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Health Disparities in Genomics and Genetics

Guest Editors: Ida J. Spruill, Jacquelyn Taylor, Irma B. Ancheta, Adebowale A. Adeyemo, Yolanda Powell-Young, and Willa Doswell

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Editorial Board

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Editorial

Health Disparities in Genomics and Genetics

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Disparities or inequities in health refer to sociodemographic group differences in the distribution of disease, health outcomes, or access to health care [1]. In order to eliminate health disparities, more efforts are needed to address social issues directly contributing to the healthy inequities observed across racial and ethnic groups. With broad support from many federal agencies, alleviating health disparities in the United States remains a goal of Healthy People 2010 [2]. However, genetic research also has a significant role to play in alleviating and understanding disparities. With tremendous advances in technology and increased investigation into human genetic variations, genomics is poised to play a valuable role in bolstering efforts to find new treatments and preventions for chronic conditions that disparately affect certain ethnic groups. The recent statement regarding the future of genomics from the National Human Genome Research Institute (NHGRI) [3] indicated that the need to develop genome-based tools to address health disparities remains a "grand challenge". The statement acknowledges that social and economic factors contribute significantly to disparities but nevertheless assert the need for extensive research to better understand the contribution of genetics.

In this special issue, we present five promising studies conducted by nurse scientists focused on understanding the genetic underpinnings of diseases such as epigenetic markers of renal function in African Americans and the association between KIF6 single nucleotides in Filipino women. Other studies reported on participation of African Americans into research, attitudes toward genetic testing for hypertension, and, lastly, the role of epigenetics in health disparity among Native Americans. All have characterized the importance of their work in its capacity to advance our understanding of health disparities research.

The resounding success of the human genome project (HGP), international hap map project, and the 1000 genomes project has brought clinical translation closer than ever. Although some prevention programs now exist to reduce disparities by targeting specific genetic disorders, publications by ethnic minorities of nurses remains underrepresented in genetic research. In seeking articles for this special edition, the editors actively engage authors to submit publications addressing health disparities in genomics and genetics. Although there is an interest in the topic and it is evident globally with Journal of Nursing Scholarship, it is still an underresearched area. Nevertheless, it is a topic that is acknowledged to have great importance to understanding the underpinnings of disparity in genetics and genomics.

More specifically, this special edition is dedicated to genetic/genomic research conducted by nurse scientist like V. Ancheta et al. entitled "*the association between KIF6 single*

nucleotide polymorphism rs20455 and serum lipids in Filipino-American women." Their results showed that the majority of Filipino women are either heterozygous or homozygous carriers of the risk allele of the rs20455 SNP in the KIF6 gene. More importantly, even with borderline LDL-C levels, many FA would benefit from statin treatment, and many participants did not exhibit guideline recommended LDL-C levels including those on statin drugs.

On the other hand, work by S. M. Bomotti et al. entitled "*epigenetic markers of renal function in African Americans*" focused on chronic kidney disease (CKD) in African Americans. The authors sought to understand how DNA methylation plays a role in CKD. To better understand the role of these methylation markers, they measured 26,428 DNA methylation sites in 972 African Americans from the genetic epidemiology network of arteriopathy (GENOA) study to evaluate (1) whether epigenetic markers (EMs) are associated with estimated glomerular filtration rate (eGFR), (2) whether the significantly associated markers are also associated with traditional risk factors and/or novel biomarkers for eGFR, and (3) how much additional variation in eGFR is explained by epigenetic markers. The authors concluded that at least six EM were identified to predict eGFR.

Most importantly, innovative work by T. M. Brockie et al. entitled "*a framework to examine the role of epigenetics in health disparities among Native Americans*" offers an interesting insight to stress among Native Americans. They reported that Native Americans disproportionately experience adverse childhood experiences (ACEs) as well as health disparities, including high rates of posttraumatic stress, depression, and substance abuse, and that many ACEs have been linked to methylation changes in genes that regulate the stress response, thus suggesting that these molecular changes may underlie the risk for psychiatric disorders. The authors reviewed published studies to provide evidence that ACEs-related methylation changes contribute to health disparities and that this framework may be adapted to understand how ACEs may result in health disparities in other racial/ethnic groups.

S. M. Underwood et al. were interested in factors that may prevent involvement of African Americans' participation in genetic research. Their study entitled"*enhancing the participation of African Americans in health-related genetic research: findings of a collaborative academic and community-based research study*" sought to identify factors inhibiting the participation of African Americans in health-related research. They employed an exploratory study of factors presumed to be associated with participation in health-related research, among a nonprobability sample of African Americans from a large urban community in the Midwest. The results revealed that knowledge, beliefs, and perceptions about genetics and the involvement of providers were associated with willingness to engage in health-related genetic research. The most interesting, however, was that 88.7% of the participants reported that "they had never been asked" to participate in research.

Similarly, J. Y. Taylor et al. were concerned about the high prevalence of hypertension among African American women. Their study entitled "*attitudes toward genetic testing for hypertension among African American women and girls*" sought to understand perceived barriers and benefits for genetic testing of multigenerational triads. They employed the health belief model as a guide to examine attitudes toward perceived barriers and benefits of genetic testing and to determine whether they differed by generation, age, education, or income level. Results indicated that increased age and education were associated with significant differences in attitudes regarding benefits. They highlighted the need for increased outreach to younger generations regarding benefits of genetic services.

These articles illustrate and summarize many of the ways in which nurse scientists are involved in disparities around genetics and genomic research. Understandably, some medical research adopts well-established perspective that racial discrimination and poverty are the major contributors to unequal health status. Therefore programs advocating social justices must be supported.

The editors of this special topic issue hope that these studies will stimulate additional discussion around genetic variation between and among population groups, the role of epigenetics in understanding genes expression of chronic diseases, and the inequalities observed among "racialized groups."

The overall intention of the editors is that other nurses will become proactive in understanding the relationship between gene expression, environment, and illness. It is critical that research on the genetics of human health and disease continue and bring to clinical fruition the vast storehouse of basic and observational research that the human genome project has produced.

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Research Article

The Association between KIF6 Single Nucleotide Polymorphism rs20455 and Serum Lipids in Filipino-American Women

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The Trp719Arg allele of KIF6 rs20455, a putative risk factor for CHD especially in those with elevated low-density lipoprotein cholesterol (LDL-C), was investigated in Filipino-American women (FAW, $n = 235$) participating in health screenings in four cities. The rs20455 genotype of each subject was determined by a multiplex assay using a Luminex-OLA procedure. The risk allele Trp719Arg was present in 77% of the subjects. The genotype distribution was 23% Trp/Trp, 51% Arg/Trp, and 26% Arg/Arg. Genotype did not predict the presence of CHD risk factors. Moreover, LDL-C, HDL-C, and triglycerides mean values did not vary as a function of genotype. However, those with the Arg/Arg genotype on statin medication exhibited a significantly higher mean triglycerides level ($P < 0.01$). Approximately 60% of participants regardless of genotype exhibited LDL-C levels ≥100 mg/dL but were not taking medication. Approximately 43% of those with the Trp719Arg risk allele on statins exhibited elevated LDL-C levels. Our study suggests that the Trp719Arg allele of KIF 6 rs20455 is common among Filipino-American women; thus, even with borderline LDL-C levels would benefit from statin treatment. Secondly, many participants did not exhibit guideline recommended LDL-C levels including many who were on statin drugs.

1. Introduction

Coronary heart disease is a multifactorial and complex disease resulting from the interaction of genes and environmental factors [1–5]. Genetics plays an important role in determining the inherent CHD vulnerability and in determining how a person responds to statin therapy. KIF6 is a member of the kinesin family, a class of motor proteins that are involved in the intracellular transport of membrane organelles, messenger RNA's and other protein complexes along microtubules [6–10]. Several studies focusing on Caucasians have reported that the Trp719Arg allele of single nucleotide polymorphism (SNP) rs20455 in the *KIF6* gene is associated with CHD and that carriers who carry one or two copies of the risk allele respond better to statin therapy than noncarriers [10–15]. The prevalence among Caucasians is about 59% and the risk ratio has been stated to be 1.22 (95% confidence intervals 1.12–1.32) [15]. This association with increased risk was present in African Americans, who have very high numbers (~90%) with the risk allele [15]. Chinese and Japanese also have been reported to have a high frequency (~70%) of the risk allele [15]. Other investigators were unable to detect the association of the rs20455 allele with increased CHD risk [16] even with a large sample size [17]. Ference et al. [18] carried out a large meta-analysis with 88,535 subjects in 8 randomized statin trials examining the role of KIF6 Trp719Arg allele in CHD and concluded that the risk allele increases the vulnerability to elevated LDL-C cholesterol. Thus, the KIF6 genetic variant may be a predictor

of CHD in those who have elevated LDL-C. Indeed, carriers of the Trp719Argallele may have a greater increase in CHD risk per unit increase in LDL and a greater reduction in CHD risk per unit decrease in LDL, compared to noncarriers [18]. Indeed, the number needed to treat with statins to prevent a single CHD event ranged from 10 to 20 for the Trp719Arg carriers compared to >80 for noncarriers in a large meta-analysis [15]. Furthermore, the results of Ference et al. [18] suggest that the differential vulnerability to LDL-C based upon genotype may explain the reason for the prior disagreement in studies.

To the best of our knowledge despite a plethora of evidence, no studies of KIF6 Trp719Arg allele have been conducted in the Filipino population. Filipinos are the second largest minority population of Asians in America [19] and have the highest prevalence of hypertension [20], type 2 diabetes [21, 22], and metabolic syndrome [23] compared with other Asian subgroups. The heterogeneity of CHD risk factors among Asians is well established [24] and the allelic frequency distribution of rs20455 has been shown to vary across populations [15]. Scientific gaps still exist regarding the role of genetics in the predisposition to CHD in various populations. Since the rs20455 variant has not been studied in those of Filipino descent, we investigated its prevalence in association with plasma lipid levels in a cohort of Filipino American women, who attended community-based health screenings at various locations throughout the USA.

2. Materials and Methods

2.1. Study Design, Population, and Recruitment Procedure. Approval of the University Institutional Research Board (IRB) was obtained prior to the conduct of this study. This study is a descriptive, cross-sectional study of Filipino American women ($n = 234$), who participated in a cardiovascular health screening at their places of worship or cultural centers during 2011–2013. Subjects were between 40 and 65 years of age. Health screenings were performed in various US cities: Jacksonville, FL; Chicago, IL; Tampa, FL, and San Francisco, CA. In order to recruit a wide variety of subjects, advertisements were posted in church bulletins and at community organizations and stores frequently visited by Filipino women. These flyers specified inclusion and exclusion criteria and the need to be fasting for at least 12 hours on the day of the study. Participants were instructed to bring all prescription and nonprescription medications to the study site.

Participants were enrolled if they were women, self-identified as Filipino by ethnicity, agreed to volunteer for the study, and were fasting for 12 hours. Women who had severe arthritis, any autoimmune disorder, and a recent cancer diagnosis and/or presented with any infection or severe inflammation were excluded from the study important for the measurement of inflammatory markers. Informed consent was obtained after the study purpose was carefully explained. Prior to giving consent, participants were given ample time to ask questions or state concerns regarding the study. Total time for participation in the study was approximately 45 min per participant.

2.2. Screening Protocol. A demographic and clinical information questionnaire, including participants' medical history and current medications, was completed for each subject by trained research staff. The clinical information questionnaire included family history, smoking history, participant's medical history, and current medications. Upon completion of the survey, blood pressure was obtained using a standard Omron digital HEM-705CP sphygmomanometer on the nondominant arm, after the participant had been seated for 10 min. Measurements were repeated three times with 5 min in between each reading. Subsequently, weight and height were measured using a standard Tanita weighing scale (WB-3000). Waist circumference was measured using a tension tape guided by the NHANES measurement protocol. Lastly, a licensed phlebotomist drew 5 mL of blood via venipuncture from each participant for a series of tests including the standard lipid panel, hemoglobin A1C, serum glucose, a cardiovascular inflammatory biomarker (high-sensitivity C-reactive protein or hs-CRP), and genetic assays. The blood was captured in three test tubes containing EDTA. Sample tubes were labeled with numbers only, centrifuged, and immediately frozen. At the end of each screening session, frozen samples were sent to a CLIA certified laboratory where assays were performed using standard clinical protocols. The lipid panel assay included total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL-C) (Roche Modular methodology) performed at Berkeley Heartlab, Inc. (Alameda, CA). Plasma hs-CRP concentration was determined using an automated immunoturbidimetric assay (Roche Modular methodology, Berkeley Heartlab Inc.).

2.3. Cardiovascular Risk Factors. The operational definition for CHD risk factors for the study was based on the following guidelines. For blood pressure values, the American Heart Association and the Joint National Committee for the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) guidelines for the normal range of blood pressure were used (≥120 mmHg systolic and/or diastolic blood pressure of ≤80 mmHg). We used the criteria of the World Health Organization regarding cut-off for the body mass index (BMI). The National Cholesterol Education Program (NCEP) Adult Treatment Panel III guidelines were used to define the cholesterol normal reference values, namely, total cholesterol (TC) ≥200 mg/dL, triglycerides (TGL) ≥150 mg/dL, high-density lipoproteins cholesterol (HDL-C) ≤50 mg/dL, low-density lipoprotein cholesterol (LDL-C) ≥100 mg/dL, fasting blood glucose (FBG) ≥100 mg/dL (5.6 mmol/L), and waist circumference ≥35 inches (88 cm). The American Diabetes Association criteria for diabetes were used: hemoglobin A1C ≥6.5% and fasting plasma glucose ≥126 mg/dL. Metabolic syndrome was defined using both the International Diabetes federation and the National Cholesterol Education Program-Adult Treatment Panel III criteria.

Table 1: Demographic characteristics of the Filipino-American women subjects.

Demographic characteristics	Mean ± SD (n = 234)	Frequency (%)
Age	51.5 ± 7	—
No. of years in the USA	24.0 ± 13	—
Age—arrival in USA	31.0 ± 11	—
Marital status		
Single		8
Married		73
Divorced/widow		19
Philippine-born		98
Residency in the USA		
<5 years		11
5–10 years		6
10–20 years		26
Over 20 years		57
Income		
<$12,000/year		17
$13,000–$40,000		47
$41,000–$69,000		20
$70,000 and above		16
Education		
High school		18
Some college		21
4 year degree		52
Graduate degree		9
Occupation		
Health occupations		34
Healthcare insurance		
With insurance		87

Table 2: Morphometric measurements and cardiovascular risk factors of the Filipino-American women participants (n = 235).

Measurement	Means ± SD	Percent of group (risk level)
Weight (lbs.)	151 ± 22	
Height (inches)	61 ± 2	
Body mass index	29 ± 4	37 (≥25 kg/m^2) 15 (≥30 kg/m^2)
Waist circumference	40 ± 4	79 (≥35 inches)
Systolic blood pressure	131 ± 19	64 (≥120 mmHg)
Diastolic blood pressure	87 ± 10	61 (≤80 mmHg)
Fasting blood glucose	101 ± 25	38 (≥100 mg/dL)
Hemoglobin A1C	6.0 ± 0.8	36 (≥6.5%)
Total cholesterol	201 ± 45	44 (≥200 mg/dL)
Triglycerides	116 ± 67	19 (≥150 mg/dL)
Low-density lipoprotein-C	122 ± 35	61 (≥100 mg/dl)
High-density lipoprotein-C	62 ± 15	21 (≤50 mg/dL)
High-Sensitivity C-Reactive protein	1.97 ± 3.0	13 (<3.0 mg/L)
Metabolic syndrome		56
Smoking		3
Family history		48

Note: metabolic syndrome was defined by both the International Diabetes Federation (IDF) and the National Cholesterol Education Program—Adult Treatment Panel III (NCEP/ATP III) criteria.

2.4. Genetic Analysis. Celera research reagents were used to genotype the rs20455 SNP of each subject in a single-tube assay using a Luminex-OLA procedure as described previously [25]. The procedure included amplification of genomic DNA (~3 ng) by multiplex PCR followed by multiplex OLA. The resulting ligation products were hybridized to Luminex xMAP microspheres and labeled by the reporter molecule SA-PE. The xMAP microspheres were analyzed on a Luminex 100 or a Luminex 200 systems. Genotypes were subsequently determined using the Celera allele calling software as described in Iannone et al. [25].

2.5. Data Analysis. A confidential, password-secured database was used for data entry, management, and analysis. Inconsistencies were checked, and the data descriptions were verified by the principal investigator and the statistician. Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 19) and GraphPad Prism 5 software. Means ± standard deviations were determined for all continuous variables and number and percentage were determined for categorical variables. Analysis of variance (ANOVA) was used to compare group means and multiple stepwise linear regression determined whether risk alleles significantly predicted CHD risk factors, controlling for alternative predictors of CHD risks. Dichotomous variables were analyzed using Fisher's chi-square tests for independence with Yates continuity correction. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Demographic Characteristics. Demographic characteristics of the nonrelated Filipino-American women (n = 234) enrolled in the study are shown in Table 1. The mean age of the women was 55.4 ± 7.1 years old and the majority (98%) were born in the Philippines with the mean length of residency in USA of 24.1 ± 13.1 years. The mean age upon arrival in the USA was 31.1 ± 10.9 years. The majority (75%) were married and many had completed a college degree and were employed in health-related professions. Most of the participants (88%) had some sort of insurance including private insurance (49%).

