High blood pressure in pregnancy, DNA methylation, and later blood pressure in African American women enrolled in the InterGEN Study

Veronica Barcelona PhD, MSN, MPH, RN, PHNA-BC[a], Zeyuan Wang MSPH[b], Cindy Crusto PhD[c], Qin Hui PhD[b], Yan V. Sun PhD[b] and Jacquelyn Y. Taylor PhD, PNP-BC, RN, FAHA, FAAN[d]

Veronica Barcelona (corresponding author) [a]

Assistant Professor

Yale School of Nursing

400 West Campus Drive

Orange, CT 06477

Phone: (203) 737-6707

Fax: (203) 737-8298

Email: veronica.barcelona@yale.edu

[b] Graduate Research Assistant (Wang)

Data Analyst (Hui)

Associate Professor (Sun)

Emory University Rollins School of Public Health

201 Dowman Drive, Atlanta, Georgia 30322

[c] Associate Professor of Psychiatry

Yale University School of Medicine

300 George St, New Haven, CT, 06511

University of Pretoria

South Africa

[d] Professor of Nursing

Columbia University School of Nursing

560 W 168th St, New York, NY 10032

Revised word count: 2564

Funding: This work was supported by the National Institute for Nursing Research [R01NR013520, K01NR017010].

The InterGEN study received Institutional Review Board approval from both Yale University (#1311012986) and Columbia University (#AAAS9653), and written, informed consent was obtained from all participants.

Abstract

Background: Few studies have examined the effects of high blood pressure (BP) in pregnancy, preeclampsia or eclampsia on later BP, and the epigenetics of this phenomenon is similarly poorly understood, especially among African Americans. The purpose of this study was to examine the association between high BP in pregnancy, epigenomics, and later BP in African American women in the InterGEN Study (n=250).

Methods: In cross-sectional analyses, regression and linear mixed effects models were employed to examine the effects of high BP in pregnancy on: 1) epigenetic associations (DNA methylation), and 2) BP 3-5 years after birth. The 850K Illumina EPIC BeadChip was used for evaluating epigenome-wide DNA methylation. High BP in pregnancy, preeclampsia, or

eclampsia was self-reported by women, and BP was measured 3-5 years after birth, per JNC-7 guidelines. DNA methylation and clinical BP were the main outcomes.

Results: Mean age of enrolled women was 31.2 years, 21.8% were smokers, 58% had some college or higher education, 46.6% reported an annual income <$15,000, and 13.6% reported high BP in pregnancy. After adjustment for obesity, smoking, and age, women with a history of high BP in pregnancy had significantly higher BP than those who did not report this complication (5.39±2.4 mmHg, $p = 0.030$). Epigenome-wide analysis revealed no significant sites after multiple testing correction.

Conclusions: We observed a small, but clinically significant increase in BP in women who reported high BP in pregnancy 3-5 years after that pregnancy. Future studies with larger sample sizes should examine epigenetic contributions to this finding.

Keywords: DNA methylation, preeclampsia, high blood pressure, pregnancy, epigenomics, African Americans, women

1. Introduction

High blood pressure (BP) affects more than 85 million Americans and is a major risk factor for cardiovascular disease.[1] African Americans carry a disproportionately high burden of high BP (41.2%), compared to Caucasians (28.0%), Asians (24.9%), and Hispanic adults (25.9%).[2] For reproductive aged African American women, the risk of high BP is exacerbated by pregnancy. Preeclampsia is defined as new onset hypertension (systolic ≥140 or diastolic ≥90) after 20 weeks gestation, plus new onset proteinuria.[3] In pregnancy, approximately 2-8% of

women are diagnosed with preeclampsia,[4] 5% with chronic hypertension,[3] and 2-3% with gestational hypertension,[5] placing them at higher risk for later high BP.[6] African American women have higher rates (5.5%) of preeclampsia than Caucasians (3.3%).[7] The links between reproductive and later cardiovascular health are beginning to be recognized, and the American Heart Association now recommends inclusion of pregnancy-related complications such as preeclampsia when screening for cardiovascular diseases.[8]

There are several known maternal and fetal sequelae of preeclampsia that increase risk of later cardiovascular disease across the life course.[9] Children born to mothers with preeclampsia are at higher risk of preterm birth,[10] a leading cause of mortality and morbidity worldwide, low birthweight,[11] and stillbirth.[12] Preeclampsia is a leading cause of maternal mortality[13] and is associated with increased risk of high BP later in life (hazard ratio: 2.1, 95% CI: 1.80–2.40).[14] Other risks for children exposed to preeclampsia in utero include increased risk of diabetes,[15] higher BP,[16,17] stroke,[18] and systemic vascular dysfunction[19] over the life course.

