years of age, and those who are undiagnosed, which will allow for identification of risk over time. The psychological measures of stress in this study may

change over time and directly influence blood pressure, therefore, a longitudinal study design is ideal to examine these issues. This paper may serve as a guide to other researchers interested in replicating or conducting multidisciplinary work on African American mother/child dyads that examine the interactive effects of psychological and genetic/genomic factors contributing to hypertension risk in this population.

## **Methods**

### ***Design***

InterGEN is a multidisciplinary, ongoing, longitudinal study, based in Southwest and Central Connecticut. The study partners with 12 Early Care and Education (ECE) Centers which provide preschool education for qualifying low-income children in ethnically and racially diverse communities. The majority of study participants are recruited via ECEs, and remaining participants are recruited from community events with similar target populations. ECEs are provided with study materials such as brochures, business cards, and flyers for distribution to potential families.

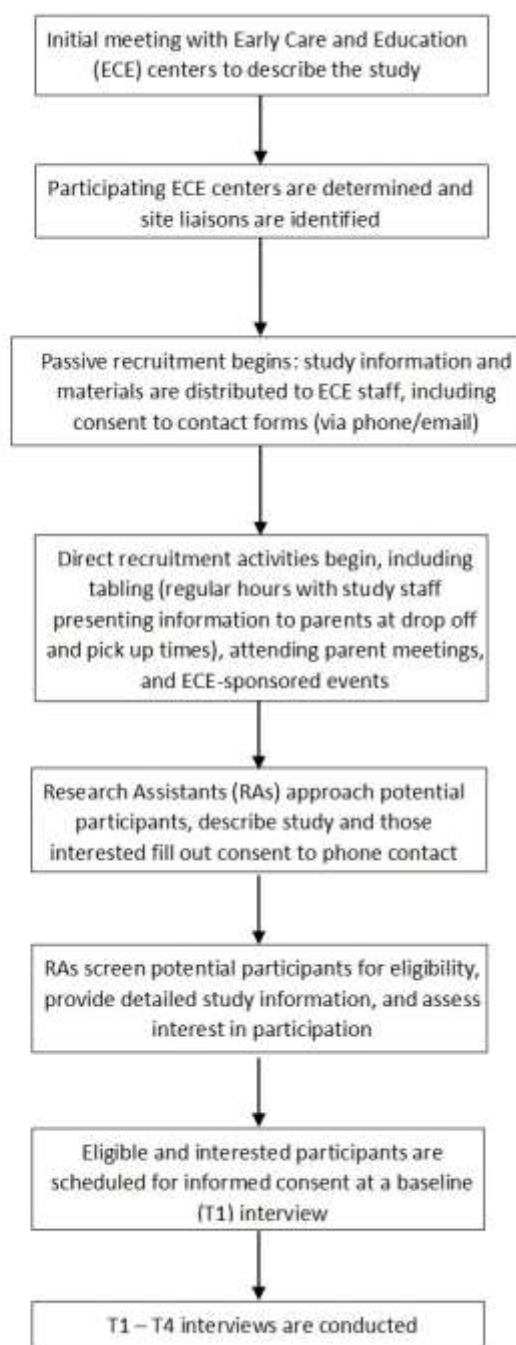
### ***Procedures***

Recruitment for the study began in April 2015, with an enrollment goal of 250 mother-child dyads, and the community engagement and recruitment protocol is summarized in Figure 3. Briefly, women are approached by study staff in partnering ECEs, informed of the study aims and procedures, and consent to be contacted for screening. Telephone screening is then done to ensure eligibility criteria is met, and then an appointment is scheduled for the first interview. Families will be interviewed at four time points (T) in the study: (T1- enrollment, T2- six months post-enrollment, T3- 12 months post-enrollment, and T4- 18 months post-enrollment). Written



informed consent is obtained at T1, and all study procedures have been approved by Yale University's Institutional Review Board. Clinical data are measured at each interview (T1-T4),

**Figure 3.** Community engagement and recruitment protocol, The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure Study (InterGEN).



and DNA is collected at T1 only. Demographic information and psychological data are collected using Audio Computer-Assisted Self-Interviewing (ACASI) (T1-T4). Eligibility for participation in the study is restricted to women who (1) are  $\geq 21$  years old, (2) identify as African American or Black (via self-report), (3) speak English, (4) do not have a psychiatric or cognitive disorder which may limit accuracy of reporting of study data and (5) have a biological child three to five years old.