3.2. Cardiovascular Risk Factors. Clinical and morphometric measurements are shown in Table 2. Participants had a mean height of 5′1″ and mean weight of 151 lbs. and 50% of participants were considered either overweight or obese as indicated by BMI. The majority were classified as prehypertensive because blood pressure was over the criterion

Table 3: Selective cardiovascular disease risk factors as a function of the rs20455 genotype and statin medication usage.

Risk factors	AA Trp/Trp $n = 53$ 23%	AG Arg/Trp $n = 120$ 51%	GG Arg/Arg $n = 62$ 26%	ANOVA P value
Age				
Total	52 ± 7	54 ± 7	52 ± 6.9	0.09
No statins	51 ± 10	52 ± 9	50 ± 10	0.28
Statins	52 ± 4	54 ± 6	52 ± 7	
HDL-C				
Total	60 ± 14	64 ± 15	61 ± 16	0.21
No statins	60 ± 16	65 ± 7	63 ± 15	0.06
Statins	56 ± 11	61 ± 15	52 ± 14	
LDL-C				
Total	114 ± 36	113 ± 38	113 ± 37	0.71
No statins	118 ± 39	119 ± 39	116 ± 34	0.59
Statins	106 ± 32	101 ± 36	113 ± 40	
TG				
Total	119 ± 58	109 ± 67	130 ± 74[1,2]	0.009
No statins	118 ± 60	107 ± 74	111 ± 54	
Statins	121 ± 50	113 ± 39	177 ± 97[3]	0.002
Waist Circ				
Total	37 ± 7	37 ± 6	36 ± 7	
No statins	36 ± 6	37 ± 4	37 ± 4	0.93
Statins	37 ± 4	36 ± 7	37 ± 3	
BMI				
Total	26 ± 4	28 ± 4	26 ± 4	0.06
No statins	26 ± 4	27 ± 4	25 ± 5	
Statins	30 ± 5	30 ± 4	27 ± 5	0.15
Hs-CRP				
Total	1.41 ± 1.5	1.86 ± 2.2	1.55 ± 2.1	0.45
No statins	2.1 ± 3.9	2.2 ± 3.3	1.6 ± 1.7	
Statins	1.6 ± 2.9	1.4 ± 1.9	2.2 ± 3.0	0.87
HbA1c				
Total	5.8 ± 0.46	5.8 ± 0.49	6.01 ± 0.97	0.39
No statins	5.8 ± 0.48	5.8 ± 0.48	5.9 ± 0.66	
Statins	6.3 ± 1.2	6.1 ± 0.54	6.4 ± 1.5	0.17

Note: HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein-cholesterol; TG: triglycerides; Waist Circ: waist circumference; BMI: body mass index; hs-CRP: high-sensitivity C-reactive protein; HbA1c: Hemoglobin A1c. [1]$P < 0.05$ Trp/Trp versus Arg/Arg, [2]$P < 0.01$ Arg/Trp versus Arg/Arg, and [3]Arg/Arg versus all other groups $P < 0.001$; AA no statins $n = 22$, statins $n = 12$; AG no statins $n = 48$, statins $n = 21$; GG no statins $n = 26$, statins $n = 16$.

set forth by the JNC 7. Approximately 36% of the women had diabetes as indicated by the levels of hemoglobin A1C. A high percentage (61%) of the participants had elevated LDL-C but the percent of the group (21%) with unhealthy HDL and elevated triglycerides (19%) was much smaller. Elevated hs-CRP was seen in 13% of the group. Approximately 50% of participants were classified as having metabolic syndrome. About 50% of participants self-reported a family history of heart disease, indicated by stroke and/or CHD events of parents and immediate siblings. Only 3% of the participants smoked and thus smoking was not thought to be a confounder in this study.

Table 4: Multiple regression analysis of cardiovascular risk factors.

Variables	TG T ratio (P value)	LDL T ratio (P value)	HDL T ratio (P value)
Genotype	0.57 (0.27)	0.18 (0.86)	1.11 (0.24)
HbA1c	3.42 (0.0008)	0.75 (0.45)	3.36 (0.001)
hs-CRP	0.97 (0.34)	0.41 (0.68)	1.92 (0.06)
Age	0.46 (0.64)	2.32 (0.02)	1.15 (0.25)
BMI	0.56 (0.57)	1.23 (0.22)	0.22 (0.86)

Note: HbA1c: hemoglobin A1c; hs-CRP: high-sensitivity C-reactive protein.

3.3. KIF6 rs20455 Genotype and CHD Risk Factors. The distribution of genotypes is as follows: 23% AA (Trp/Trp), 51% AG (Arg/Trp), and 26% GG (Arg/Arg). Thus, a majority (77%) of participants have at least one copy of the risk allele. This SNP was found to be in Hardy Weinberg equilibrium. There were no differences in mean age, BMI, waist circumference, hs-CRP, or HbA1c between the groups (Table 3).

Differences in lipid risk factors as a function of the rs20455 genotype and statin use are shown in Table 3. The mean value of TG was significantly elevated in the GG genotype (Arg/Arg) group ($P < 0.009$) but further analysis indicated that this elevation was only seen in those of this genotype taking statin medication. Multiple regression analyses confirmed a lack of association of genotype with TG levels but indicated a strong association with diabetes ($P = 0.0008$) indicated by HbA1c (Table 4). The prevalence of high TG (≥150 mg/dL) as a function of genotype was 10% of AA (Trp/Trp), 16% of AG (Trp/Arg), and 38% of GG (Arg/Arg) genotype (Fisher's exact $P < 0.001$) indicating that those subjects with the GG (Arg/Arg) genotype were more likely to have elevated TG levels. Similar to TG, there was a lack of association of genotype with HDL-C levels but an association with diabetes ($P = 0.001$). No differences in mean values of HDL-C between groups were apparent. No association of LDL-C with genotype nor differences in mean values were seen as a function of genotype or statin usage but an association of LDL-C with age was seen ($P = 0.02$).

We also investigated the prevalence of elevated LDL-C as a function of genotype and statin use. Many participants (60–65%; Table 5) had elevated LDL-C and were not being treated with statins including 65% of those with the Trp719Arg allele. Moreover, many in each genotype who were on statin medication exhibited elevated LDL-C including 43% of those with the Trp719Arg allele.

4. Discussion

The major finding of this study is a high prevalence (0.70) of the Trp719Arg allele in the Filipino-American women participants. Moreover, a high percent of women with the risk

Table 5: Percent of participants with elevated LDL as a function of genotype and statin usage.

Medications	Trp/Trp (AA)	Arg/Trp (AG)	Arg/Arg (GG)	Arg/Trp Arg/Arg (AG + GG)	Fisher's exact test *P* Value
No statins	$n = 22$	$n = 48$	$n = 26$	$n = 74$	
% elevated LDL-C	69%	67%	62%	65%	0.41
Statins	$n = 12$	$n = 21$	$n = 16$	$n = 37$	
% elevated LDL-C	58%	33%	56%	43%	0.31

allele and elevated LDL-C levels were not being treated with statin medications and a significant number of those with the risk allele on statin medications still exhibited elevated LDL-C levels. Results from the current study are novel since an extensive search in the literature revealed no studies describing the prevalence of rs20455 SNP of KIF6 and its association with lipid levels in Filipino-American women. Our study may be the first in which the CVD rs20455 risk allele and its association to LDL-C were determined in this group of understudied minority women. Our current results showed that the majority of the FAW participants carried a single (heterozygote) or double (homozygote) copy of the risk allele. The carrier frequency seen for Filipino-American women is similar to those reported by Li et al. [15] for Asians (Japanese and Chinese) which was 72% based upon HapMap and Celera data. Thus, the carrier frequency may be higher in Asians than Caucasians who had a prevalence of 59% in one study [15]. Peng et al. [9] have reported differences in the rs20455 allele frequencies across populations.

Another finding of our study is that TG levels were significantly elevated in individuals with the Arg/Arg genotype and on statin medication. This result could be interpreted as those with Arg/Arg genotype have higher TG levels when they have elevated LDL-C but, given the small subject number, further study is warranted. Wu et al. [10] in a case-control study evaluated the association of KIF6 rs20455 SNP with angiographic CAD and serum lipid levels in the Han Chinese population of northern China. Their results demonstrated no significant differences in genotype and allele frequency between the cases (angiographic CAD) and controls ($P > 0.05$). However, further analysis showed that nonfatal MI risk and TG levels were significantly higher in 719Arg carriers compared to noncarriers ($P < 0.05$) [10].

Our findings demonstrated that there were no differences in HDL or LDL-C levels as a function of genotype; however, this finding must be viewed with caution because the sample size may not be sufficiently powered to discern an association of genotype with lipid levels. Importantly, there were a significant number of Filipino-American women, including many with the Trp719Arg allele, who had elevated LDL-C but were not being treated with statins. Others were being treated but had not achieved recommended levels of LDL-C. Although studies regarding KIF6's polymorphism and risk for CHD are equivocal, the KIF6 genetic variant may be a predictor of CHD in those who have elevated LDL-C as described in the Introduction [10–18]. Given that the number needed to treat with statins to prevent a single CHD event ranged from 10 to 20 for the Trp719Arg carriers, it is imperative that those with this allele be treated for elevated LDL-C [15]. The elevated LDL-C in participants on statin medication could indicate noncompliance with statin medications due to muscle pains or other side effects of the drugs; however, we did not capture data about nonadherence to prescribed cholesterol-lowering medication [26, 27]. Additionally, elevated LDL-C levels despite use of statins in this population may be conceivably due to genetic differences of this population [9]. These results may also be due to the differential performance of lipophilic and hydrophilic statins [28, 29]. Bonsu et al. [28] conducted a systematic review and meta-analysis of lipophilic and hydrophilic statins on heart failure patients. The investigators claimed that there are differences in the pleotropic effects of statin medications due to differences in lipophilicity in the type of statin medications. Lipophilic statins utilize passive diffusion for entering cells while hydrophilic statins use carrier-mediated process for uptake in the liver cells. Although there are controversial outcomes on the effects of lipophilic statins, this group of statins appear to show more beneficial effects than those of hydrophilic statins, though improved outcomes have been reported for rosuvastatin in the Controlled Rosuvastatin in Multinational Trial in Heart Failure (CORONA) and the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Heart Failure (GISSI-HF) trials [29]. Regardless of cause, further research is required to determine why LDL-C is not at optimal levels among Filipino-American women even when taking cholesterol-lowering medications. Our study indicates that this population needs to be more carefully monitored and treated for elevated LDL-C. Specific statin medications may have adverse effects in Asian populations [30]. In 2005, the US Food and Drug Administration provided additional safety label warning for rosuvastatin since a pharmacokinetic study that included a diverse population of Asians showed that rosuvastatin drug levels were found to be approximately twofold higher when compared with the Caucasian control group. This in effect may produce increased risk of muscle myopathies and risk of kidney failure in Asians [30].

Many clinical trials have shown the benefit of lowering cholesterol levels even in those with relatively normal cholesterol levels and no previous myocardial infarctions [31–33]. One clinical trial of participants with myocardial infarction and plasma total cholesterol <240 mg/dL and LDL-C of 115–174 mg/dL randomized to 40 mg of pravastatin

per day or placebo had 10.2% events in the pravastatin group and 13% events in the placebo group indicating that cholesterol-lowering treatment is beneficial for CAD patients with borderline cholesterol levels [31]. Pravastatin lowered cholesterol by 20% and LDL-C by 26% and reduced MI incidence in men with hypercholesterolemia and no previous MI [32]. These studies also suggest the need for cholesterol lowering in carriers of the rs20455 allele. For example rs20455 allele carriers had a hazard ratio of 1.50 in the CARE trial [31] and odds ratio of 1.55 (95%CI 1.14–2.09) in WOSCOPS trial [32]. For the rs20455 carriers the absolute risk reduction by pravastatin was 4.89% in the CARE trial and 5.49% in the WOSCOPS trial. In the PROSPER [33] study among the elderly population with previous CVD disease on pravastatin therapy, there was an absolute risk reduction of 6.3% in KIF6 carriers versus 1.2% in noncarriers with a 33.6% reduction of relative risk among carriers with the number needed to treat with pravastatin significantly lower in carriers compared to noncarriers (16 versus 83). Thus, these three trials demonstrated that KIF6 risk allele carriers had an increased risk of developing coronary events and the use of pravastatin significantly reduced the risk. Observational studies with multiple populations also found increased risk in allele carriers [34–38], Another study of 539 participants showed that the KIF6 risk variant was associated with cardiac events with a hazard ratio of 1.33 after adjustment for age, sex race, high-sensitivity C-reactive protein, and LDL-C [34]. In the ARIC [35, 36] study, carriers of the KIF6 719Arg risk variant had a higher incidence of CHD with a hazard ratio of 1.22 after model was adjusted for age and sex. In the WHS [38] study, carriers of the KIF67 719Arg risk variant had a greater CHD risk with a hazard ratio of 1.24 and a hazard ratio of 1.34 associated with risk for MI.

In contrast, other large studies have not found an association of Kif6 allele with statin response [39, 40]. The Heart Protection Study (HPS) with 18,348 randomized patients found that there was no impact of KIF6 genotype on statin therapy and vascular events across all genotypes [39, 40]. In The JUPITER Trial, Caucasian participants with low LDL-C <130 mg/dL and elevated high-sensitivity C-reactive protein ≥2 mg/L were randomly assigned to rosuvastatin or placebo and followed for first major CVD event [12]. There were no differences in vascular event rates in KIF6 risk allele carriers compared to noncarriers (hazard ratio of 0.91) nor amount of LDL-C reduction. Differences in study design between the earlier statin trials and more recent trials such as HPS [39, 40] and JUPITER [12] could help explain the different findings. In the HPS placebo patients have been intensively treated with 40 mg simvastatin for 6 weeks prior to randomization which substantially reduced LDL-C levels and according to Ference et al. [18] could attenuate risk associated with KIF6 and therefore explain the lack of a differential response to statin therapy in those with KIF6 genotype. And, similarly, in JUPITER only participants with low LDL-C levels were enrolled in the study and were treated with high-dose rosuvastatin. Such contradictory findings necessitate further investigation of the effect of KIF6 polymorphism on cardiovascular health.

4.1. Limitations. Certain study limitations merit comment. First, the study was based upon a small number of subjects and the significant genetic admixture of Filipinos also presents a challenge when small population subsets are studied. The exclusion of Filipino-American men was another limitation to the study, although it should be noted that most studies include only men and women have been an understudied gender. Moreover, we have developed a successful method of recruiting women for these sorts of studies in community settings. It is not clear if this method would work for Filipino-American men.

Third, since this study was only conducted on the Filipino population in the United States, the generalizability to Filipinos residing in other countries is not known. Fourth, the use of the cross-sectional design limits examining risk factors for heart disease at a single point in time without the ability to measure cardiovascular outcomes.

5. Conclusion

Our results showed that 70% of a cohort of nonrelated Filipino-American women are carriers of the Trp719Arg risk allele (rs20455 SNP of the KIF6 gene). Importantly, many of these women, even those treated with statins, did not achieve guideline recommended LDL-C levels. Although larger population studies are needed to confirm our findings, the results indicate that Filipino-American women need to be more carefully monitored and treated for elevated cholesterol in order to reduce the incidence of CHD in this population.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Epigenetic Markers of Renal Function in African Americans

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Chronic kidney disease (CKD) is an increasing concern in the United States due to its rapidly rising prevalence, particularly among African Americans. Epigenetic DNA methylation markers are becoming important biomarkers of chronic diseases such as CKD. To better understand how these methylation markers play a role in kidney function, we measured 26,428 DNA methylation sites in 972 African Americans from the Genetic Epidemiology Network of Arteriopathy (GENOA) study. We then evaluated (1) whether epigenetic markers are associated with estimated glomerular filtration rate (eGFR), (2) whether the significantly associated markers are also associated with traditional risk factors and/or novel biomarkers for eGFR, and (3) how much additional variation in eGFR is explained by epigenetic markers beyond established risk factors and biomarkers. The majority of methylation markers most significantly associated with eGFR (24 out of the top 30) appeared to function, at least in part, through pathways related to aging, inflammation, or cholesterol. However, six epigenetic markers were still able to significantly predict eGFR after adjustment for other risk factors. This work shows that epigenetic markers may offer valuable new insight into the complex pathophysiology of CKD in African Americans.

1. Introduction

Chronic kidney disease (CKD) is an increasing public health concern in the United States due to its rapidly rising incidence and prevalence, particularly among older individuals. While about 20 million United States adults over the age of 20 (10%) currently have CKD, the prevalence of CKD among those 60 and older is approximately 25% [1]. Further, the incidence of CKD among those aged 65 and older more than doubled between 2000 and 2008 [1]. As a result, health care costs related to the most severe form of CKD, End-Stage Renal Disease (ESRD), have also nearly doubled in the past decade to over $40 billion per year [1]. Individuals over 60 are almost 6 times more likely to develop CKD than those aged 20–39, and females are 1.4 times more likely than males to develop it [2]. Further, African Americans are at higher risk for ESRD than other races. While African Americans accounted for only 12% of the US population in 2009, they accounted for nearly one-third of kidney failure cases [3].