Several risk factors have been established for preeclampsia, including those related to pregnancy such as nulliparity, multiple gestation, hydatidiform mole, and prior pregnancy with preeclampsia.[20] Other risk factors such as obesity, diabetes, and high BP are similar to those for cardiovascular diseases.[21] In addition, maternal age, smoking, and antiphospholipid antibody syndrome[22] have been associated with preeclampsia. Genome-wide association studies have begun to examine genetic risk factors for preeclampsia,[23,24] however, few genes have been consistently identified in this complex disorder. Preeclampsia risk is influenced by both maternal[25] and fetal genetic factors.[26] In recent years, research in this area has expanded to epigenetics, as changes to the DNA sequence are heritable, potentially reversible, and known

to influence gene expression. The most frequently studied epigenetic mechanism, DNA methylation [DNAm], has been shown to be a valuable target for preeclampsia research, as alterations in DNAm have been reported in the placenta,[27] cord blood,[28] peripheral blood,[29] and omental blood vessels.[30,31] Many studies have examined epigenetic effects of preeclampsia on offspring,[17] but few have investigated effects on mothers, especially African American women.

Researchers have reported short- and long-term effects of preeclampsia. Increased rates of hypertension and proteinuria have been reported to persist in women at six months postpartum.[32] Few studies have examined the effects of high BP in pregnancy and DNAm on BP in the short-term, especially in an all-African American cohort. The purpose of this study was to investigate the short-term effects (3 to 5 years postpartum) of high BP in pregnancy on DNAm and later BP in African American women enrolled in the Intergenerational Effects of Genetic and Psychological Factors on Blood Pressure (InterGEN) study. In this study, women indicated if they had high BP in pregnancy, preeclampsia, or eclampsia during their pregnancy.

2. Methods

InterGEN was a longitudinal cohort study in Connecticut (2014-2019), which enrolled 250 mother/child dyads (N=500) from the community with the purpose of studying gene-environment interactions on blood pressure. Eligibility criteria included women (≥21 years old) who self-identified as African American or Black, spoke English, had no mental illness that could interfere with psychological measures, and who enrolled with a biological child (3-5 years old). At the first study visit, demographic, clinical (height, weight, blood pressure, saliva for DNA analysis), and psychological data were collected (i.e. parenting stress, perceived racism and discrimination, and symptoms of depression). Detailed information regarding study

procedures have been published elsewhere.[33] Women self-reported pregnancy complications and smoking behaviors (current smoker: yes/no) using Audio Computer Assisted Self-Interview (ACASI) software.

The study exposure was assessed by maternal self-report to the following survey question during the computer-based interview at Time 2: “When you were pregnant with the enrolled child, did a doctor or health care professional tell you that you had high blood pressure (eclampsia or preeclampsia)?”. Women were asked this single question and chose their response, indicating that they had high BP in pregnancy, eclampsia, or preeclampsia. This question did not allow women to indicate which of these distinct disease processes were present. Body mass index (BMI) was calculated from height measured by portable stadiometer (Model 214 Road Rod, Seca Corporation, Hanover, MD) and weight by electronic scale (BWB/807 Tanita Tokyo, Japan) by trained research staff. Blood pressure was measured three times at each visit, according to JNC-7 guidelines.[34] We treated the outcome variable of BP as a continuous variable (systolic and diastolic BPs) and categorical variable (Normal/Elevated vs. Stage 1/Stage 2) according to the 2017 American Heart Association Hypertension guidelines.[35] The InterGEN study received Institutional Review Board approval from involved universities, and written, informed consent was obtained from all participants.

DNA was obtained through saliva collection using the Oragene (OG)-500 format tubes,[36] according to established collection and analysis procedures.[37] Once transported from the field to the research laboratory, saliva samples were refrigerated at 4ºC. ReliaPrep kits were used for DNA extraction and purification, and epigenome-wide DNAm measurement was done using the Illumina Infinium Methylation EPIC (850K) BeadChip. 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 SNPs (n = 15,341), or were listed in the recent Illumina product quality notice. Quality control procedures and all analyses were uniformly performed among autosomal and X-chromosome sites due to a unisex (all female) sample. A total of 831,219 autosomal and 18,895 X-chromosomal CpG sites were included in the association analyses as previously described.[38,39]

Two statistical procedures were carried out for the present analysis. First, linear and logistic regression of the effects of high BP in pregnancy on later BP was conducted for women enrolled in the InterGEN study. Covariates included smoking, age, and BMI. Secondly, we conducted epigenome-wide analysis (EWAS) of high BP in pregnancy to predict systolic and diastolic BP separately in linear mixed effects models, controlling for smoking, age, BMI, and antihypertensive medication use, as well as batch effects and potential heterogeneity in cell proportions from saliva using the reference-free EWAS method.[40] False Discovery Rate (FDR) was used to control for multiple comparisons, and the Benjamini Hochberg method was used to calculate the FDR-corrected q-value.[41] Statistical significance was set at FDR-corrected $q < 0.05$ for CpG sites, and the R statistical environment version 3.4.1 was used for genetic analyses [http://www.r-project.org/].