### ***Primary outcome (dependent) variables***

The primary study outcome (dependent) variables are systolic and diastolic blood pressure. At each study visit (T1-T4), blood pressure measurements are obtained from the participating mother and her child by trained data collectors following the study protocol and according to JNC-7 guidelines (Chobanian et al., 2003; National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Before the T1 interview, mothers are sent a letter reminding them that blood pressure will be measured three times during the interview. The mother is informed that this is resting measurement, and that the child should not be too active for about five minutes before his or her blood pressure is taken. At each interview, manual BP is measured three times in the left arm of seated participants. Children who have difficulty remaining seated are given a tablet to play with to ensure they are at rest.

### ***Clinical variables***

Additional clinical data collection include DNA (T1 only), height and weight. Further methods on DNA collection, clinical variable collection (BP, BMI), laboratory analysis and data analysis structure is described in a companion paper (Taylor et al., 2016).

**Table 1.** Variables and measurement for psychological data collection, The Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure Study (InterGEN).

Variable	Conceptual Definition	Description/Measure	Procedure*
Parenting Stress Index, Short Form (PSI/Short Form)	Characteristics associated with parenting stress and dysfunctional parenting	Parenting Stress Index- Short Form, 3rd edition (PSI-SF) (Abidin, 1995). 36-item questionnaire; five-point Likert scale with three subscales which are summed to produce a total stress score. Reliability ( $\alpha$ ) range: 0.85-0.87 for subscales and $\alpha=0.91$ for total stress scale (Abidin, 1995). The PSI-SF has been validated in an ethnically diverse sample of caregivers at a Head Start program with good internal consistency ( <i>Total Stress</i> : $\alpha=0.94$ ) (Roberts, Campbell, Ferguson, & Crusto, 2013).	ACASI: T1 – T4
Parent Behavior Checklist (PBC)	Focuses on multiple aspects of childrearing, including developmental expectations, discipline and nurturing	Parent Behavior Checklist (PBC) consists of 100 items scored on a Likert scale. Demonstrates good to excellent reliability (range= 0.82-0.97) (Fox, 1994).	ACASI: T1 – T4
Parenting Styles and Dimension Questionnaire (PSDQ)	Assesses parenting practices within three parenting typologies: Authoritative, Authoritarian and Permissive	Parenting Styles and Dimension Questionnaire (PSDQ) consists of 62 statements scored on a Likert scale. Has adequate internal consistency in each of the subscales ( <i>Authoritative</i> , $\alpha=0.84$ ; <i>Authoritarian</i> , $\alpha=0.74$ ; <i>Permissive</i> , $\alpha=0.73$ ) (Robinson et al., 2001).	ACASI: T1 – T4
Experiences of Discrimination (EOD)	Measures self-reported experiences of discrimination in adults (women only, children's experiences of discrimination not assessed in this study) in 11 areas including gender, race, age, and skin color	Krieger's Experiences of Discrimination (EOD) scale contains nine items, and has good internal consistency in a diverse sample (26% Black) ( $\alpha=0.74$ or higher) (Krieger, Smith, Naishadham, Hartman, & Barbeau, 2005).	ACASI: T1 – T4
Race-Related Events Scale (RES)	Assesses exposure to stressful and potentially traumatizing experiences of race-related stress in adults (women only, children's experiences of discrimination not assessed in this study)	Race-Related Events Scale (RES) (Waelde et al., 2010) has 23 items. Respondents indicate if they have experienced an event (yes/no). The RES had good reliability ( $\alpha=0.78-0.88$ ) in a sample of ethnically diverse caregivers of children enrolled in Head Start (Crusto, Dantzler, Roberts, & Hooper, 2015).	ACASI: T1 – T4
Stress Overload Scale (SOS)	Measures feelings of powerlessness and the burden of demands	The Stress Overload Scale (SOS) measures stress in the last week via 24 questions scored on a five-point Likert scale, ranging from "not at all" to "a lot" (Amirkhan, 2012). SOS has high reliability in a diverse sample ( $\alpha=0.95$ ) (Amirkhan, 2012).	ACASI: T1 – T4
Coping Strategy	Measures three main coping strategies: Seeking support, Problem solving, and	Coping Strategy Indicator (CSI) (Amirkhan, 1990) scale contains 33 items rated on a Likert scale. Higher scores indicate greater use of the	ACASI: T1 – T4