Level of kidney function is assessed by the glomerular filtration rate (GFR), the rate at which blood passes through the kidney's filtering mechanisms. GFR levels below 60 mL/min/1.732 m^2 are used, in conjunction with markers of kidney damage such as proteinuria, to diagnose CKD and determine its severity [4]. GFR is difficult to measure directly, but it can be estimated using blood markers such as creatinine in combination with demographic factors (age, sex, and ethnicity). Since the early stages of CKD generally have few or no symptoms, disease detection is difficult before progressive kidney damage has already occurred [5].

Several risk factors have been implicated in CKD etiology. The most common are diabetes mellitus, hypertension, obesity, elevated cholesterol, smoking, and cardiovascular disease (CVD) [3, 5]. Approximately 20% and 35% of US adults with diabetes and hypertension, respectively, have CKD [3]. Further, a recent study showed that hypertension, smoking, obesity, and low HDL cholesterol were associated with reduced kidney function [6]. While risk prediction of CKD is in its infancy, studies have found moderate prediction capability for CKD development and progression using models that include hypertension, diabetes, history of CVD, body mass index, and other variables [7].

The identification of new biomarkers for early detection of CKD is crucial to developing novel prevention strategies. It is particularly important to identify markers within high-risk populations in order to reduce social disparities in CKD and ESRD incidence. Researchers have been exploring the use of inflammatory markers as potential biomarkers of kidney function in recent years. Several such markers have been shown to be strongly associated with renal function. Specifically, C-reactive protein, fibrinogen, homocysteine, and several other markers of inflammation are elevated in individuals with decreased kidney function [8–11].

Epigenetic markers are now also being considered as potentially viable predictors of kidney function [12]. Epigenetics refers to mitotically heritable genomic modifications that do not alter the underlying DNA sequence. DNA methylation is one type of epigenetic modification that alters gene transcription [13], potentially influencing initiation and progression of chronic diseases such as CKD. Alterations in DNA methylation have already been shown to be associated with a variety of chronic diseases including CKD, cardiovascular disease, cancer, diabetes, and systemic lupus erythematosus [10, 12–21]. For example, a recent study found that patients with Stage 5 CKD with inflammation and hyperhomocysteinemia exhibited global DNA hypermethylation in blood leukocytes compared to patients with no inflammation and typical homocysteine levels [10]. In spite of the progress in this research area, little is still known about the relationship between epigenetics and kidney disease.

In order to better understand how epigenetics, inflammation, and traditional risk factors can explain the variation in kidney function, we conducted a study that evaluated three key questions: (1) is DNA methylation in peripheral blood cells associated with eGFR? (2) are the significantly associated epigenetic markers also associated with traditional risk factors and/or novel biomarkers for eGFR? and (3) how much additional variation in eGFR is explained by the epigenetic markers beyond these risk factors and biomarkers? Although peripheral blood cells may not be fully representative of kidney epigenetic patterns, leukocytes (a key component of peripheral blood cells) orchestrate the inflammatory responses within the kidney and are therefore likely to play a role in the pathophysiology of CKD. Juxtaposing the epigenetic biomarkers in peripheral blood cells against more traditional risk factors will allow us to identify new interconnections between genome biology and CKD precursors.

2. Methods

2.1. Sample. The Genetic Epidemiology Network of Arteriopathy (GENOA) study is a community-based study investigating the genetics of hypertension and its arteriosclerotic complications in non-Hispanic whites from Rochester, Minnesota, and African Americans from Jackson, Mississippi [22]. In this study, we investigated the relationship between DNA methylation and eGFR in GENOA African Americans. African American sibships in which at least two siblings had been diagnosed with primary hypertension before the age of 60 ($N = 1,854$) were recruited for an initial examination (Phase I: 1996–1999) that included standardized interviews concerning demographic and medical history, as well as a physical examination and blood sample collection. The second examination (Phase II: 2000–2004) comprised 1,482 participants returning from Phase I. This exam included reassessment of the original interview, a physical examination, and a blood draw, as well as additional measurements of arteriosclerotic target-organ damage of the kidney, heart, brain, and peripheral arteries.

2.2. Measurement of Traditional Risk Factors. Height was measured by stadiometer and weight by electronic balance, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Resting systolic blood pressure and diastolic blood pressure were measured by a random zero sphygmomanometer. The diagnosis of hypertension was established based on BP levels measured at the study visit (>140 mmHg average systolic BP or >90 mmHg average diastolic BP) or a prior diagnosis of hypertension and current treatment with antihypertensive medications.

Blood was drawn by venipuncture after an overnight fast. Serum triglycerides (TG), creatinine, total cholesterol, glucose, and high-density lipoprotein (HDL) cholesterol were measured by standard enzymatic methods on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Estimated GFR (eGFR) was calculated for each participant from serum creatinine values and relevant demographic factors (age, sex, and race) using the CKD-EPI creatinine equation [23]. Diagnosis of diabetes was established based on fasting glucose levels >126 mg/dL measured at the study visit or current treatment with diabetes medications. C-reactive protein was measured by a highly sensitive immunoturbidimetric assay [24], fibrinogen was measured by the Clauss (clotting time based) method [25], and plasma homocysteine was measured by high-pressure liquid chromatography.

2.3. Measurement of DNA Methylation. DNA methylation was quantified on 1,008 Phase II participants using stored blood samples collected during the second examination. Samples were prepared and DNA methylation was measured according to previously published methods [26–28]. Briefly, the Illumina Infinium HumanMethylation27 BeadChip microarray was used to measure DNA methylation at 27,578 CpG sites. To reduce batch and chip effects, the correlation structure among 56 control probes was evaluated

within channel to identify the most parsimonious subset of probes that explained the maximum amount of batch and chip variation across samples (5 probes in the red channel and 8 probes in the green channel). Normalization was conducted by linearly regressing the 13 selected probes onto the intensity signals from the methylated (**M**) and unmethylated (**U**) bead types separately across each CpG site.

The M-value is a commonly used measurement in microarray analysis that was recently adapted for use with DNA methylation array data. We chose to assess DNA methylation using the M-value because the statistical distributions of M-values for individual CpG sites conform to modeling assumptions more often than do those of other standard metrics, such as the Beta value [29, 30]. The M-value for each individual i at a single site, k, is calculated as follows: $M\text{-value}_{ik} = \log_2 [(\max(\mathbf{M}_{ik}, 0) + 1)/(\max(\mathbf{U}_{ik}, 0) + 1)]$ [30]. M-values that are <−2.0 are considered to be unmethylated, M-values that are >2.0 are considered methylated, and M-values that are between −2.0 and 2.0 are considered semimethylated.

Prior to statistical analysis, we removed samples that had poor bisulfite conversion ($N = 7$), as determined by bisulfite-conversion control fluorescence intensity of <4,000. An additional 29 samples were removed from the analysis due to extreme control probe values, assessed as having at least one control probe with a value of greater than 4 standard deviations from its mean value. This resulted in a total sample size of 972 individuals.

In this study, we analyzed only autosomal CpG sites. A total of 58 CpG sites were removed from the analysis because they were found to be multimodal based on the Dip Test proposed by J. A. Hartigan and P. M. Hartigan [31] using a cut-off of $P < 0.001$ on the signal intensities of the methylated and/or unmethylated bead types. This resulted in a total number of 26,428 CpG sites included in our analysis.

2.4. Statistical Analyses

2.4.1. Linear Mixed Modeling. We used linear mixed modeling to identify the top 30 CpG sites that were most significantly associated with eGFR, prior to adjustment for any risk factors. Rather than adjusting for age and sex prior to any statistical analysis, each of the 26,428 CpG sites were modeled individually as covariates against eGFR so that we were not failing to detect sites that act as mediators of age and sex on eGFR *a priori*. The linear mixed model $\text{eGFR}_{ij} = \beta_0 + \beta_1 \cdot \text{CpG}_{ij} + W_j + \varepsilon_{ij}$ (Model 1) was estimated using the nlme package in the statistical R software version 2.14.0 [32]. CpG_{ij} is the M-value of the epigenetic marker for participant i in sibship j, and W_j is the random effect for sibship j.

2.4.2. Linear Modeling. In order to determine the risk factors most highly associated with eGFR, we performed forward selection using traditional linear modeling. We then checked the robustness of the modeling using linear mixed modeling to ensure that accounting for family structure did not influence the inferences of the linear modeling. We used traditional linear modeling instead of linear mixed modeling to facilitate the comparison of R^2 values among nested models. Univariable linear regression models of the generic form $\text{eGFR}_i = \beta_0 + \beta_1 \cdot \text{Risk Factor}_i + \varepsilon_i$ (Model 2) were used to evaluate the relationships between eGFR and traditional risk factors as well as novel risk factors. Traditional risk factors including diabetes, hypertension, cholesterol, blood pressure, age, sex, and anthropometric measures were considered in addition to novel inflammatory biomarkers such as homocysteine, fibrinogen, and C-reactive protein. All significantly associated risk factors were then processed in forward selection methods with an entry significance level of $P < 0.05$ in SASv9.3 (SAS Institute Inc., Cary, NC) to determine the amount of variation in eGFR explained by all of the risk factors in the model.

Univariable linear models of the generic form $\text{Risk Factor}_i = \beta_0 + \beta_1 \cdot \text{CpG}_i + \varepsilon_i$ (Model 3) were used to determine whether there were any associations between the risk factors identified in Model 2 and each of the top 30 significant CpG sites for eGFR identified in Model 1. Once all of the CpG sites significantly associated with each risk factor were determined, bivariable linear models of the form $\text{eGFR}_i = \beta_0 + \beta_1 \cdot \text{Risk Factor}_i + \beta_2 \cdot \text{CpG}_i + \varepsilon_i$ (Model 4) were used to estimate the contribution of each CpG site that remained significantly associated with eGFR after the adjustment for each risk factor.

The multivariable model $\text{eGFR}_i = \beta_0 + \sum_{m=1}^{p} \beta_m \cdot \text{Risk Factor}_{mi} + \varepsilon_i$ (Model 5) estimated by forward selection procedures (previously described) allowed us to estimate how much of the eGFR variation could be explained by risk factors. The multivariable model $\text{eGFR}_i = \beta_0 + \sum_{m=1}^{p} \beta_m \cdot \text{Risk Factor}_{mi} + \beta_{p+1} \cdot \text{CpG}_i + \varepsilon_i$ (Model 6) was then used to investigate whether each individual epigenetic marker added additional predictive information about eGFR. The final model combining risk factors and epigenetic markers was $\text{eGFR}_i = \beta_0 + \sum_{m=1}^{p} \beta_m \cdot \text{Risk Factor}_{mi} + \sum_{q=p+1}^{p+r} \beta_q \cdot \text{CpG}_{qi} + \varepsilon_i$ (Model 7). This model was estimated using forward regression techniques (keeping the risk factors constant from previous models).

3. Results

3.1. Descriptive Statistics. A majority of the study population was female (71%), with a mean age of 66.3 years. Many of the participants had hypertension (83%) and/or diabetes (31%). Less than half of the participants had ever smoked (42%) and the mean BMI was 31.2 kg/m^2 (Table 1). Women had a higher average BMI and higher levels of cholesterol, HDL cholesterol, C-reactive protein, and fibrinogen than men, and they had lower diastolic blood pressure and homocysteine levels. A majority of the 26,428 CpG sites evaluated in this study had mean values that were considered unmethylated: 58% of CpG sites (15,217 sites) had average M-Values < −2.0 (see Figure 1 in Supplementary material available online at http://dx.doi.org/10.1155/2013/687519).

3.2. Top 30 CpG Sites Associated with eGFR. Nineteen methylation markers were significantly associated with eGFR

Table 1: Descriptive statistics of traits studied in GENOA African Americans.

	Total $N = 972$ Mean (SD)	Males $N = 285$ Mean (SD)	Females $N = 687$ Mean (SD)	P value
Estimated glomerular filtration rate (mL/min per 1.732 m^2)	85 (21)	83 (21)	86 (21)	0.0646[a]
Age (years)	66 (8)	67 (8)	66 (8)	0.2179[a]
Height (cm)	167 (9)	178 (6)	164 (6)	$3.1E-153$[a]
Weight (kg)	88 (18)	92 (17)	86 (18)	$3.3E-05$[a]
Waist circumference (cm)	104 (14)	104 (13)	103 (15)	0.7565[a]
Hip circumference (cm)	116 (14)	110 (11)	118 (15)	$1.3E-17$[a]
Body mass index (kg/m^2)	31 (6)	29 (5)	32 (7)	$1.7E-15$[a]
C-reactive protein (mg/L)	0.7 (1)	0.6 (1)	0.7 (1)	0.00003[b]
Fibrinogen (mg/dL)	369 (82)	354 (87)	376 (80)	0.0002[a]
Homocysteine (μmol/L)	11 (5)	12 (5)	10 (4)	$4.7E-09$[b]
Serum cholesterol (mg/dL)	204 (42)	192 (40)	209 (42)	$1.8E-08$[a]
Serum glucose (mg/dL)	113 (44)	116 (51)	112 (40)	0.3147[b]
Serum triglycerides (mg/dL)	120 (64)	120 (74)	120 (60)	0.3292[b]
Systolic blood pressure (mm Hg)	140 (21)	138 (21)	141 (22)	0.0396[a]
Diastolic blood pressure (mm Hg)	78 (11)	80 (11)	78 (11)	0.0005[a]
Combined high-density lipoprotein (mg/dL)	58 (18)	50 (15)	62 (18)	$5.6E-24$[b]
	N (%)	N (%)	N (%)	P value[c]
Hypertension	802 (83)	228 (80)	574 (84)	0.1845
Diabetes	298 (31)	86 (30)	212 (31)	0.8334

[a] P value from an independent t-test of means between males and females for normally distributed continuous variables.
[b] P value from a two-sided Z-test of a nonparametric Wilcoxon test for nonnormally distributed continuous variables.
[c] P value from a chi-square test for categorical variables.

at the Bonferroni-corrected P value for an alpha level of 0.05 (1.89×10^{-6}) (see Supplementary Figure 2 for a Q-Q plot of the results from the association between genome-wide CpG sites and eGFR). However, the highly intercorrelated nature of the epigenomic markers renders Bonferroni correction too conservative. Most of the top 30 CpG sites were positively associated with eGFR (Table 2) and the majority of these sites have at least moderate correlation >0.40 with at least one of the other 29 CpG sites (Supplementary Table 1). Thus, we chose to evaluate the top 30 most strongly associated sites, all of which had P values less than 6×10^{-6}. The top 30 CpG sites explain 13% ($R^2 = 0.13$) of the variation in eGFR collectively based on simple linears regression.

3.3. Risk Factors for eGFR. In order to compare the effects of epigenetic markers relative to traditional biomarkers/risk factors, we first performed univariable and then multivariable modeling of eGFR (Table 3). Fibrinogen, homocysteine, serum cholesterol, and age were the four risk factors that were found using forward selection to be significantly associated with eGFR at the significance threshold $\alpha = 0.05$ (Table 3). Homocysteine and age explained the largest amount of variability in eGFR ($R^2 = 0.197$ and 0.145, resp.) (Figure 1). All four risk factors together explained a total of 28.3% of the variation in eGFR.

3.4. Association of Risk Factors of eGFR with Top 30 Methylation Markers. To better understand whether the epigenetic sites are operating independently or through risk factors, the top 30 CpGs were then incorporated individually into models with the four significant risk factors as outcomes (Model 3) to examine the associations between each methylation marker and the four risk factors individually (Supplementary Table 2). Seventeen of the thirty CpG sites were significantly associated with fibrinogen, one was significantly associated with serum cholesterol, and all thirty were significantly associated with homocysteine and age, all at $\alpha = 0.05$. Since all of the top sites were found to be associated with at least one of the four risk factors for eGFR when placed in univariate models against each risk factor (Figure 1, Supplementary Table 3), the next step was to investigate whether the epigenetic sites remained significant predictors of eGFR after adjustment for the aforementioned risk factors.

3.5. Explanation of Variation in eGFR by Methylation Markers after Adjustment. Fourteen of the thirty CpG sites associated with age remained significantly associated ($P < 0.05$)

Table 2: Top 30 CpG sites most strongly associated with eGFR (Model 1).