Table 1. Baseline characteristics of women enrolled in the Intergenerational Blood Pressure Study, 2015-2019, N=250.

	n	%	*missing*
Primary exposure			
High blood pressure in pregnancy, pre-eclampsia or eclampsia			59
Yes	26	13.6	
No	159	83.3	
Refused/don't know	6	3.1	
Primary outcome variables			
Systolic blood pressure [mean, SD]	114.0	13.7	1
Diastolic blood pressure [mean, SD]	72.6	10.8	2
AHA Hypertension categories 2017			
Normal [SBP<120 and DBP<80]	165	66.3	1
Elevated [SBP=120-129 and DBP<80]	21	8.4	
Stage 1 [SBP=130-139 and DBP=80-89]	49	19.7	
Stage 2 [SBP≥140 or DBP≥90]	14	5.6	
Primary covariates			
Age			0
21-29	105	42.0	
30-39	124	49.6	
40-49	21	8.4	
Education			
< High School	13	5.2	0
High School graduate	91	36.7	
Some college	82	33.1	
Associate degree/College graduate or higher	62	25.0	
Annual household income			
≤$15,000	111	46.6	12
>$15,000-$34,999	102	42.9	
≥$35,000	25	10.5	
Health insurance type			
Private	35	14.1	10
Medicaid	154	62.1	
Government/ACA	37	14.9	

	None	14	5.7	
Ever received diagnosis of high blood pressure				2
	Yes	51	20.6	
	No	197	79.4	
Current high blood pressure medication use				2
	Yes	18	7.3	
	No	230	92.7	
Body Mass Index				
Underweight [<18.5] or normal weight [18.5-24.9]		78	31.2	0
Overweight [25-29.9]		61	24.4	
Obesity [≥30]		111	44.4	
Had a job in the last 12 months				
	Yes	164	66.1	7
	No	79	31.9	
Current smoker				
	Yes	54	21.9	3
	No	193	78.1	

SD= standard deviation
SBP= systolic blood pressure
DBP=diastolic blood pressure
*Numbers may not add to 100 due to rounding

3. Results

Of the 250 women enrolled in the study, the women's mean age was 31.2 years (SD=5.8), 58.0% had some college or more education, 46.6% reported an annual household income of less than $15,000 (46.6%), and 66.1% were employed in the past year. Less than a third of women had a BMI indicating underweight/normal weight (31.2%), and 21.8% were current smokers. Approximately one in five women had ever received a diagnosis of high blood

pressure (20.6%), and fewer reported current blood pressure medication use (7.3%) (Table 1). In unadjusted association analyses, age, current smoking status, diagnosis of high BP, current use of high BP medication, education, and body mass index were significantly associated with systolic BP. These covariates were also significantly associated with diastolic BPs, except for education (Table 2).

Table 2. Bivariate associations between blood pressure and covariates of interest, Intergenerational Blood Pressure Study, N=250

	Systolic Blood Pressure			*Diastolic Blood Pressure*		
	df	*t/F-value*	*p-value*	*df*	*t/F-value*	*p-value*
Primary covariates						
Age [continuous]	248	99.25	<.0001	247	60.91	<.0001
Current smoker [yes/no]	246	130.22	<.0001	245	104.62	<.0001
Ever had a diagnosis of high BP [yes/no]	247	131.51	<.0001	246	105.89	<.0001
Current high blood pressure medication use [yes/no]	247	131.34	<.0001	246	106.02	<.0001
Education						
< High School	3	2.85	0.0381	3	1.56	0.2004
High School graduate						
Some college						
Associate degree/college graduate						
Body mass index						
Underweight [<18.5]	3	19.18	<.0001	3	12.87	<.0001
Normal weight [18.5-24.9]						
Overweight [25-29.9]						
Obesity [≥30]						

BP=blood pressure
df=degrees of freedom

T-tests used for continuous and dichotomous variables, and one-way ANOVA used for categorical variables.

Table 3. Unadjusted and adjusted* effects of high blood pressure in pregnancy on systolic and diastolic blood pressure, Intergenerational Blood Pressure Study, 2015-2019, n=183.