Indicator (CSI)	Avoidance	strategy. Reliability in a neighborhood sample was good (Seeking support, $\alpha=0.93$ ; Problem Solving, $\alpha=0.89$ ; Avoidance, $\alpha=0.84$ ) (Amirkhan, 1990).	
Vancouver Index of Acculturation (VIA)	Acculturation is defined as the process of adaptation to customs, behaviors and attitudes of a new culture (Abraido-Lanza, White, & Vasques, 2004). Acculturation may influence the relationship between experiences of discrimination and health outcomes. It is used to assess two values associated with Heritage and behaviors associated with Mainstream culture.	The Vancouver Index of Acculturation (VIA) is a bidimensional instrument that has been validated in diverse ethnic samples. It has shown high reliability in each of its dimensions (Heritage: $\alpha=0.91$ ; Mainstream: $\alpha=0.87$ ) (Ryder, Alden, & Paulhus, 2000).	ACASI: T1 – T4
Beck Depression Inventory (BDI)	Used to assess maternal mental health, including symptoms of sadness, guilt, agitation, sleep loss and appetite loss.	The Beck Depression Inventory (BDI) (Beck, Steer, & Carbin, 1988) contains 21 items which are scored based on severity indicated for each symptom. Excellent reliability among low-income, African American medical outpatients (N = 220) ( $\alpha=0.90$ ) (Grothe et al., 2005).	ACASI: T1 – T4
Demographics	Demographic data (age, gender, education, health insurance status, cigarette smoking, income, employment status, family structure, family health history (including hypertension), birth history)		ACASI: baseline (T1), and updated as needed (T2 – T4)

\*T1=Time 1 (Enrollment); T2=Time 2 (6 months post-enrollment); T3=Time 3 (12 months post-enrollment); T4=Time 4 (18 months post-enrollment). ACASI= Audio Computer-Assisted Self-Interviewing.

### ***Psychological Measures (Primary environmental exposure (independent) variables)***

Psychological data will be collected using ACASI, which displays questions and responses on a computer screen, and simultaneously allows participants to hear the question via audio. ACASI minimizes bias that may be present in interviewer-administered surveys, and reduces discomfort that may arise when asking sensitive questions. Variables, conceptual definitions, measurement and interview time points for data collection are presented in Table 1.

### ***Potential confounding and mediating factors***

Several demographic variables may confound or mediate the relationship between psychological factors and blood pressure. Information on age, gender, education, health insurance status, cigarette smoking, income, employment status and family structure is collected at T1 and these variables will be considered as potential confounders. Confounders will be selected for inclusion in statistical models using bivariate analyses.

### **Analytic Aims**

There are three sub-aims in the study that focus on psychological (environmental) effects on blood pressure of mothers and their young children over a period of two years. The first aim focuses on the examination of the psychological effects of mothers' feelings of perceived racism and discrimination on maternal and child blood pressure. The second aim will investigate the effects of maternal depressive symptoms on blood pressure. Finally, the last aim will examine the effects of mothers' parenting behaviors on blood pressure. Statistical modeling of independent effects of environmental (psychological) exposures and blood pressure readings will be carried out using descriptive statistics and generalized estimating equations (GEE), using an

exchangeable correlation structure. GEE models account for repeated measures and correlated data within each individual (Glenn, Stewart, Links, Todd, & Schwartz, 2003; Liang & Zeger, 1986), while controlling for confounders. Models will be run separately for mothers and children, for maternal exposures and two separate continuous outcomes: systolic and diastolic blood pressure. Power was calculated as >90% based on 20% attrition for 250 families (n=200), alpha=0.05, and a clinically relevant difference of 15 mm Hg.

G×E interaction analyses will examine the synergistic effects between genetic and environmental (psychological) factors. These analyses are described by Taylor and colleagues (2016, in press) in a companion paper. Briefly, interaction effects between SNPs (Single Nucleotide Polymorphisms) in candidate genes and environmental factors will be evaluated. African ancestry is a strong predictor of high blood pressure among African Americans, therefore population admixture/structure will be controlled for in analyses using ancestry-informative marker sets according to established methods (Price et al., 2006). Linear mixed models will be used to account for interrelatedness between mothers and their children, and blood pressure will be modeled using environmental exposures and genomic markers, controlling for confounders.

### **Implications**

The InterGEN Study will investigate how both G×E interactions influence increases in blood pressure among African American women and young children. The proposed research extends nursing science by integrating genetic, epigenetic and psychological components that can contribute to understanding a more complete picture of why African Americans have the highest incidence of hypertension in the United States (Roger et al., 2011). Findings from this study may serve as a platform for development of interventions to reduce risks for hypertension

in African American women and children. Health promotion interventions targeted to families early in life, prior to diagnosis of disease would be optimal for this high-risk population. These interventions may require the expertise and collaborative efforts of multi-disciplinary health professionals including/but not limited to nurses, advanced practice nurses, mental health professionals, counselors and the like.

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