		Genetic description		M-value information		
CpG Site	Chr	Gene	Gene product	M-value mean (SD)	β_1	P-value
cg00226923	6	*FGD2*	FYVE; RhoGEF and PH domain containing 2	2.9 (0.4)	−8.8	$6.1E-09$
cg17471102	19	*FUT3*	Galactoside 3(4)-L-fucosyltransferase	0.7 (0.3)	12.4	$1.3E-08$
cg12261786	10	*ADIRF*	Adipogenesis regulatory factor	1.2 (0.3)	12.6	$2.7E-08$
cg10917602	16	*HSD3B7*	3 Beta-hydroxy-delta 5-C27-steroid oxidoreductase	0.4 (0.4)	8.3	$6.7E-08$
cg04662594	8	*EPB49*	Erythrocyte membrane protein band 4.9 (dematin)	−0.8 (0.4)	9.6	$8.8E-08$
cg15121304	22	*IGL2*	Immunoglobulin lambda locus	1.9 (0.3)	−10.7	$1.8E-07$
cg24857721	1	*RHD*	Rh blood group D antigen isoform 1	0.4 (0.4)	8.5	$2.4E-07$
cg14688272	17	*FN3KRP*	Fructosamine-3-kinase-related protein	−0.2 (0.3)	11.3	$2.9E-07$
cg19761273	17	*HCKID*	Casein kinase 1; delta isoform 1	−2.0 (0.3)	10.9	$4.6E-07$
cg24092253	20	*YTHDF1*	YTH domain family; member 1	−1.2 (0.3)	11.0	$5.8E-07$
cg10126923	19	*NKG7*	Natural killer cell group 7 sequence	−0.2 (0.5)	6.6	$6.1E-07$
cg25538571	8	*FLJ46365*	Hypothetical protein LOC401459	−0.7 (0.3)	10.8	$6.3E-07$
cg11120551	1	*CHD1L*	Chromodomain helicase DNA binding protein 1-like	−0.9 (0.4)	8.9	$7.6E-07$
cg00563932	9	*PTGDS*	Prostaglandin H2 D-isomerase	0.3 (0.3)	9.7	$8.9E-07$
cg16280667	11	*BLR1*	Burkitt lymphoma receptor 1 isoform 1	2.1 (0.4)	−9.4	$1.1E-06$
cg12125117	16	*GPR97*	G protein-coupled receptor 97	−0.8 (0.5)	7.0	$1.4E-06$
cg01820374	12	*LAG3*	Lymphocyte-activation protein 3 precursor	−0.7 (0.3)	10.2	$1.5E-06$
cg09809672	1	*EDARADD*	EDAR-associated death domain isoform B	−0.4 (0.4)	7.5	$1.7E-06$
cg14859417	10	*PTPRE*	Protein tyrosine phosphatase; receptor type; E isoform 2	−1.7 (0.4)	9.1	$1.9E-06$
cg18152830	17	*TNFRSF13B*	Tumor necrosis factor receptor 13B	2.6 (0.3)	−10.8	$2.2E-06$
cg08743392	20	*GSS*	Glutathione synthetase	−2.5 (0.4)	8.3	$2.3E-06$
cg26842024	19	*KLF2*	Kruppel-like factor 2	−2.9 (0.4)	−8.2	$2.4E-06$
cg07426848	1	*S100A3*	S100 calcium binding protein A3	2.1 (0.3)	−9.5	$2.6E-06$
cg15297650	2	*DKFZP566N034*	Hypothetical protein LOC81615	−0.04 (0.3)	10.8	$2.9E-06$
cg17589341	18	*SLC14A1*	Rsolute carrier family 14 (urea transporter), member 1	0.03 (0.3)	9.3	$2.9E-06$
cg21126943	19	*CEACAM6*	Carcinoembryonic antigen-related cell adhesion molecule 6 (nonspecific cross reacting antigen)	−0.8 (0.4)	6.9	$5.7E-06$
cg07408456	19	*PGLYRP2*	Peptidoglycan recognition protein L precursor	−0.2 (0.4)	8.3	$5.7E-06$
cg25268718	14	*PSME1*	Proteasome activator subunit 1 isoform 1	0.3 (0.2)	13.5	$6.0E-06$
cg08700306	19	*LRP3*	Low-density lipoprotein receptor-related protein 3	−0.04 (0.3)	9.7	$6.1E-06$
cg02863947	3	*NR1I2*	Pregnane X receptor isoform 2	−0.4 (0.5)	6.7	$6.3E-06$

Model 1: $\text{eGFR}_{ij} = \beta_0 + \beta_1 \cdot \text{CpG}_{ij} + W_j + \varepsilon_{ij}$.

with eGFR in the bivariable model (Figure 1, Supplementary Table 3). All of the CpG sites that were associated with the other significant risk factors (fibrinogen, serum cholesterol, and homocysteine) remained significantly associated with eGFR in the bivariable model, indicating that these epigenetic markers are independent predictors of kidney function. Individual CpG sites were able to predict an additional 1–4% of the variation in eGFR beyond individual risk factors.

3.6. Explanation of Variation in eGFR by Methylation Markers after Adjustment for Risk Factors. In order to determine whether the CpG sites remained significant predictors of eGFR after adjusting for all four risk factors, we constructed multivariable models. A multivariable model with the four risk factors and a single CpG site predicting eGFR (Model 6) was compared to the multivariable model with only the four risk factors (Model 5). This was repeated for each of the 30 CpG sites. Six CpG sites remained significantly associated with eGFR after adjustment for all four risk factors and predicted approximately 0.3–0.8% of the variation in eGFR beyond the risk factors (Figure 1, Supplementary Table 4).

We used forward selection to build a model that consisted of the four risk factors plus the CpG sites that remained significant predictors of eGFR (Model 7). Three of the six

Table 3: Univariable and multivariable linear regression for eGFR.

Risk factors ($N = 972$)	Univariable model (Model 4)[a]		Multivariable model (Model 5)[b]	
	β_1 (P value)	R^2	β_k (P value)	R^2
Homocysteine (μmol/L)	−2.0 ($4.9E-48$)	0.1965	−1.7 ($7.6E-36$)	0.2827
Age (years)	−1.0 ($8.9E-35$)	0.1445	−0.8 ($1.1E-21$)	
Fibrinogen (mg/dL)	−0.03 (0.0009)	0.0114	−0.02 (0.0160)	
Serum cholesterol (mg/dL)	−0.06 (0.0002)	0.0140	−0.03 (0.0263)	
Sex[c]	2.8 (0.0560)	0.0038	—	—
Height (cm)	−0.1 (0.1137)	0.0026	—	—
Weight (kg)	−0.03 (0.4851)	0.0005	—	—
Waist circumference (cm)	−0.06 (0.1834)	0.0018	—	—
Hip circumference (cm)	−0.01 (0.9113)	0.0000	—	—
Body mass index (kg/m^2)	0.02 (0.8402)	0.0000	—	—
C-reactive protein (mg/L)	0.5 (0.3814)	0.0008	—	—
Serum glucose (mg/dL)	0.02 (0.1869)	0.0018	—	—
Serum triglycerides (mg/dL)	−0.03 (0.0150)	0.0061	—	—
Systolic blood pressure (mm Hg)	−0.08 (0.0072)	0.0074	—	—
Diastolic blood pressure (mm Hg)	0.09 (0.1217)	0.0025	—	—
Combined high density lipoprotein (mg/dL)	0.02 (0.5416)	0.0004	—	—
Hypertension[d]	−6.4 (0.0002)	0.0141	—	—
Type 2 diabetes[e]	−1.2 (0.3992)	0.0007	—	—

[a]Model 2: $\text{eGFR}_i = \beta_0 + \beta_1 \cdot \text{Risk Factor}_i + \varepsilon_i$.
[b]Model 5: $\text{eGFR}_i = \beta_0 + \sum_{m=1}^{p} \beta_m \cdot \text{Risk Factor}_{mi} + \varepsilon_i$, where p is the number of risk factors.
[c]Female = 1, male = 0.
[d]Hypertension = 1, no hypertension = 0.
[e]Type 2 diabetes = 1, no type 2 diabetes = 0.

CpG sites (cg26842024, cg07426848, and cg17589341) remained significant in the final model in addition to the established risk factors (Supplementary Table 5). The final model was able to predict 29.8% of the variation in eGFR (Figure 1).

4. Discussion

The purpose of this study was to identify methylation sites in peripheral blood cells that were significantly associated with eGFR, to evaluate their association with CKD risk factors, and to determine whether these epigenetic sites were still predictors of eGFR after adjustment. By evaluating DNA methylation within peripheral blood cells, we were able to examine the relationship between kidney function and epigenetic processes occurring within cells that are involved in inflammatory responses in the kidney. Given the large number of correlated epigenetic sites, we focused our study on the top 30 CpG sites that were significant after adjusting for multiple testing.

We used this approach to identify the epigenetic markers of eGFR because previous studies have indicated that many epigenetic sites are associated with age and that these sites could potentially provide fundamental insights into the biology of the aging kidney. Indeed, the top 30 CpG sites were all significantly associated with age in our study. However, 14 of these 30 CpG sites remained significant predictors of eGFR after adjustment for age. These same 30 CpG sites were also significantly associated with plasma levels of homocysteine in our study, and all 30 CpG sites remained significant predictors of eGFR after adjustment for homocysteine (a significant predictor of eGFR). Only six of the 30 CpG sites remained significant predictors of eGFR after adjustment for traditional risk factors (age, fibrinogen, serum cholesterol, and homocysteine). Consequently, it appears that the majority of the epigenetic markers that we identified may affect eGFR, at least in part, through pathways related to aging, inflammation, and cholesterol.

As a final step, we put all the top epigenetic sites and risk factors into a forward selection algorithm and identified three significant, independent CpG site predictors of eGFR. The three significant CpG sites are cg26842024 in *KLF2* gene, cg07426848 in the *S100A3* gene, and cg17589341 in the *SLC14A1* gene. The *KLF2* gene encodes a Krüppel-like transcription factor. This zinc finger family of transcription factors is important in regulating cellular processes in the vasculature, including the kidney's glomerular capillary bed [33]. For example, in renal transplants with thrombotic microangiopathy, studies of gene expression in the glomerulus have demonstrated a downregulation of *KLF2* and subsequent upregulation of genes that inhibit local fibrinolysis [34]. Recently, researchers have shown that the impact of blood flow and its laminar shear stress on glomerular endothelial

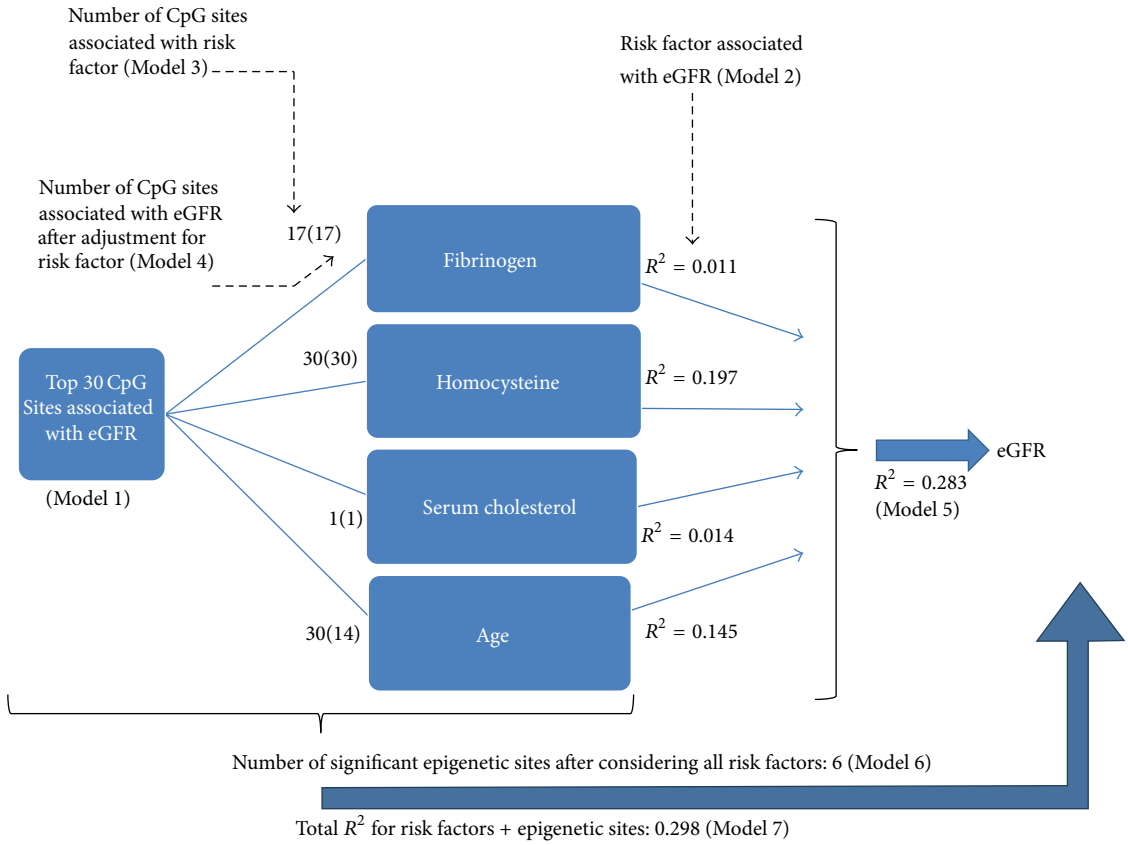

Figure 1: Flow chart of the contributions of all models used in this study and their relationships with eGFR.

cells alters expression of *KLF2*. Specifically, chronic laminar shear stress increases *KLF2* which then increases expression of endothelial nitric oxide synthase (eNOS), thrombomodulin, and nitric oxide [35]. This study also demonstrated that these changes in the glomerular endothelium associated with *KLF2* had an effect on kidney podocytes. Podocytes are cells in the glomerulus responsible for the kidney's ability to filter waste products from the blood and are intimately involved in the pathogenesis of CKD [36].

The other two significant CpG sites are within genes that are related to bladder biology and, hence, may reflect downstream consequences of variability in eGFR in our study. That is, differences in methylation within these genes may be a response to higher or lower levels of eGFR rather than influencing eGFR itself. In particular, the *S100* proteins are signaling factors that are involved in regulation of cellular processes in a wide range of cell types. The differential expression of *S100A3* has been implicated in the bladder cancers [37]. The solute carrier family 14 (urea transporter), member 1 (*SLC14A1*) gene has been studied for decades as the Kidd blood group. This urea transporter is expressed in a wide range of cell types. Recently, genetic studies have identified it as an important gene involved in the concentration of the urine in the kidney [38] as well as an important susceptibility gene for bladder cancer [39, 40].

These three genes that were significant independent predictors of eGFR point to future studies that may help to understand the mechanism underlying interindividual variation in kidney function in African Americans. We are unaware of other studies that have investigated the epigenetic predictors of eGFR on a genome-wide scale. However, a few other studies of kidney-related diseases point to the breadth of epigenetic studies of kidney phenotypes that could be conducted. For example, a study on the epigenetic markers of diabetic nephropathy among African Americans and Hispanics identified 187 genes that were differentially methylated among diabetes patients with and without nephropathy [16]. Another study found that epigenetic "metabolic memory" from prior exposure to hyperglycemia, even after glucose normalization, was implicated in End-Stage Renal Disease among African American diabetic patients [12].

Our study has several notable strengths including a large sample size, investigation of a key indicator of kidney function in a minority population, and epigenome-wide assessment of DNA methylation. However, it also has several limitations. First, the GENOA sample does have an increased prevalence of hypertension compared to an unselected population of the same age range. Hypertension is associated with measures of eGFR, and thus the distribution of eGFR in this sample differs from that of an unselected population. Also, the current study was cross-sectional rather than longitudinal, so we cannot discern the temporal relationship between changes in DNA methylation and changes in inflammatory processes that influence kidney function.

This study and other epigenetic studies support the idea that differential DNA methylation in peripheral blood cells may be an indicator of kidney function and may potentially help us understand etiological aspects of kidney disease because it provides an important link to inflammatory processes that underlie chronic diseases such as CKD. Better understanding of the role of epigenetics in kidney function, particularly among African Americans, may lead to the development of novel detection, treatment, or prevention strategies for CKD that will help to decrease the current health disparities in kidney disease.

Authors' Contribution

Samantha M. Bomotti and Jennifer A. Smith contributed equally to this work.

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Review Article

A Framework to Examine the Role of Epigenetics in Health Disparities among Native Americans

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Background. Native Americans disproportionately experience adverse childhood experiences (ACEs) as well as health disparities, including high rates of posttraumatic stress, depression, and substance abuse. Many ACEs have been linked to methylation changes in genes that regulate the stress response, suggesting that these molecular changes may underlie the risk for psychiatric disorders related to ACEs. *Methods*. We reviewed published studies to provide evidence that ACE-related methylation changes contribute to health disparities in Native Americans. This framework may be adapted to understand how ACEs may result in health disparities in other racial/ethnic groups. *Findings*. Here we provide evidence that links ACEs to methylation differences in genes that regulate the stress response. Psychiatric disorders are also associated with methylation differences in endocrine, immune, and neurotransmitter genes that serve to regulate the stress response and are linked to psychiatric symptoms and medical morbidity. We provide evidence linking ACEs to these epigenetic modifications, suggesting that ACEs contribute to the vulnerability for developing psychiatric disorders in Native Americans. *Conclusion*. Additional studies are needed to better understand how ACEs contribute to health and well-being. These studies may inform future interventions to address these serious risks and promote the health and well-being of Native Americans.