	Systolic Blood Pressure [mmHg]						Diastolic Blood Pressure [mmHg]					
	Unadjusted			*Adjusted*			*Unadjusted*			*Adjusted*		
	β	*SE*	*p-value*	*β*	*SE*	*p-value*	*β*	*SE*	*p-value*	*β*	*SE*	*p-value*
High blood pressure in pregnancy, eclampsia, or pre-eclampsia	8.21	2.7	0.003	5.39	2.4	0.030	3.87	2.2	0.091	1.21	2.1	0.570

*Model adjusted for age, smoking, body mass index, hypertension medication use

β= Beta coefficient; SE= Standard Error

Next, we examined the relationship between high BP in pregnancy and systolic and diastolic BP using unadjusted and multivariable linear regression (Table 3). In unadjusted models, women who reported high BP in pregnancy had significantly higher systolic BP (beta=8.21, SE 2.7, p=0.003) 3 to 5 years after birth than women who did not report this complication. After adjusting for age, smoking, body mass index, and antihypertensive medication use, findings were attenuated, but still statistically significant, indicating higher mean systolic BP for those women with high BP in pregnancy (beta=5.39, SE 2.4, p=0.030). High BP in pregnancy was not significantly associated with diastolic BP in either unadjusted (beta=3.87, SE 2.2, p=0.091) or adjusted models (beta=1.21, SE 2.1, p=0.570). We also used logistic regression to examine later BP as a categorical variable. We found increased odds of Stage 1 or Stage 2 hypertension compared to Normal/Elevated BP among women who reported high BP in pregnancy (aOR 1.34, 95% CI 0.49-3.61), though these findings were not statistically significant after adjustment for age, smoking, body mass index, and antihypertensive use (data not shown).

We then conducted EWAS of systolic and diastolic BP for women who reported having high BP in pregnancy (n=179). Using linear mixed effects models, and controlling for high BP in pregnancy, age, smoking, body mass index, principal components, and cell type heterogeneity, we found no significant sites associated with either systolic or diastolic BP (Figures 1 and 2).

Figure 1. Manhattan plot: Epigenomic association of systolic blood pressure and high BP in pregnancy

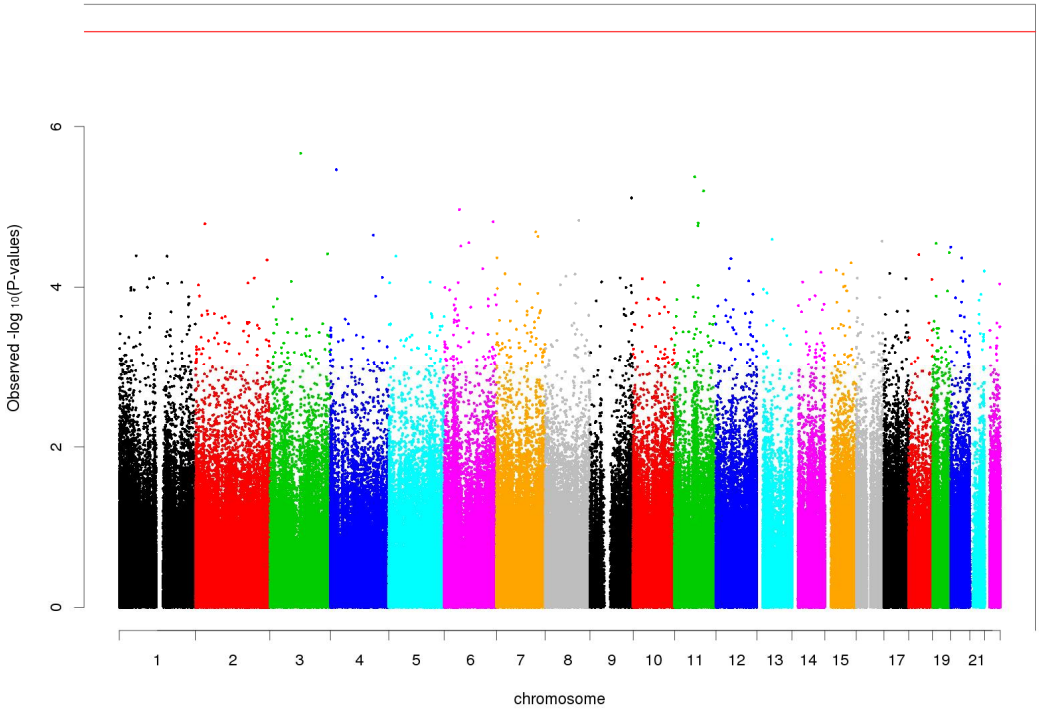
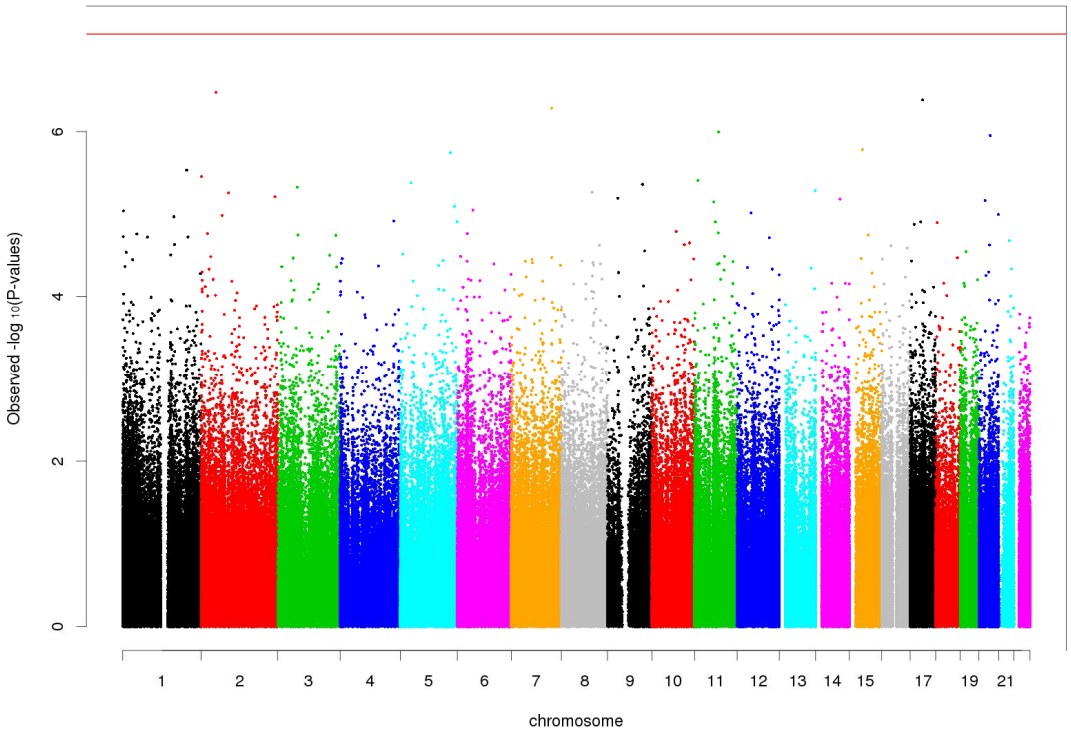


Figure 2. Manhattan plot: Epigenomic association of diastolic blood pressure and high BP in pregnancy



Finally, we ran sensitivity analyses to compare women with missing data for the self-reported measure of high BP in pregnancy to women who responded to this question. We found that women in these two groups did not differ on baseline characteristics, included ever having been diagnosed with chronic high BP, and no statistically significant differences were found (data not shown).

4. Discussion

In this cross-sectional analysis of African American women enrolled in the InterGEN study, we found that women who reported high BP in pregnancy had higher systolic blood pressures than those who did not report this complication. We did not find any significant sites associated with high BP in pregnancy in epigenome-wide analyses.

We observed higher systolic BPs 3-5 years after pregnancy in women who reported high BP in pregnancy (beta=5.39, SE 2.4, p=0.030) compared to women who did not report this complication. In logistic regression models, women who reported high BP in pregnancy were 33% more likely to have Stage 1 or Stage 2 hypertension than those who did not identify high BP in pregnancy, however these findings were not statistically significant (OR 1.33, 95% CI 0.49-3.61), (data not shown). Our finding of increased BP among African American women who report high BP in pregnancy and in the short-term afterwards is supported by previous studies. Most studies [42] looked at cardiovascular outcomes such as heart disease, myocardial infarction, or death, and not a more short-term outcome like hypertension. Haas and colleagues (2019) reported findings of a prospective cohort study of 4484 women followed for two to seven years after their first pregnancy.[43] Women in this study were recruited from eight centers across the United States, were primarily white (62.1%), and few were African American

(13.8%). In that study, authors reported that women with any hypertensive disorder in pregnancy were more than two times as likely to have hypertension at follow-up (RR 2.7, 95% CI 2.0-3.6).[43] Others found significant increases in high BP as soon as two years postpartum in women with hypertensive disorders of pregnancy, though that sample consisted of primarily White women (from the UK) as well.[44] Another study reported a significant increase in hypertension risk 12 months postpartum for obese women with preeclampsia, (OR 2.35, 95% CI 1.63-3.41).[45] Less than a third of this sample were African American women (28%).[45] Our study is consistent with previous work in that we found an increased risk for high BP in women years after experiencing high BP in pregnancy, despite limited participation of African American women in existing studies.