1. Introduction

Reservation-based Native Americans live in pervasively adverse social and physical environments that place them at increased risk of exposure to a myriad of stressors during childhood which impact their psychological and physical health over their lifetimes [1]. About 1 of 2.9 million Native Americans that identify as Native American alone resides on reservations [2]. Indian reservations were established by treaty during the Removal and Relocation (1827–1887) period and are lands set aside for tribes in exchange for ceded land and resources. Today there exist 275 Indian land areas in the USA administered as Indian reservations [3]. Of the ten poorest counties in America, five are home to an Indian reservation [4]. Concentrated poverty results in higher crime rates, underperforming public schools, poor housing, and poor health and limits access to many services and job opportunities [5]. Adverse childhood experiences (ACEs) that are substantial contributors to health disparities include childhood physical and sexual abuse, witnessing violence, poverty, and racism. The concept that these experiences become biologically embedded has gained substantial support and provides an explanatory mechanism for health disparities [6]. ACEs are linked to differences in the function of the stress-response system including the neuroendocrine system, the parasympathetic nervous system, and the immune system. These changes likely have substantial long and short term impacts on health and well-being [7]. It is likely that these changes are shaped by epigenetic modifications which alter the function but not the structure of the gene. Epigenetic modifications are considered to be an individual's molecular response to the environment and occur in an effort to preserve the health of the individual by increasing the accessibility of genes for transcription and translation that relate to immediate survival [8]. These genes code for proteins that prepare the individual to be able to respond to the stressor

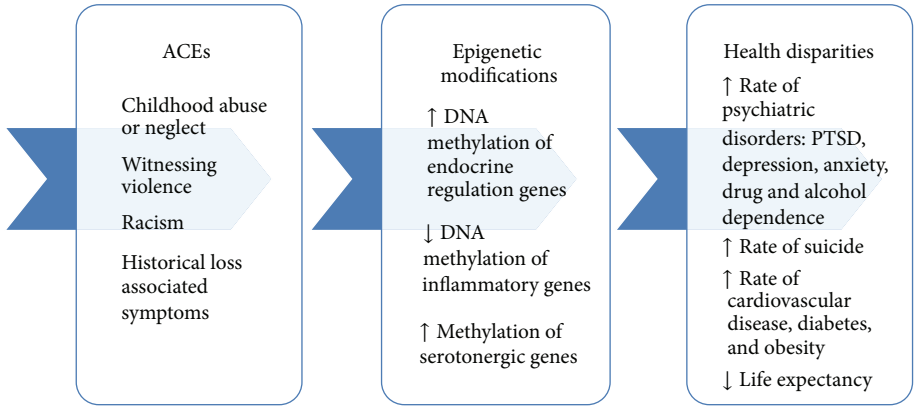

Figure 1: The mediating relationship of epigenetics on the risk for health disparities in Native Americans with childhood adversity.

through a fight or flight response; yet, in Native Americans living on reservations, the stressors most encountered are chronic, not acute. Thus, this adaptive response likely results in overactivation of this stress-response system, and this excessive activity has substantial negative consequences on the health and well-being of Native Americans, individually and across generations. Here we provide a conceptual review of how nurses and other health care professionals can examine health disparities in Native Americans through epigenetic modifications that likely result from ACEs (see Figure 1), including historical trauma, the residual of which is assumed to be historical loss associated symptoms. We expect this conceptual framework to have implications for or be relevant to the mechanisms of health disparities in other racial or ethnic groups.

2. ACEs and Psychiatric Risks in Native Americans

A neighborhood's safety and access to quality health care, economic opportunities, social connections, and social capital are all key determinants of the health of its residents over time [9–12]. Reservations are often characterized by low economic status and segregation, both of which limit access and are risk factors for higher rates of morbidity and mortality [13, 14]. Chronic stress such as that which accompanies experiences of racism and poverty over a lifetime places individuals at risk for posttraumatic stress disorder (PTSD). This vulnerability can be solved in part by ethnic connectedness [15].

Unique to Native Americans is the race-based stress associated with historical trauma [16], as well as discrimination [17–20]. Historical trauma is defined here as the "collective experience of violence perpetrated against Indigenous Peoples in the process of colonizing the Americas resulting in an unresolved humanitarian crisis for reservation communities." The effects of historical trauma are proposed as being transmitted across generations with historical loss associated symptoms currently exhibited [21–23] and include symptoms of complicated bereavement and complex PTSD [24]. This type of trauma has been linked to impaired individual and collective tribal identity [16, 24], which likely also relates to stress and morbidity risk. Over 50% of Native Americans indicate that they think about loss related to historical trauma, such as loss of language, loss of culture, and loss of land, at least occasionally, and which caused them psychological distress [17, 25]. Discrimination has been associated with early substance use among Native American children, and suicidal behavior, and anger, and aggression among adolescents [18, 20, 26]. Thus, this stress combined with other ACEs may be a significant contributor to health disparities.

Native Americans are disproportionally affected by trauma in childhood, including abuse, neglect, and exposure to intimate partner violence (IPV) [27–29]. Approximately half of Native American adolescents and young adults have been exposed to one or more severe traumatic events [30], and 98% have experienced a traumatic event of any severity [31]. Native American adolescents are more likely than other adolescents to witness violence or to have been physically abused, sexually abused, or neglected as a child, resulting in rates of PTSD that are twice that of the estimated rates in the general U.S. population [31].

Assaultive trauma in childhood is linked to the highest risk for PTSD, suggesting that this ACE is specifically linked to this high risk for psychiatric disorders [31]. Specifically, trauma that involves physical or sexual assault prior to adolescence places an individual at five to ten times the risk for PTSD onset compared to an individual without this experience [32, 33]. Witnessing abuse during childhood, as well as residing in a high-crime area, is also linked to a far greater risk for PTSD [34, 35]; however, assaultive trauma at an early age is the ACE most linked to PTSD onset.

ACEs have also been linked to increased risk of depression onset [36–38]. These studies link physical abuse, witnessing domestic violence, and parental alcohol and drug abuse to a vulnerability for depression symptom onset [36, 37]. In addition, residing in an urban, socioeconomically disadvantaged area has also been linked to risk of depression onset as well as drug use [39]. Exposure to trauma also increases the risk for the early onset of substance use and the onset of substance use disorder [40]. Other studies have found similar results, including that ACEs increase the risk for drug use and early alcohol abuse and increased the rates

of initiating these behaviors during adolescence by a factor of two to four [41, 42]. Thus, ACES in general are linked to psychiatric disorder vulnerability, with high degree of comorbidity among these disorders.

3. Health Disparities in Native Americans

Reservation-based Native Americans die at higher rates than other Americans from tuberculosis (750% higher), alcoholism (524% higher), diabetes (293% higher), unintentional injuries (153% higher), homicide (103.3% higher), and suicide (66% higher) (2002–2004, rates adjusted for misreporting of race on state death certificates) [43]. Not only do Native Americans bear a disproportionate burden of disease, but they also experience a lower life expectancy. Life expectancy is an overall measure of quality of life [44] and is one of the indicators used to measure the magnitude of the burden of health disparities [45]. In general, Native Americans born in 2000–2002 have a life expectancy that is about 2.4 years less than the overall US population rate: 76.9 years compared to 74.5 years for Native Americans [43]. However, when this average is disaggregated by IHS Area, the life expectancy ranges from 64.8 years (11 years less than for the U.S.) in the Aberdeen Area to 76.4 years (greater than the U.S. average of 75.8 years) in the California Area (adjusted for race miscoding) using 1994–1996 data [46]; thus highlighting the within group differences. Additionally, the Indian Health Service, using 2000 census data, found 25.7% of all Native Americans were living below the poverty level, compared to 12.4% of the U.S. population overall [43]. The Bureau of Justice, in the first comprehensive statistical analysis of "American Indians and Crime," reports Native American are the victims of violent crimes at two times the rate of the U.S. population overall, and about 7 in 10 violent victimizations involved an offender who was reported by the victim to be a person of another race [47]. However, this may not apply to all communities, especially those that are more remote and isolated where few non-Native American people live. Another report by the Department of Justice, disclosed Native Americans sustain rates of violent victimization (rape, sexual assault, robbery, aggravated assault, and simple assault) at rates that are 2 times higher than African Americans, 2.5 times that of Hispanics, 3 times that of Caucasians, and 6.5 times that of Asians [48]. PTSD is the anxiety disorder most linked to trauma and its prevalence in Native Americans adults is 4.4 times the national average [25, 49]. There is little research regarding the impact that adversity has on tribal communities, so it remains poorly understood.

The adverse childhood experiences (ACE) study suggests that certain adversities are major risk factors for morbidity and mortality [50]. The study established a relationship between adversity in childhood and suicide attempt [51], prescription drug use [52], alcoholism and alcohol abuse [53, 54], illicit drug use [42], obesity [55], and depressive disorders [38]. Among adolescents and young adults, childhood adversity was also associated with a greater risk for interpersonal violence perpetration [56], poor perceived health, more medical care visits, and additional somatic concerns [57]. Therefore, current studies link ACEs to risks to health and well-being; however, the mechanisms underlying these risks have not yet been well described.

4. Genetic Inheritance and Influences on ACEs and Health

In some cases, genetic predisposition may explain some of the enduring effects of ACEs; however, the evidence for this link remains poorly understood. Genetic inheritance provides information encoded in DNA which is transcribed to various types of RNA molecules which likely shape the response of the individual to stressors such as ACEs. One important concept related to phenotypic variation is heritability, which estimates the extent of which genetic inheritance contributes to the phenotypic variance in a population [58]. Heritability is the percent of variation in the genome responsible for the difference in the phenotype. Another parameter used to estimate the contribution of genomic factors in phenotypes is relative risk, which refers to an individual's risk of developing a condition with a family history compared to those without a history [58]. When the heritability estimate or relative risk of a phenotype is low, the influence of the genome sequence is considered to be relatively smaller than the influence of other factors such as environment, and the genomic influence can be easily masked or have a negligible impact. Since most human diseases involve many genes, their interactions, and nongenetic factors, an approach termed "endophenotypes" is used to characterize the disease in a molecular or genetic manner, rather than using a clinical diagnosis to define the phenotype.

Polymorphisms in Native Americans have been linked to a greater vulnerability for alcohol abuse [59], as well as obesity [60]. In general, U.S. samples of trauma exposed participants link endocrine gene (FKBP5) polymorphisms to a greater risk for PTSD development [59, 61]; yet, these are small and do not include Native Americans. Thus, it is essential to consider unique genetic inheritance features in Native Americans which interact with epigenetic modifications and likely contribute to health disparities.

4.1. ACEs and the Biological Stress Response. The stress-response system provides the individual protection from acute stressors through an activation of interactive biological systems [6]. One biological system that is central to this response and is linked to ACEs is the hypothalamic-pituitary-adrenal (HPA) axis, with the end result of activation of this system being the production of cortisol. In addition to playing a pivotal role in activating the stress response, the HPA axis also influences biological functions related to mood, growth, immune function, metabolism, and regulation of biological systems on circadian rhythm [62]. The sympathetic nervous system (SNS) also is activated by stress providing neuronal focus and energy to muscles in order to escape the stressor. Although these systems are effective in adapting to acute stress, chronic activation is linked to negative consequences. Both the HPA axis and SNS impact immune function, and chronic stress is linked to a risk for inflammation [62].

Overactivation of the HPA axis results in disruptions of functioning at rest and following stressors, and these changes have been linked to ACEs. HPA axis alterations are linked to health disparities through mechanisms that include impaired neuronal growth and survival, inflammation, reductions in neuropeptide activity, and accelerated cellular aging [63–65]. SNS function changes have also been linked to health disparities, with one of the most pivotal mechanisms being a lack of circadian variation in blood pressure, a key risk factor for myocardial infarctions [66].

5. Epigenetic Modifications Resulting from ACEs

Evidence is accumulating that environmental influences early in development remain pervasive into adulthood, a relationship that is attributed to an interaction of gene function and environment. Both genetic and environmental factors are critical to developmental processes and even minor changes in either type of factor can result in trajectories of resilience or vulnerability [67]; however, it is the interaction between these factors that may provide the most vital information to understand the heterogeneous response to trauma. This leads us and others to question how future research can address this critical issue.

Epigenetics refers to changes in an individual's phenotype independent of genotype. These changes occur through mechanisms such as histone modification, methylation, acetylation, and noncoding ribonucleic acids which alter the accessibility of genes for transcription. The resulting transcription modifications and protein production result from factors such as environmental challenges including, but not limited to, ACEs [68, 69]. An individual's genome interacts with internal and external factors to create phenotypes such as height, physical appearance, personality, and alterations in the stress-response system [70].

Preclinical models illustrate how ACEs result in epigenetic modifications in neurons, thereby increasing the risk for psychiatric symptoms. To illustrate this link, a study reports that the offspring of high-licking canine mothers exhibit reduced methylation of the glucocorticoid receptor gene [71] and endocrine regulation of a subsequent stressor [72]. In contrast, offspring that face early adversity exhibit endocrine dysregulation [73], as well as reductions in neuronal plasticity in the prefrontal cortex (PFC) that persist into adulthood [74]. In studies of rats who exhibit PTSD-like behavior, there is evidence of increased methylation of stress-response genes including brain-derived neurotrophic factor and nuclear protein phosphate-1 [75] in neurons [76]. Although these studies provide additional evidence linking ACEs to methylation changes in neurons, these studies are not able to clearly determine psychiatric symptoms. Therefore, these studies are limited by not being able to determine the comprehensive risks that relate to ACEs.

The ACE most linked to epigenetic differences and vulnerability for health disparities is that of child abuse. To illustrate this link, in a hallmark study by Labonté in suicide completers, ACEs were linked to increased DNA methylation of the glucocorticoid receptor in the hippocampus, and this differential methylation was particularly linked to childhood abuse [77]. This study provides further support for the McGowan et al. study, whose subject group was also suicide completers, which reported that childhood abuse was associated with greater methylation levels at CpG sites in the $\text{exon}1_{\text{F}}$ of the promoter region of the glucocorticoid receptor gene [78]. These studies had the distinct advantage of examining epigenetic modifications in neurons, which is not available in other studies. Epigenetic patterns differ among cell types, even differing among brain regions [79, 80]. Thus, an additional challenge to understanding the impact of ACEs on health disparities is to determine how epigenetic alterations in the brain differ from those in peripheral tissues and how to advance despite this methodological challenge. In addition, these few studies are not able to determine the role of preexisting methylation in this risk or to measure other factors that may contribute to methylation changes.

Epigenetic changes resulting from ACEs can also be observed in studies that use peripheral blood in living participants, which show that HPA-regulating genes are often impacted. A study by Klengel et al. linked ACEs to reduced methylation of the FKBP5 gene, an essential regulator of the stress response, as well as to changes in the function of the HPA axis under stress, and to reduced cognitive ability [81]. Direct physical abuse and observing the abuse of a mother have also been associated with greater methylation levels at CpG sites in the $\text{exon}1_{\text{F}}$ of the promoter region of the glucocorticoid receptor gene in leukocytes [82]. Similar methylation profiles are also reported in the peripheral blood of babies whose mothers were depressed during the third trimester of pregnancy, and these methylation changes were related to salivary cortisol elevations at three months of age [83]. Although glucocorticoid receptors in peripheral tissue may differ from those on the HPA axis, the link between methylation of the glucocorticoid gene in the periphery and the function of the HPA axis has been demonstrated in multiple studies in addition to those of Oberlander et al., 2008. Additional studies that include analysis of blood samples collected closer to the time of the ACE may provide additional insights into the individual variation in response to ACEs.

Other studies link ACEs to hypomethylation of inflammatory genes, suggesting that these experiences result in a greater inflammation later in life. In a recent study of children who were removed from their parents due to abuse or neglect, a reduction in methylation of NR3C1, an inflammatory regulation gene, as well as differential methylation of cancer related pathways was found in children with ACEs compared to controls [84]. A study of adults linked child abuse to reduced methylation of IGF2AS, an antisense transcript of the insulin-like growth factor gene, which encodes for the inflammatory cytokine family of growth factor beta [85]. Borghol et al. linked childhood poverty to differential methylation of genes related to metabolism and inflammation, and these changes were different from those in participants who experience poverty only during adulthood [86]. Together these studies provide evidence that a variety of ACEs result in methylation changes, suggesting that these

molecular changes likely contribute to health disparities; however, additional, larger, and more representative studies are needed to determine relationships.

Altered serotonergic neurotransmission is also postulated to result from ACEs and provides a mechanistic link to increased vulnerability for psychiatric disorders. The Iowa adoption study demonstrated a link between hypermethylation of the serotonin gene SLC64A to childhood sex abuse [87], and this molecular change mediated the development of antisocial personality disorder [88]. This group was also able to relate differences in gene expression of serotonin related genes to methylation, and that genotype influenced methylation at cg22584138 [89]. Additional studies are needed to determine the role of other ACEs in serotonergic gene methylation and to determine how ACEs contribute to psychiatric risks.