Women frequently reported being current smokers in our sample (21.9%). This rate is higher than overall smoking rates for African American adults (14.9%) in 2017.[46] More women reported smoking in pregnancy in our sample (7.8%) than in national samples of African American women in pregnancy (6.0%).[47] It is difficult to disentangle whether these higher rates of smoking led to lower rates of high BP in their pregnancies, as we were not able to distinguish between the types of high BP in pregnancy.

In the present analysis, we found no significant CpG sites associated with history of high BP in pregnancy. This could be attributed to a lack of large, long-term impact of high BP in pregnancy on DNAm, in addition to limited power. There is a paucity of research on high BP in pregnancy, epigenomics, and later BP, and no studies were identified that examined this question in African American women. Previous work has reported differences in methylation in maternal leukocytes with women who had preeclampsia, however, this study used an all-

Caucasian sample.[48] Another study conducted in India described increased global DNAm levels among pregnant women with preeclampsia, compared to those with normal BP.[27] None of top ten associations in our study (Supplemental Table) were replicated in other placental or cord blood methylation studies.[49-51] Our study used saliva as the tissue for DNA analyses, however, peripheral blood and saliva have been shown to be concordant in methylation studies.[39]

Like any study, ours had limitations. Our modest sample size may have affected our ability to detect both later clinical BP and epigenetic differences between women with high BP in pregnancy and those without this complication. For example, we observed a statistically significant increase in later BP when measured continuously, but not as a categorical outcome. This was likely due to lack of power; however, the direction of findings is the same. Another limitation of this study is potential recall bias, as women were reporting being told they had high BP in pregnancies that occurred three to five years prior. Previous research, however, has found that maternal self-report of pregnancy complications such as high BP is modest in accuracy, at four years after birth.[52] There also is the potential for misclassification of hypertension in pregnancy, as we did not have a more precise ascertainment of the sub-type of high blood pressure mothers had during pregnancy. The fact that we could not discriminate between the different subtypes of high BP in pregnancy (i.e. pregnancy-induced hypertension, preeclampsia, preeclampsia superimposed on chronic hypertension, and chronic hypertension), with unique etiologies, is a limitation. However, preeclampsia and eclampsia are relatively rare complications of pregnancy. Therefore, it is plausible that most of the respondents in our sample had pregnancy-induced or chronic hypertension, though we were unable to confirm this

with objective medical record data.

Strengths of this analysis include an all-African American cohort, epigenomic and phenotype data, and despite the limited sample size, replication of previous work showing increased BP in women who report high BP in pregnancy. Further studies are necessary to more finely examine and differentiate risks and epigenetic mechanisms related to each sub-type of high BP in pregnancy. It is unclear if high BP in pregnancy is an independent risk factor for later cardiovascular disease or an early marker for high-risk women, and this is another important area for further investigation. Future research should also include prospective data collection from medical records for more precise exposure assessment.

There may be clinical implications of these findings as well. Women with high BP in pregnancy had increased BP 3-5 years later both before and after adjustment for common risk factors such as high body mass index, age, and smoking. This may indicate that counseling for tobacco cessation and weight loss may not be enough to mitigate the effects of high BP in pregnancy and later cardiovascular risk in this population. Inclusion of pregnancy history for women in primary care settings is warranted and should guide women's health care throughout the lifespan. Health promotion messaging to women should also include education on the importance of this linkage.

In conclusion, our findings are clinically important, as we observed a small, but significant increase in BP in African American women three to five years after reporting high BP in pregnancy. This presents a challenge and opportunity to focus screening and prevention efforts, as well as to expand research to be inclusive of this high-risk population and study the mechanisms and prevention of cardiovascular disease. This is especially important as African

American women bear some of the highest burdens of both adverse reproductive outcomes and cardiovascular disease, a cycle of which cannot be separated, and must be investigated within the context of the lifecourse.

References

1. Benjamin EJ, Virani SS, Callaway CW, et al. Heart disease and stroke statistics-2018 update: A report from the American Heart Association. *Circulation*. 2018;137(12):e67-e492.

2. Yoon SS, Carroll MD, Fryar CD. Hypertension prevalence and control among adults: United States, 2011-2014. *NCHS Data Brief*. 2015;(220)(220):1-8.

3. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. *Obstet Gynecol*. 2013;122(5):1122-1131.

4. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol*. 2009;33(3):130-137.

5. Wallis AB, Saftlas AF, Hsia J, Atrash HK. Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004. *Am J Hypertens*. 2008;21(5):521-526.

6. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. *Hypertension*. 2009;53(6):944-951.

7. Breathett K, Muhlestein D, Foraker R, Gulati M. Differences in preeclampsia rates between African American and Caucasian women: Trends from the national hospital discharge survey. *J Womens Health (Larchmt)*. 2014;23(11):886-893.

8. Mosca L, Benjamin EJ, Berra K, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: A guideline from the American Heart Association. *J Am Coll Cardiol*. 2011;57(12):1404-1423.

9. Garovic VD, Bailey KR, Boerwinkle E, et al. Hypertension in pregnancy as a risk factor for cardiovascular disease later in life. *J Hypertens*. 2010;28(4):826-833.

10. Davies EL, Bell JS, Bhattacharya S. Preeclampsia and preterm delivery: A population-based case-control study. *Hypertens Pregnancy*. 2016;35(4):510-519.

11. Odegard RA, Vatten LJ, Nilsen ST, Salvesen KA, Austgulen R. Preeclampsia and fetal growth. *Obstet Gynecol*. 2000;96(6):950-955.

12. Harmon QE, Huang L, Umbach DM, et al. Risk of fetal death with preeclampsia. *Obstet Gynecol*. 2015;125(3):628-635.

13. Creanga AA, Berg CJ, Syverson C, Seed K, Bruce FC, Callaghan WM. Pregnancy-related mortality in the United States, 2006-2010. *Obstet Gynecol*. 2015;125(1):5-12.

14. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): Population-based retrospective cohort study. *Lancet*. 2005;366(9499):1797-1803.

15. Henry EB, Patterson CC, Cardwell CR. A meta-analysis of the association between pre-eclampsia and childhood-onset type 1 diabetes mellitus. *Diabet Med*. 2011;28(8):900-905.

16. Oglaend B, Forman MR, Romundstad PR, Nilsen ST, Vatten LJ. Blood pressure in early adolescence in the offspring of preeclamptic and normotensive pregnancies. *J Hypertens*. 2009;27(10):2051-2054.

17. Davis EF, Newton L, Lewandowski AJ, et al. Pre-eclampsia and offspring cardiovascular health: Mechanistic insights from experimental studies. *Clin Sci (Lond)*. 2012;123(2):53-72.

18. Kajantie E, Eriksson JG, Osmond C, Thornburg K, Barker DJ. Pre-eclampsia is associated with increased risk of stroke in the adult offspring: The Helsinki birth cohort study. *Stroke*. 2009;40(4):1176-1180.

19. Jayet PY, Rimoldi SF, Stuber T, et al. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. *Circulation*. 2010;122(5):488-494.

20. Jeyabalan A. Epidemiology of preeclampsia: Impact of obesity. *Nutr Rev*. 2013;71 Suppl 1:S18-25.

21. Shih T, Peneva D, Xu X, et al. The rising burden of preeclampsia in the United States impacts both maternal and child health. *Am J Perinatol*. 2016;33(4):329-338.

22. Hutcheon JA, Lisonkova S, Joseph KS. Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2011;25(4):391-403.

23. Zhao L, Triche EW, Walsh KM, et al. Genome-wide association study identifies a maternal copy-number deletion in PSG11 enriched among preeclampsia patients. *BMC Pregnancy Childbirth*. 2012;12:61-2393-12-61.

24. Johnson MP, Brennecke SP, East CE, et al. Genome-wide association scan identifies a risk locus for preeclampsia on 2q14, near the inhibin, beta B gene. *PLoS One*. 2012;7(3):e33666.

25. Gray KJ, Kovacheva VP, Mirzakhani H, et al. Gene-centric analysis of preeclampsia identifies maternal association at PLEKHG1. *Hypertension*. 2018;72(2):408-416.

26. Gray KJ, Saxena R, Karumanchi SA. Genetic predisposition to preeclampsia is conferred by fetal DNA variants near FLT1, a gene involved in the regulation of angiogenesis. *Am J Obstet Gynecol*. 2018;218(2):211-218.

27. Kulkarni A, Chavan-Gautam P, Mehendale S, Yadav H, Joshi S. Global DNA methylation patterns in placenta and its association with maternal hypertension in pre-eclampsia. *DNA Cell Biol*. 2011;30(2):79-84.

28. Ching T, Ha J, Song MA, et al. Genome-scale hypomethylation in the cord blood DNAs associated with early onset preeclampsia. *Clin Epigenetics*. 2015;7:21-015-0052-x. eCollection 2015.

29. Julian CG, Pedersen BS, Salmon CS, et al. Unique DNA methylation patterns in offspring of hypertensive pregnancy. *Clin Transl Sci*. 2015;8(6):740-745.

30. He J, Zhang A, Fang M, et al. Methylation levels at IGF2 and GNAS DMRs in infants born to preeclamptic pregnancies. *BMC Genomics*. 2013;14:472-2164-14-472.