6. Methylation Changes Associated with ACEs Increase the Risk for Psychiatric Disorder Onset

Clinical studies are restricted to examining differential methylation in samples of peripheral fluids, but these studies do provide some key insights into how these molecular changes relate to PTSD, depression, and drug abuse risk. For instance, PTSD is associated with changes in the methylation of inflammatory (toll-like receptors 1 & 3, IL-8, chemokine ligand 1, and others) and endocrine genes FK506 binding protein-5 (FKBP5) [90]. Another study that measured DNA methylation reported that postdeployment hypomethylation of LINE-1 was associated with PTSD onset following deployment [91]. Differential methylation of neurotransmitter genes is also linked to PTSD risk. Two studies utilized samples of civilians from the Detroit Neighborhood Health Study. One study determined that serotonin transporter gene (SLC6A4) methylation levels were modified by the effect of the number of traumatic events on PTSD after controlling for SLC6A4 genotype, such that persons with more traumatic events were at increased risk for PTSD, but only at lower methylation levels [92]. The other study found that the candidate gene MAN2C1 showed a significant methylation × trauma experience interaction, such that those with both higher MAN2C1 methylation and greater exposure to traumatic events showed an increase in risk of lifetime PTSD [93]. Thus, there is evidence that PTSD is associated with similar methylation differences in immune, endocrine, and neurotransmitter genes to those linked to ACEs; suggesting that these chronic differences may be a result of ACEs, yet additional prospective studies are needed to better describe these relationships.

Studies in individuals with depression have also shown differential DNA methylation. Sabunciyan et al. carried out the first genome-wide DNA methylation scan in major depressive disorder patients. The study pinpointed 224 candidate regions, primarily involved in neuronal growth and development genes, which showed differential methylation; PRIMA1 showed the greatest differences [94]. Specific genes that have been shown to be differentially methylated in individuals with depression include those that code for angiotensin converting enzyme [95], brain-derived neurotrophic factor [96], orexin A [97], and gamma-aminobutyric acid receptor alpha1 [98].

In addition to observing differential DNA methylation in PTSD and depression, many studies have observed methylation differences in those that suffer from drug abuse as compared to healthy controls. Increases in DNA methylation of the OPRM1 gene that codes for opioid receptors have been reported in individuals with chronic opioid use [99–102] and alcohol dependence [103], and global methylation differences have also been reported for these two populations [99, 104]. The proopiomelanocortin gene promoter [105], dopamine transporter gene promoter [106], homocysteine-induced endoplasmic reticulum protein promoter [107], and alpha synuclein promoter [108] were found to be differentially methylated in individuals with alcoholism compared to healthy controls. Methylation at the monoamine oxidate A locus was also significantly associated with nicotine and alcohol dependence in women, but not in men [109]. Together these studies show that psychiatric disorders related to ACEs are associated with methylation changes that may be reflective of ACEs or psychiatric symptoms; however, there are no prospective studies to elucidate the possible mediating role of methylation on these psychiatric risks in individuals that experience ACEs.

7. Conclusion

Reservation-based Native Americans disproportionately experience ACEs and health disparities, significantly impacting long-term physical and psychological health. In addition to these experiences, the persistence of stress associated with discrimination and historical trauma converges to add immeasurably to these challenges. Here we provide evidence to suggest that ACEs result in methylation differences in genes that regulate the stress response and that these changes may contribute to an increased vulnerability for developing psychiatric disorders, as depicted in Figure 1. Although we postulate these relationships, the lack of prospective studies in this at-risk group prevents us and others from concluding this causality, as well as more studies that include Native Americans. Thus, additional studies are needed to better understand the mechanisms through which ACEs contribute to health and well-being. These studies may inform future interventions to address these serious risks and promote the health and well-being of Native Americans.

Conflict of Interests

All authors have no conflict of interests.

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Research Article

Enhancing the Participation of African Americans in Health-Related Genetic Research: Findings of a Collaborative Academic and Community-Based Research Study

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The involvement of African Americans in research has long been expressed as a concern by the scientific community. While efforts have been undertaken to identify factors inhibiting the participation of African Americans in health-related research, few efforts have been undertaken to have highlight factors associated with their engagement of health-related research. An exploratory study of factors presumed to be associated with participation in health-related research was conducted among a nonprobability sample of African Americans ($n = 212$) from a large urban community in the Midwest. The study was guided by a framework that hypothesized the influence of knowledge, beliefs, and perceptions about genetics and the involvement of providers in decision-making on willingness to participate in health-related genetic research. The results revealed that knowledge, beliefs, and perceptions about genetics and the involvement of providers were associated with willingness to engage in health-related genetic research ($P < .05$). The most interesting, however, was that 88.7% of the participants who had not previously been involved in a health-related study who expressed a willingness to participate reported that they "had never been asked." Study findings suggest the need for research that further examines factors associated with the involvement of African Americans in health-related genetic research.

1. Introduction

In April 2003 the directors of the Human Genome Project (HGP), an international scientific research project coordinated by the United States Department of Energy and the National Institutes of Health National Human Genome Research Institute, announced that the first draft of the map of the human genome had been completed [1]. It was anticipated that mapping the complete set of DNA would revolutionize health care and lay the groundwork for the development of clinical markers with predictive capabilities and, thereby, shift the disease-treatment trajectory and lead to preventive interventions, tailored treatments, and averted deaths (Figure 1). Likewise, it was anticipated that the map would lead to a better understanding of the causes of cardiac disease, cancer, diabetes, Alzheimer's disease, mental disorders, and other common and rare diseases; the development of diagnostic tests to detect errant genes; the development of new classes of medicines based on gene sequence and protein structure function; and the development of therapies which use genes in treating genetic and acquired diseases [2, 3].

For population groups known to experience excess disease-related morbidity, and mortality it was believed that the ability to use genetics to predict, prevent, detect, and more effectively treat disease held tremendous promise. Ten years after completion of the map, HGP leaders report several accomplishments of the HGP. They report the identification of approximately 1,800 disease genes [4, 5], the development of more than 2,000 genetic tests for various human diseases/conditions [6], and the development and ongoing

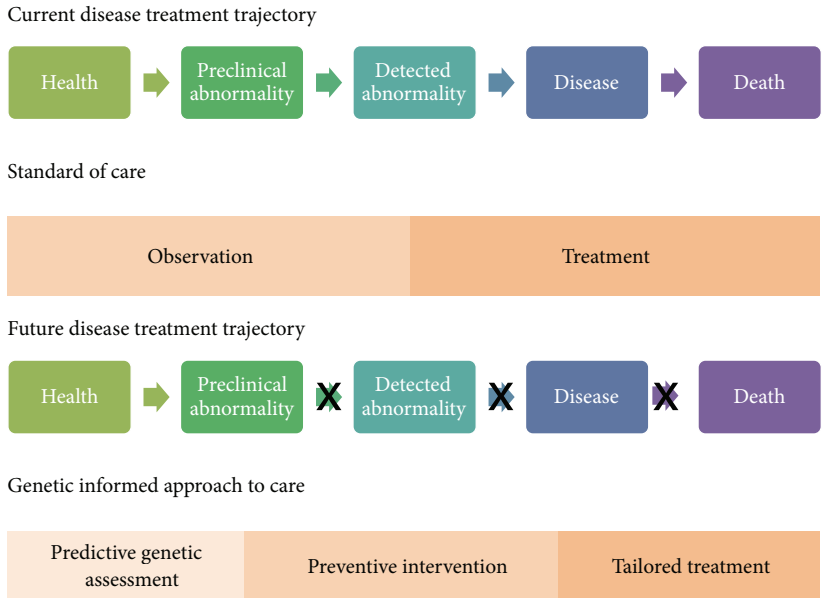

Figure 1: Disease treatment trajectory.

testing of more than 350 biotechnology-based genetic products [4]. Yet, in a more subdued voice, they note that the anticipated clinical benefit from the HGP has yet to unfold [3].

Several public and private entities across the country have been established and have begun collecting and storing tissue specimens for genetic testing to support and expand the program of study initiated by the HGP. According to reports authored by respected scientists in the field of genetics, in 1999 close to 300 million tissue samples—most of which were collected during routine clinical and surgical procedures—were stored in public health departments, blood banks, pathology archives, and researchers' laboratories within the United States [7–10]. It has been estimated that the number of tissue samples collected since that time has increased by more than 20 million a year [11].

Many in the scientific, medical, and advocacy arena, concerned about the excess disease-related morbidity and mortality and health disparities experienced by African Americans and other racial/ethnic minority populations, believe that the inclusion of biological specimens (and other health-related data) from African American and other racial/ethnic minority populations is essential [12–14]. However, an ever increasing number of reports allude to the limited inclusion of biological specimens (and other health-related data) from ethnic/racial minorities in biorepositories [15–23].

2. Purpose

The manner and degree to which African Americans have been involved in medical research—a population often cited as being unduly burdened by disease—have long been expressed as a concern by leaders in the scientific community. While several efforts have been undertaken to identify factors inhibiting the participation of African Americans in health-related research, few efforts have been undertaken to have highlight factors associated with the engagement of African Americans in health-related research and gene testing [15, 22–30]. A focused study of factors presumed to be associated with the participation of African Americans in health-related genetic research was therefore proposed. The study was conducted by a team of nurse scientists, health educators, clinicians, and biostatisticians using principles of community engagement and community-based research [31, 32]. The study was designed to assess factors associated with the engagement of African Americans in health-related research among a targeted group of African-American men and women. More specifically, the study was designed to assess the influence of knowledge about genetics; beliefs regarding benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and the involvement of health care providers in decisions regarding participation in health-related genetic research and their willingness to participate in health-related research that involved gene analysis.

The organizing framework designed for the study included constructs deemed to be essential to informed decision-making and engagement in health-related genetic research [4, 33–36] (Figure 2). In applying the framework in this study, the independent process variables were knowledge about genetics; beliefs regarding the benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and involvement of health care providers in health care decisions. The dependent outcome variable was willingness to participate in health-related genetic research. More specifically, the organizing framework hypothesized the influence of knowledge about genetics; beliefs regarding benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and directive and nondirective involvement of health care providers on decisions made relative to participation in health-related genetics research and willingness to participate in health-related genetic research.

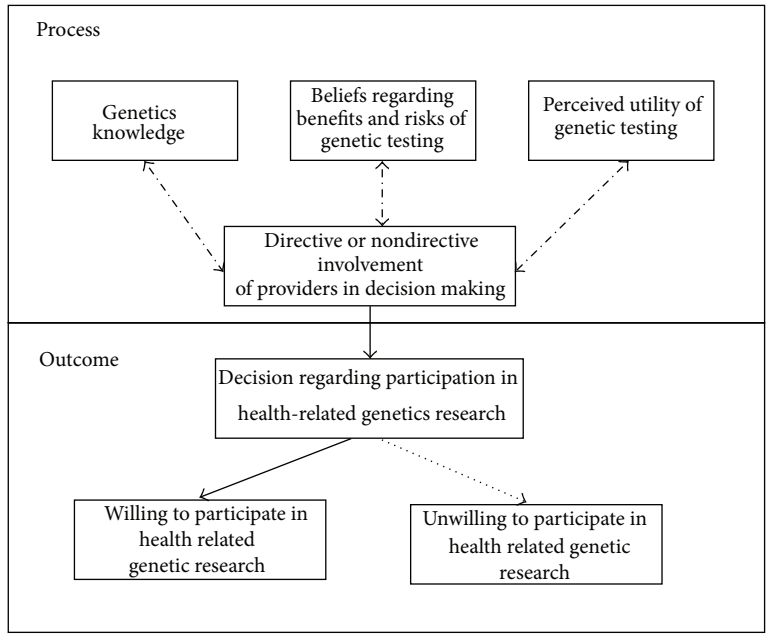

Figure 2: Organizing framework.

3. Methods

3.1. Design. An exploratory cross-sectional study design was used to examine the influence of knowledge about genetics; beliefs regarding benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and the involvement of health care providers on decisions made relative to participation in health-related genetics research and willingness to participate in health-related genetic research among a targeted group of African American men and women.

3.2. Sample, Setting, and Data Collection. A nonprobability sample of African American men and women who resided in a large, densely populated, economically, socially, and culturally diverse urban community in the Midwest was recruited to the study. Prospective participants who were 18 years of age or older; able to communicate in English; willing to complete a questionnaire about gene testing and genetic research; and able and willing to consent to participate in the study were invited to participate in the study by members of the research team. The investigative team worked in collaboration with the Black Health Coalition of Wisconsin (BHCW) to recruit study participants. Four community leaders, identified by the coalition director, were hired to and served as facilitators and recruiters for the study. After completing an online module on the protection of human subjects, each study facilitator was provided an overview of the study protocol and an overview of the procedures and methods used for data collection. Flyers and announcements explaining the project and providing contact information were posted by the study facilitators at community centers, heath centers, social service centers, and other public venues frequented by diverse groups of African American men and women.

Individuals expressing an interest in participating in the study were contacted by a study facilitator. After which, a meeting was arranged to further discuss the purpose of the study, to describe the procedures to be used in gathering study data, and to obtain their written consent to participate in the study. To facilitate the collection of the study data, the questionnaire was administered by the study facilitators. Prospective participants were informed that completion of the study questionnaire was voluntary and that receipt of services and/or support at the recruitment sites was not contingent on their participation. Prospective participants were informed that no names or personal identifiers would be requested or recorded. Data were collected between June 2010 and January 2012.

3.3. Measures. An investigator-designed questionnaire was developed to collect the study data. Included in the questionnaire were quantitative measures relevant to involvement of providers in health care decisions and decisions made by regarding participation in health related genetic research. Quantitative measures developed, validated and used in national cohort studies to assess genetics knowledge, beliefs regarding the merits and risks of genetic testing, and perceptions regarding the utility of genetic testing [37–42] were also included.

(i) Knowledge about Genetics. Seventeen items were used to assess knowledge about genetics. Eleven true-false items were used to assess knowledge of genetics principles and implications; two items were used in which participants were asked to describe how much they felt they knew about genetics; and four forced-choice items were used to assess knowledge of ongoing health-related genetic studies being conducted within the region.

(ii) Beliefs Regarding the Benefits and Risks of Gene Testing. Nineteen items were used to assess benefits and risks of gene testing. The items included statements suggesting the benefits, anticipated consequences, and negative impact of gene testing in which participants were asked to describe their beliefs using a 5-point Likert scale (1 = strongly disagree to 5 = strongly agree).

(iii) Perceived Utility of Gene Testing. Six items were used to assess perceptions regarding the utility of gene testing. Items inferring the importance of gene testing for risk assessment, screening, early detection, and treatment were included.

(iv) Involvement of Health-Care Providers in Health Care Decisions. Twenty items were used to assess involvement of health care providers in decision making regarding gene testing. The items included statements in which the participants were asked to characterize the "extent to which they discuss health concerns with providers," the "extent to which their provider understand their background, needs, concerns and values," and the "medical judgments made by the providers on their behalf" using a 4-point Likert scale (1 = strongly disagree to 4 = strongly agree). Also an item in which participants were asked if they believed their provider would recommend genetic testing if he or she believed it would cause them harm was included.

(v) Willingness to Participate in Health-Related Research That Includes Gene Analysis. Twenty one items were used to assess willingness to participate in health-related research that included gene analysis. Items in which participants were asked if they had ever participated in medical research were included. If participants reported that they had never participated in medical research, they were queried as about their willingness to participate and their willingness to provide personal, social, occupational and medical information, and biological specimens for genetic analysis.

(vi) Demographic Characteristics. Seventeen items were included within the questionnaire to elicit data reflective of the participant's gender, age, education, marital status, employment status, income, personal or family history of a chronic disease/condition, previous involvement in health-related research, insurance status, primary source of health care, and perceived health status.

Content validity and appropriateness of the questionnaire for use among the targeted population were assessed by the study investigators prior to initiation of the study.

3.4. Data Analysis. Descriptive and inferential statistics, computed using SPSS-PC version 20 (SPSS Inc., Chicago, IL), were used to analyze the study data. Descriptive statistics (including frequency, percentages, measures of central tendency, and measures of variability) were used to describe the characteristics of the study sample. Inferential statistics (including cross tabulations and chi-square analyses) were used to identify factors associated with willingness to participate in health-related genetic research.

3.5. Ethical Considerations. The research protocol of the study was reviewed and approved by the Institutional Review Board for the Protection of Human Subjects at the University of Wisconsin Milwaukee.

4. Results

4.1. Participant Profile. Of the 212 study participants, 45.8% (n = 97) were men and 54.2% (n = 115) were women (Table 1). The mean age of the study participants was 43.04 years (SD = 6.14; range 19–95). The majority of the study participants were single (62.3%, n = 132); employed full or part time (50.5%, n = 107); and had attended or completed college (60.8%, n = 129). Sixty four percent (n = 135) reported incomes of $29,999 or less and 80.7% percent (n = 171) reported that they were insured. When asked to describe their health status, 73.6% (n = 156) described their health status as "good," "very good," or "excellent," and 43.4% (n = 92) reported a history of a chronic disease/condition.

4.2. Knowledge of Principles and Implications of Genetics. Ninety four percent (n = 192) of the African American men and women involved in the study reported that they knew little about genetics. However, data suggested that most were aware that healthy parents could bear a child with a hereditary disease; were aware of the implications of consanguinity and late parity on the expression of heritable disease/conditions and birth outcomes; and were aware of the influence of the environment on multifactorial genetic disorders (e.g., asthma, congestive heart disease, diabetes) (Table 2). In addition, when questioned about tests used for genetic analysis, most indicated that they were aware of tests used in newborn screening.