31. Mousa AA, Archer KJ, Cappello R, et al. DNA methylation is altered in maternal blood vessels of women with preeclampsia. *Reprod Sci*. 2012;19(12):1332-1342.

32. Girsberger M, Muff C, Hosli I, Dickenmann MJ. Short term sequelae of preeclampsia: A single center cohort study. *BMC Pregnancy Childbirth*. 2018;18(1):177-018-1796-z.

33. Crusto CA, Barcelona de Mendoza V, Connell CM, Sun YV, Taylor JY. The intergenerational impact of genetic and psychological factors on blood pressure study (InterGEN): Design and methods for recruitment and psychological measures. *Nurs Res*. 2016;65(4):331-338.

34. Chobanian AV, Bakris GL, Black HR, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The JNC 7 report. *JAMA*. 2003;289(19):2560-2572.

35. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: Executive summary: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension*. 2018;71(6):1269-1324.

36. Bahlo M, Stankovich J, Danoy P, et al. Saliva-derived DNA performs well in large-scale, high-density single-nucleotide polymorphism microarray studies. *Cancer Epidemiol Biomarkers Prev*. 2010;19(3):794-798.

37. Taylor JY, Wright ML, Crusto CA, Sun YV. The intergenerational impact of genetic and psychological factors on blood pressure (InterGEN) study: Design and methods for complex DNA analysis. *Biol Res Nurs*. 2016.

38. Klebaner D, Huang Y, Hui Q, et al. X chromosome-wide analysis identifies DNA methylation sites influenced by cigarette smoking. *Clin Epigenetics*. 2016;8:20-016-0189-2. eCollection 2016.

39. Barcelona V, Huang Y, Brown K, et al. Novel DNA methylation sites associated with cigarette smoking among African Americans. *Epigenetics*. 2019;14(4):383-391.

40. Houseman EA, Molitor J, Marsit CJ. Reference-free cell mixture adjustments in analysis of DNA methylation data. *Bioinformatics*. 2014;30(10):1431-1439.

41. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*. 1995;57(1):289-300.

42. Wu P, Haththotuwa R, Kwok CS, et al. Preeclampsia and future cardiovascular health: A systematic review and meta-analysis. *Circ Cardiovasc Qual Outcomes*. 2017;10(2):10.1161/CIRCOUTCOMES.116.003497. Epub 2017 Feb 22.

43. Haas DM, Parker CB, Marsh DJ, et al. Association of adverse pregnancy outcomes with hypertension 2 to 7 years postpartum. *J Am Heart Assoc*. 2019;8(19):e013092.

44. Melchiorre K, Sutherland GR, Liberati M, Thilaganathan B. Preeclampsia is associated with persistent postpartum cardiovascular impairment. *Hypertension*. 2011;58(4):709-715.

45. Hauspurg A, Countouris ME, Jeyabalan A, et al. Risk of hypertension and abnormal biomarkers in the first year postpartum associated with hypertensive disorders of pregnancy among overweight and obese women. *Pregnancy Hypertens*. 2019;15:1-6.

46. Wang TW, Asman K, Gentzke AS, et al. Tobacco product use among adults - United States, 2017. *MMWR Morb Mortal Wkly Rep*. 2018;67(44):1225-1232.

47. Martin JA, Hamilton BE, Osterman MJK, Driscoll AK, Drake P. Births: Final data for 2016. *Natl Vital Stat Rep*. 2018;67(1):1-55.

48. Anderson CM, Ralph JL, Wright ML, Linggi B, Ohm JE. DNA methylation as a biomarker for preeclampsia. *Biol Res Nurs*. 2014;16(4):409-420.

49. Dwi Putra SE, Reichetzeder C, Meixner M, Liere K, Slowinski T, Hocher B. DNA methylation of the glucocorticoid receptor gene promoter in the placenta is associated with blood pressure regulation in human pregnancy. *J Hypertens*. 2017;35(11):2276-2286.

50. Ching T, Ha J, Song MA, et al. Genome-scale hypomethylation in the cord blood DNAs associated with early onset preeclampsia. *Clin Epigenetics*. 2015;7:21-015-0052-x. eCollection 2015.

51. Del Gobbo GF, Price EM, Hanna CW, Robinson WP. No evidence for association of MTHFR 677C>T and 1298A>C variants with placental DNA methylation. *Clin Epigenetics*. 2018;10:34-018-0468-1. eCollection 2018.

52. Carter EB, Stuart JJ, Farland LV, et al. Pregnancy complications as markers for subsequent maternal cardiovascular disease: Validation of a maternal recall questionnaire. *J Womens Health (Larchmt)*. 2015;24(9):702-712.