4.3. Beliefs Regarding the Benefits and Risks of Genetic Testing. Study participants had varied beliefs about the benefits, consequences, and risks associated with genetic testing (Table 2). Gene testing is currently used in the health care arena for carrier screening, preimplantation genetic diagnosis, prenatal diagnostic testing, newborn screening, presymptomatic testing for predicting adult-onset disorders, presymptomatic testing for estimating the risk of developing adult-onset disorders, and confirmatory diagnosis [43]. Most study participants reported that they believed that genetic testing would lead to improved treatments and improve health outcomes. Yet, several study participants expressed concerns about adverse consequences that could result from the diagnosis of a genetically-linked condition/disease (e.g., potential breech of their privacy, emotional trauma, stigma, and discrimination).

Fifty seven percent (n = 121) of the study participants expressed beliefs and concerns that the diagnosis of a genetically-linked condition would not remain confidential. Fifty two percent (n = 111) expressed beliefs and concerns about the effect of a genetically-liked condition on their family. Twenty two percent (n = 47) of the study participants expressed beliefs and concerns that they would not be able to emotionally handle the diagnosis of a genetically-linked condition. Thirteen percent (n = 28) reported that if they were found to carry a genetically-linked condition "others

Table 1: Profile of the study participants ($N = 212$).

Background characteristics	n	(%)	
Age in years			Mean = 43.04; SD = 6.14
19–29	38	17.9	
30–39	47	22.2	
40–49	61	28.8	
50≥	66	31.1	
Gender			
Male	97	45.8	
Female	115	54.2	
Marital status			
Married	34	20.2	
Partnered	9	4.2	
Single	133	63.5	
Widowed	9	4.2	
Divorced/separated	24	11.3	
Education			
High school or less	83	39.2	
Some college	84	39.6	
College graduate	31	14.6	
Graduate degree	14	6.6	
Employment			
Full time	80	37.7	
Part time	27	15.1	
Unemployed	59	30.2	
Disabled, not able to work	36	17.0	
Income			Median: $20,000–$29,999
<$5,000–$9,999	60	28.3	
$10,000–$29,999	75	35.4	
$30,000–$49,999	36	17.0	
$50,000–$69,999	21	5.7	
$70,000+	5	2.4	
Insurance status			
Insured	171	80.7	
Uninsured	36	17.0	
Perceived health status			
Excellent	29	13.7	
Very good	55	25.9	
Good	81	38.2	
Fair	35	16.5	
Poor	12	5.7	
History of chronic disease			
Yes	92	43.4	
No	115	54.2	
Regular healthcare provider			
Yes	165	77.8	
No	46	21.7	

would view them negatively and 8.0% ($n = 17$) reported that it would cause them to feel ashamed.

4.4. Perceived Utility of Gene Testing. Most study participants perceived that the results of gene testing would be useful to providers attempting to make patient care decisions and to individuals attempting to improve their overall health status (Table 2). When questioned about the utility of gene testing 93.9% ($n = 199$) reported that they believed that gene testing would be useful to providers when assessing patient's health risks; 93.9% ($n = 199$) reported that they believed that gene testing would help providers in their attempts to validate (or rule out) the presence of indolent disease; and 90.1% ($n = 191$) of the study participants reported that they believed that gene testing would aid providers in their attempts to provide personalized treatments and health care. Similarly, 93.4% ($n = 198$) of the study participants reported the belief that the results of gene testing would help them make decisions about how to live a healthier life.

4.5. Involvement of Health Care Providers in Health Care Decisions. The merits of directive and nondirective involvement of health-care providers in decision making about gene testing have been widely reported in the literature [33, 35, 36, 44–46]. Nondirective involvement of health-care providers (relative to gene testing) implies that the patient is given relevant information about a genetic test by the health care provider and is left to make his or her own choice about testing. Directive involvement of health care providers (relative to gene testing) implies that the health-care provider reviews relevant information about a genetic test and makes the decision for the patient about testing and the patient concurs.

Most health care providers and genetic counselors when discussing gene testing, in an effort to distance gene testing from any association with "eugenics," tend to be more inclined toward nondirective approaches. Yet, while there has been much debate about whether any discussion with patients is completely nondirective, research suggests that most clients, attempting to make decision about genetic testing and genetics related research expect information, advice and help in making decisions [35, 36].

Review of the study data revealed that the majority of the participants valued their health-care provider's knowledge and advice when making health decisions. Seventy percent ($n = 148$) of the study participants reported that they completely trusted their provider's judgment about their medical care; 89.6% ($n = 190$) reported that the medical information they received from providers was accurate and up-to-date, and 81.1% ($n = 172$) reported that they always tried to follow the provider's advice. When probed further, 67.5% ($n = 143$) reported that their providers put their medical needs above costs and all other considerations and 89.6% ($n = 190$) reported that they trusted that their provider would refer them for specialized testing if there was a need.

4.6. Willingness to Participate in Health-Related Research That Included Gene Analysis. Twenty percent ($n = 43$) of the study

Table 2: Knowledge, perceptions, beliefs, and willingness to engage in health-related genetic research ($N = 212$).

Variable	n	(%)	
Knowledge about principles and implications of genetics			Range = 3–11; mean = 8.49; SD = 1.63
Knowledge of implications of consanguinity on heritable diseases/conditions	197	92.9	
Knowledge of implications of late parity on heritable diseases/conditions	167	78.8	
Knowledge of influence of the environment on genetic disorders	186	87.7	
Knowledge of tests and procedures used in genetic testing			
Breast tumor analysis for BRCA1/BRCA2 mutations	61	28.8	
Amniotic fluid analysis for fetal defects	174	82.1	
Capillary blood analysis of newborns genetic diseases in newborns	144	67.9	
Beliefs about advantages of genetic testing			Range = 0–6; mean = 3.4; SD = 2.2
Believe that testing would provide important information	112	52.8	
Believe that testing would lead to improved risk assessment	170	80.2	
Believe that testing would lead to improved treatment	140	66.0	
Beliefs about disadvantages of genetic testing			Range = 13–53; mean = 34.3; SD = 7.14
Believe that testing would breach privacy and confidentiality	121	57.1	
Believe that abnormality would have negative impact on the family	111	52.3	
Believe that testing would cause emotional trauma	47	22.2	
Believe that results would cause other to view them negatively	28	13.2	
Believe that results would result in feelings of being "singled out"	19	9.0	
Believe that genetic abnormality would cause a sense of shame	17	8.0	
Perceived utility of gene testing			Range = 0–6; mean = 5.6; SD = 1.1
Believe that gene testing would provide information about disease risk	199	93.9	
Believe that gene testing would personalize screening/diagnostics	199	93.9	
Believe that gene testing would lead to personalize treatments	191	90.1	
Believe that gene testing would provide health information for lifestyle intervention	198	93.4	
Provider trust and involvement in health care decisions			Range = 31–80; mean = 60.5; SD = 9.1
Trust the provider's medical judgment	148	69.8	
Trust that providers place their needs above other considerations	143	67.5	
Providers refer for specialized testing as needed	190	89.6	
Follow provider's medical advice	172	81.1	
Willing to participate in health-related study that included gene analysis	116	54.7	
Willing to complete detailed questionnaires about family history	113	55.3	
Willing to allow the collection of environmental samples	94	44.3	
Willing to provide biological specimens for gene analysis	109	51.4	
Willing to participate in longitudinal studies	94	44.3	

participants reported that they had been previously involved in a health-related research study. Among those who indicated that they had not been previously involved in a health-related research study, 50.3% ($n = 169$) reported a willingness to participate. Fifty one percent ($n = 85$) reported that they would be willing to complete detailed questionnaires about their family health history, social history, occupational history, psychological, and/or emotional health. Fifty three percent ($n = 70$) reported that they would be willing to allow researchers to collect environmental samples from their home. Fifty two percent ($n = 82$) reported that they would be willing to provide researchers biological specimens (e.g., tissue, blood, saliva, hair, nail clippings) for genetic analysis. Fifty four percent ($n = 70$) reported that they would be willing to participate even if the study were longitudinal and required the collection of data over several years. However 88.7% ($n = 149$) reported that they "had never been asked."

4.7. Factors Associated with Willingness to Participate in Health-Related Genetics Research. Study findings implicate the impact of knowledge, perceptions, and beliefs on the willingness of African American men and women to participate in health-related genetics research (Table 3). Participants with higher levels of knowledge about genetics and heritable diseases; with understanding of the benefits, risks, and utility of genetic testing; and who had previous involvement in a health-related research study were more likely to report a willingness to engage in health-related genetic research than study participants with lesser knowledge about genetics, those with lesser knowledge, and those who had not previously been involved in a health-related research study. Participants reporting higher levels of trust in their provider's knowledge and judgment and participants reporting that their providers "listened well" to their concerns about their health and well-being were more likely to report a willingness

Table 3: Factors associated with willingness to engage in health-related genetic research ($N = 212$).

Variable	χ^2	df	P
Knowledge about principles and implications of genetics			
Awareness of implications of late parity on heritable diseases	7.346	2	.025
Understanding of patterns of transmission of heritable disorders	4.07	1	.045
Awareness of tests and procedures used in genetic testing	4.343	1	.037
Understanding of benefits and risks of genetic testing	49.787	35	.050
Perceptions regarding utility of genetic testing	4.176	1	.042
Provider trust and involvement in health care decisions	8.648	3	.034
Previous involvement in health-related research	4.226	1	.040

to engage in health-related research than those who did not. In addition, as might be expected, participants reporting that they had been informed and offered the opportunity to participate in a study were more likely to express a willingness to engage in a health-related research study than those who were not.

5. Discussion

The use of nonprobability sampling and self-reported measures used in this exploratory study limit the generalizability of the findings. Yet, in spite of these constraints, the findings warrant careful consideration.

Increasing the involvement of minority participants in health-related research has long been identified as a national health priority. Accounts of events that have negatively impacted the engagement of African Americans have been widely reported in the scientific literature. Also are codes, guidelines, procedures, and regulations are reported to prevent these and other unethical behaviors conducted in the name of science [47, 48].

On June 10, 1993, the NIH Revitalization Act of 1993, PL 103-4 was signed into law [49]. The legislation directed the National Institutes of Health to establish guidelines for inclusion of women and minority groups and their subpopulations in NIH-funded clinical research, unless a clear and compelling rationale and justification that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Despite the enactment of legislation specific to the inclusion of minorities in health-related research and the adoption of codes, guidelines, procedures, and regulations to prevent unethical behaviors in research and protect the rights and well-being of persons involved, the contribution of specimens to biorepositories by African Americans and the participation of African Americans in health-related genetic research are limited.

This study was designed to assess factors deemed to be essential to the engagement of African Americans in health-related research that involves gene testing. More specifically, the study was designed to assess the influence of knowledge about genetics; beliefs regarding benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and the involvement of health care providers on decisions regarding participation in health-related genetic research. The results of this study support hypotheses proposed in the organizing framework relative to influence of knowledge about genetics; beliefs regarding benefits and risks of gene testing; perceptions regarding the utility of genetic testing; and the involvement of health care providers on decisions relative to participation in health-related genetics research and the willingness to engage in health-related genetic research. As hypothesized, study findings revealed that knowledge about genetic research, perceptions regarding the utility of genetic testing, beliefs regarding the benefits and risks of genetic testing, and previous involvement in research are associated with participant's willingness to engage in health-related genetic research. Study participants reporting higher levels of trust and engagement with providers were more likely to report a willingness to engage in health related research than those who did not. The most surprising were the findings that the vast majority of the African American men and women involved in the study reported support of health-related genetic research; however, most indicated that they had "never been asked."

This study is unique in that it attempted to highlight factors associated with the participation of African Americans in health-related research as well the processes, outcomes, and principles of informed decision making. The findings suggest the need for research that further examines factors presumed to be associated with the involvement of African Americans in health-related genetic research. The most important appears to be the need for research that explores the manner and extent to which African Americans are informed about the benefits, risks, and utility of genetic testing; research that explores the manner and extent to which African Americans are informed and afforded the opportunity to participate in health-related genetic research; and research that explores the manner and extent to which the decisions of African American men and women relative to participation in health-related genetic research (as assured during the consenting process) are supported. The findings also suggest that the need for strategies to better inform African Americans of opportunities to contribute health information and biological specimens to biorepositories and to better engage researchers and health care providers in efforts to inform, recruit, and support African American men and women willing to participate in health-related genetic research. Without the design of interventions to better inform, recruit, engage, and support the decisions of African Americans (and persons representing other minority population groups) willing to participate in health-related genetic research it would be

reasonable to anticipate that current trends relative to their involvement will remain unchanged.

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Research Article

Attitudes toward Genetic Testing for Hypertension among African American Women and Girls

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Introduction. Although African American (AA) women have the highest prevalence of hypertension and many genetic studies have been conducted to examine this disparity, no published studies have investigated their attitudes toward genetic testing for hypertension. The purpose of the present study was to use the health belief model as a guide to examine attitudes toward perceived barriers and benefits of genetic testing held by AA multigenerational triads and to determine whether they differed by generation, age, education, or income level. *Methods*. A descriptive correlational research design were used with 183 African American women and girls from Detroit. Correlations between triad membership, age, income, and education level were examined for association with attitudes toward genetic testing. *Results*. Increasing age and education were associated with significant differences in attitudes regarding benefits ($F[2, 160] = 5.19$, $P = 0.007$, $d = 0.06$) and awareness ($F[2, 160] = 6.49$, $P = 0.002$, $d = 0.08$). No statistically significant differences existed on the three subscales when compared by income levels or triad membership. *Conclusions*. This highlights the need for increased outreach to younger generations regarding benefits of genetic services. Further research is necessary to determine whether rural and male populations have similar beliefs.

1. Introduction

Cardiovascular disease (CVD) carries the highest mortality rate for women in the United States. The American Heart Association (AHA) reports that more than 47% of African American women, of age 20 years and older, have been diagnosed with CVD as of 2008 [1]. Additionally, African American women also have the highest reported death rates from CVD [1]. Hypertension is a significant risk factor for the development of CVD [2]. Among African American women, 45.7% have hypertension marking them as possessing the highest incidence and prevalence rates amid all ethnic and racial groups in the United States [1]. In 2008, deaths related to hypertension among African American women totaled 7,002 with African American women's death rate being two and a half times more than that of Caucasian women [1, 3].

Research has shown that genetic factors contribute significantly to the susceptibility of developing hypertension [4–7]. A study of Caucasian and African American children revealed the T235 allele on the angiotensinogen gene to be more common in African American children compared to Caucasian. This is meaningful such that the T235 allele is positively correlated with increased serum angiotensinogen levels and hypertension in African American boys and girls, when compared to Caucasian children ($P < 0.01$) [8]. Other groups addressing African Americans have found that single-nucleotide polymorphisms (SNPs) on *SLC4A5*, a sodium bicarbonate transporter gene found on chromosome 2, were also significantly associated with hypertension [9–14]. Conversely, the presence of certain SNPs (such as SLC4A5 rs8179526) may be protective against the development of hypertension among African American women even when dietary sodium is elevated [13].

2. Genetic Testing

Genetic testing is a useful screening tool that can help identify people at high risk for developing disease and has the potential to enhance health and wellbeing [15]. However, research

shows that few African Americans seek genetic services [16–19]. Studies have shown that fewer African American women seek genetic testing services compared to Caucasian women [20–22]. Underutilization of genetic services (including counseling and testing) reduces this group's ability to benefit from genetic testing, including early detection and intervention to prevent illness. To reduce disparities in the use of genetic services for heritable and preventable diseases, such as hypertension, efforts must be made to identify perceived barriers and attitudes towards genetic testing to ensure equal distribution of resources. By elucidating these barriers and attitudes among African American women and girls, the scientific and healthcare community can garner greater understanding to improve assessment and treatment of hypertension in this at-risk population. Currently, genetic testing for essential hypertension is not routinely conducted in the clinical setting. However, with-direct-to-consumer genetic testing for many disorders currently available, there is a growing potential for genetic testing to be used by the consumers to better inform their healthcare decision making for hypertension and many other diseases. The present study assesses the attitudes toward genetic testing for hypertension at a time when it is moving from a research question to more of a clinically relevant measure of health.

3. Conceptual Model

To understand the factors that contribute to African American women and girls' decision to participate in genetic testing, the present study used aspects of the Health Belief Model (HBM) to guide its design. The HBM was originally developed to explain why individuals failed to participate in programs to detect and prevent disease (Figure 1) [23]. Researchers use the HBM to understand an individual's decision to take preventative measures, such as genetic testing, for personal health promotion [24]. The HBM considers an individual's perceived susceptibility to disease development as well as disease severity, personal demographic variables (gender, age, socioeconomic status, education, and knowledge), cues to action, and benefits and barriers regarding illness prevention [25]. Mobilization to act and take preventative measures is based on a cost-benefit analysis of all the components.

The HBM includes four primary aspects that explain the infrequent acceptance of preventive practices and preillness screening tests: perceived susceptibility, perceived severity, perceived benefits, and perceived barriers [26]. Of the four components, Strecher and Rosenstock (1997) believe that perceived benefits and barriers are stronger predictors of the behavior change when the perceived threat is high [27]. Hypertension is a well-established risk factor for developing cardiovascular disease, a disease with high morbidity and mortality. Thus, the present study focused primarily on examining participants' perceived benefits, awareness, and outcomes of genetic testing, while considering certain demographic variables.

The rationale for this approach was based on several components of the HBM [27]. First, a woman highly susceptible to hypertension may not undergo preventative actions, such as genetic testing, if the actions are not perceived to be efficacious. Likewise, if an action such as taking an antihypertensive medication is not believed to have positive consequences (e.g., decreasing risk for cardiovascular accident, heart attack, stroke, kidney disease, etc.), an action to take on other related lifestyle preventative measure (e.g., weight loss measures, increased physical activity, and dietary changes) may also be impacted. Finally, select demographic variables may have an indirect effect on health behaviors, thereby influencing personal perceptions of whether or not to implement preventative action. When personal demographics and perceived barriers are combined, they may affect a woman's decision.

Investigators have sought to understand reasons why African Americans participate less frequently in genetic testing than other ethnic groups by using the HBM, focusing primarily on two aspects—personal demographics and perceived barriers and benefits [24, 28]. Currently, no study exists which takes into account demographics such as age and education to assess perceived barriers and benefits associated with genetic testing for chronic diseases such as hypertension. The present study is also the first to address three generations of African American women and girls' attitudes toward benefits and barriers of genetic testing specific to hypertension. While research has been conducted addressing cancer and congenital issues with perceived barriers and benefits to genetic testing, the present study fills a gap in the literature by addressing a common complex disease such as hypertension in an at-risk population.

Through phone interviews with over 800 African American and Caucasian participants (with known or unknown risk genetic risk for disease), Furr (2002) found that while age, gender, income, and educational achievement of African Americans held no influence on attitudes toward genetic testing in general, African Americans had increased negative perceptions regarding genetic testing when compared to European Americans [29]. Although studies have assessed attitudes/barriers to genetic testing for diseases such as ovarian, breast and colon cancer, the present study is the first study to examine participants' perceived benefits, awareness, and outcomes related to genetic testing for a chronic disease such as hypertension. Conversely in a later study, Forman and Hall (2009) found multiple socioeconomic barriers to genetic testing in women with breast and ovarian cancer [16]. Significant barriers included potential time constraints, limited access to knowledgeable providers, geographic barriers, limited awareness, language/cultural barriers, high cost, and ineligibility for Medicare/Medicaid. In yet another study, Sussner et al. (2009) found that foreign-born women of African descent reported more anticipation of negative emotional reactions about genetic testing for BRCA1/2 compared with US-born African American women [20]. Acculturation was suggested as being associated with perceived barriers and concerns regarding genetic testing. Acculturation is defined as the extent to which a majority culture is adopted by a minority culture thereby exchanging cultural elements, measured by length of time living in the US [20]. As these studies suggest, perceived barriers and benefits are significant in attitudes towards genetic testing.

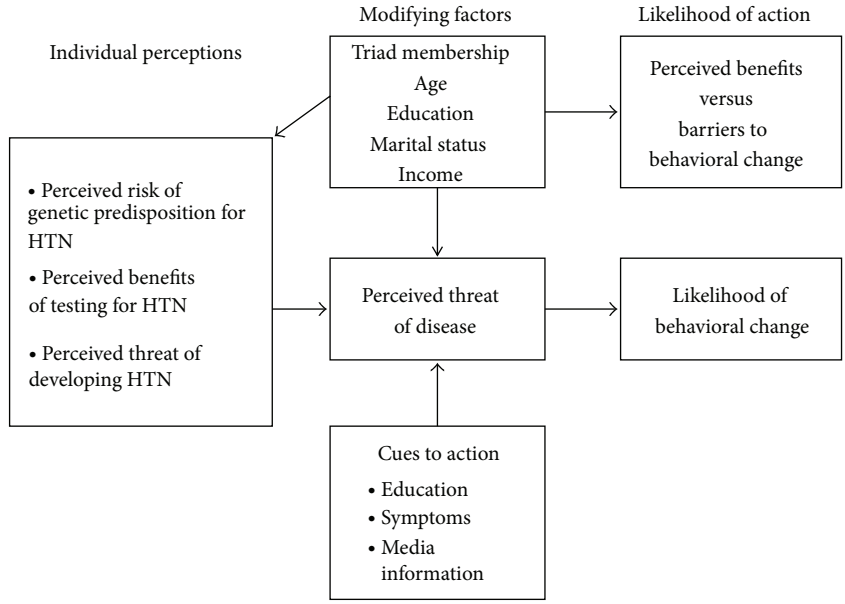

Figure 1: Health belief model.

Common themes among African Americans in regards to genetic testing have been elucidated in recent research with lack of knowledge and/or negative attitudes towards genetic testing standing out as a predominant theme [20, 22, 29–31]. Thompson et al. (2002) assessed beliefs and attitudes towards breast cancer genetics research and found that knowledge of breast cancer genetics plays a major role in women's decision to undergo genetic testing [32]. This study was unique as authors viewed genetic testing as a process that began with counseling and ended with testing [32]. Authors recognized that refusal to participate in genetic testing could happen at any step (either before or after counseling) and that attitudes toward genetic testing may change between points of refusal.

In another cancer study, specifically focusing on hereditary colorectal carcinoma susceptibility, Kinney et al. (2001) used focus group interviews to obtain insight into beliefs, attitudes, and informational needs regarding genetic testing for patients with colorectal carcinoma or those with first-degree relatives diagnosed with this disease [33]. Though African Americans were undersampled, results revealed a general lack of knowledge regarding cancer genetics, genetic testing, concern over confidentiality issues, concern for other family members (specifically children), and the need to have a primary care provider be cognizant of these issues. In another focus group study, Murphy and Thompson (2009) studied predominantly African American participants with a history of anxiety or depression to assess attitudes, beliefs, and knowledge regarding genetic testing for psychiatric disorders [18]. Although a majority of participants lacked true understanding of genetic testing, participants had strong opinions concerning genetic use. Consensus was reached that genetic testing was beneficial, yet feared the process of testing could be harmful and painful.

Current literature supports conflicting attitudes and beliefs regarding genetic testing among African American women [34]. A review by Rew et al. (2009) noted few studies that focus directly on genetic testing in adolescents, remarking that of the limited available literature, educated Caucasian adolescents girls are oversampled [19]. Authors further argue that while ethical issues of genetic testing have been studied, empirical results to explain attitudes, beliefs, and knowledge of people who are participating in genetic testing are lacking especially in multigenerational families. As evidenced by the above-mentioned studies, methodologies used to assess psychosocial barriers to genetic testing vary. The present study uses one-on-one in-person interviews to obtain quantitative information.

While many studies have explored the psychosocial barriers to genetic testing for heritable diseases such as breast cancer, none have examined attitudes towards genetic testing for hypertension. The present study used several components of the Health Belief Model to better understand factors that influence African American women and girls when making a decision to undergo genetic testing for hypertension. Using specific personal demographic variables and perceptions of benefits and barriers associated with the health belief model, the present study examined attitudes toward genetic testing for hypertension among African American multigenerational triads (daughter-mother-grandmother). The research questions were as follows: (a) what are three generations of African American women and girls' attitudes toward genetic testing for hypertension? and (b) do African American women and girls' attitudes towards genetic testing for hypertension differ by triad (generation), age, education, or income level?

4. Methods

4.1. Design. The study employed a descriptive correlational research design to address the research questions. Correlations between variables (triad membership, age, income,

and education) were examined to assess the strength and direction of the association with attitudes toward genetic testing (benefits, awareness, and outcomes).

4.2. Sample and Setting. Participants in the present study included 183 African American women and girls from the Detroit Metropolitan area, originally involved in a parent study titled, "Hypertension and heredity: hypertension genetic polymorphisms in three generations of African American women" [35].

4.3. Human Subjects. Recruitment and the process of consent began after approval by University of Michigan and Wayne State University, Institutional Review Boards (IRB). Three generations of maternally, blood-related women and girls were recruited to examine genetic markers known to increase susceptibility to hypertension. Details of the research methods for the parent study are described elsewhere [35–38]. To meet the inclusion criteria for the parent study, participants were required to self-identify as African American and have a living family of at least three generations to constitute the triad of daughter-mother-grandmother. All participants who were recruited resided in urban and suburban neighborhoods in a large midwestern urban area. For those with a diagnosis of hypertension, their blood pressure had to average 140/90 or higher (stage 1 or 2 hypertension) without medication. Participants in the study also included: women diagnosed with diabetes, those who were on antihypertensive medication, or women who were normotensive and girls (offspring) not diagnosed with hypertension. Exclusion criteria consisted of having comorbidities of substance abuse, mental illness, end-stage cancer, end-stage renal disease, or other terminal illness. The researchers in the parent study excluded children under 12 years of age from completing the AGT survey because of the nature of the questions asked in the survey.

4.4. Instruments

4.4.1. Demographic Survey. Participants completed a research-developed questionnaire that collected information on family triad relationship (daughter, mother, grandmother), age, educational level, marital status, household income, and sources of income.

4.4.2. Perceived Benefits and Barriers of Genetic Testing. The attitudes toward genetic testing (AGT) was developed by the PI of the present study. Ten items were used to measure participants' attitudes toward genetic testing for hypertension. The items were rated using a 4-point Likert-type scale ranging from 1 for strongly agree to 4 for strongly disagree. A principal components factor analysis with a varimax rotation was used to determine if subscales would emerge on the AGT. To be retained on a subscale, the factor loadings had to be greater than 0.40 and not load on more than one subscale. Three subscales (benefits, awareness, and outcomes) were emerged from the factor analysis, explaining 58.91% of the variance in AGT (see Table 1). The three subscales had eigenvalues greater than 1.00, indicating that they were each explaining statistically significant amounts of variance. Mean scores were obtained for each subscale and total scale for each participant by summing the responses and dividing by the number of items with valid responses. Lower scores were indicative of more positive attitudes toward genetic testing. The internal consistency of the AGT was tested using Cronbach's alpha. The obtained coefficient of 0.66 provided evidence that the instrument had adequate internal consistency. Face validity of the instrument was determined by having three experts on genetic testing review the instrument. They indicated that the instrument had good face validity.

4.5. Statistical Analysis. A one-way multivariate analysis of variance (MANOVA) and a one-way analysis of variance (ANOVA) were used to determine if there was any significant difference between triad members (daughters, mothers, grandmothers) on their attitudes toward genetic testing.

Data collected from the included triads were analyzed using PASW Statistics, ver. 17.0 (SPSS, Inc., Chicago, IL, USA). The data analysis included descriptive statistics to summarize responses to age, number of children and grandchildren, marital status, level of education, household income, and sources of income. Measures of central tendency summarized demographic information.

A one-way analysis of variance (ANOVA) was used to determine if any differences could be found among the triad members' (daughters, mothers, grandmothers) attitudes regarding genetic testing. The three subscales (benefits, awareness, and outcomes) from the AGT survey were used as dependent variables in a one-way multivariate analysis of variance (MANOVA) to determine if there were differences among the triads by income, educational level, and age. All decisions on the statistical significance of the findings were made using an alpha level of 0.05. Given the sample size ($N = 183$), power analysis showed that we had >80% power to detect effect sizes as low as 0.23 at a significance level of 0.05. The "d" is the dimension of the group means, or an estimate of the effect size, that represents the practical significance. The "d" ranges from 0 to 1, with numbers closer to 1 representing a stronger effect. Practical significance provides the reader with the importance of the findings to clinical practice based on effect size even when the results may not be statistically significant. Statistical significance has a greater dependence on sample size than effect size and is the standard method of determining important differences between variables in research studies. When conducting research in nursing science, it is important to represent both of these statistics in the findings.

5. Results

5.1. Age. A total of 183 participants were included in the study. Of this number, 45 (24.6%) were grandmothers, 69 (37.7%) were mothers, and 69 (37.7%) were daughters. The participants ranged in age from 12 years to 93 years. The mean age of the grandmothers was 65.64 years (SD = 12.30). The mothers had a mean age of 46.39 years (SD = 15.17). The

Table 1: Principal components factor analysis of "Attitudes toward genetic testing (AGT)".

Item	Factor		
	Benefits	Awareness	Outcomes
(1) You are confident that the results of your genetic tests will be kept confidential.	0.58		
(2) Genetic testing is relevant to you and/or your child's health.	0.57		
(3) Genetic testing is beneficial in the prevention of the disease.	0.85		
(4) Genetic testing is beneficial in the treatment of the disease.	0.86		
(5) Genetic testing is beneficial in preventing the disease.	0.68		
(6) You would like to know if you and/or your child tests positive for a genetic disorder.		0.78	
(7) If you or your child tested positive for a genetic disorder, you would seek medical care immediately to minimize you or your child's chances of developing the disease.		0.89	
(8) If you or your child tested positive for a genetic disorder, would you wait until you and/or your child experienced signs and symptoms of the disease before obtaining medical care?			0.63
(9) If you or your child tested positive for a genetic disorder, you believe that you and/or your child would be treated differently by healthcare providers.			0.73
(10) If you test positive for a genetic disorder, it is likely that your child is at risk for testing positive for the same disease.			0.60
Percent of explained variation	26.86	18.47	13.58
Eigenvalues	2.69	1.85	1.36

average of the granddaughters' age was 21.95 years (SD = 16.13).

5.2. Educational Level. The educational levels of the grandmothers were generally high school (n = 11, 24.4%) or some college (n = 12, 26.7%). In contrast, the largest group of mothers (n = 27, 39.1%) had completed some college, with 16 (23.3%) indicating they had attained bachelor degrees. The granddaughters' ages indicated that a substantial proportion had not yet completed high school (n = 28, 40.7%). One (2.2%) grandmother and 2 (2.9%) granddaughters had obtained doctorate degrees (see Table 2).

5.3. Marital Status. Grandmothers were more likely to be widowed (n = 15, 33.3%) or married (n = 11, 24.5%), while mothers tended to be either married (n = 23, 33.3%) or single (n = 20, 29.1%). Most of the granddaughters tended to be single (n = 54, 78.4%).

5.4. Income. The household incomes of the grandmothers ranged from less than $10,000 to over $80,000. The largest group of grandmothers had incomes between $10,000 and $40,000 ($n$ = 23, 51.2%). The largest group of mothers had incomes between $40,000 and $60,000 ($n$ = 14, 20.4%). The largest group of granddaughters who were working had income levels that ranged from $40,000 to $60,000 ($n$ = 15, 21.8%).

Participants were provided with a list of possible sources of income and were asked to indicate all that applied. As a result, the number of responses was greater than the number of participants. The sources of income for grandmothers were mostly from Social Security (n = 24, 53.3%) or retirement/pension (n = 20, 44.4%). Sixteen (35.6%) grandmothers received income from working. The majority of the mothers were working (n = 47, 68.1%), with 14 (20.3%) indicating they were receiving Social Security. Twenty-nine (43.9%) of the granddaughters indicated they were receiving income from working.

5.5. ANOVA by Triad Membership. Results of the one-way analysis of variance comparing total scores on the AGT by triad membership were not statistically significant (F [2, 160] = 1.27, P = 0.283). This finding provided support that attitudes toward genetic testing did not differ relative to the generation being asked. Grandmothers (M = 1.65, SD = 0.36), mothers (M = 1.54, SD = 0.34), and granddaughters (M = 1.61, SD = 0.39) had similar positive attitudes toward genetic testing.

5.6. MANOVA by Age. The comparison of the three subscales (benefits, awareness, and outcomes) associated with attitudes toward genetic testing were used as dependent variables in a one-way MANOVA, with age of the participants used as the independent variable (see Table 3). The results of this analysis were statistically significant, F [6, 316] = 3.90, P = 0.001, d = 0.07. When the three subscales were examined separately, benefits, F [2, 160] = 5.19, P = 0.007, d = 0.06, and awareness, F [2, 160] = 6.49, P = 0.002, d = 0.08, differed significantly. The participants who were between 22 and 50 years of age (M = 1.28, SD = 0.40) had significantly more

Table 2: Personal characteristics by triad membership.

Personal characteristics	Triad membership						Total (N = 183)	
	Grandmother (n = 45)		Mother (n = 69)		Granddaughter (n = 69)			
	N	%	N	%	N	%	N	%
Age (years)								
≤18	0	0	0	0	35	50.7	35	19.1
19–24	0	0	1	1.5	12	17.3	13	7.1
25–34	0	0	17	24.7	5	7.2	22	12.0
35–44	1	2.2	19	27.5	8	11.6	28	15.3
45–54	10	22.2	15	21.7	7	10.1	32	17.5
55–64	9	20.0	8	11.6	2	2.9	19	10.4
65+	25	55.6	9	13.0	0	0	34	18.6
Educational level								
Less than high school	5	11.1	2	2.9	28	40.7	35	19.1
High school/GED	11	24.4	9	13.0	11	15.9	31	16.9
Some college	12	26.7	27	39.1	8	11.6	47	25.8
Associate degree	7	15.6	3	4.3	4	5.8	14	7.7
Bachelor degree	4	8.9	16	23.3	13	18.8	33	18.0
Master degree	4	8.9	12	17.4	2	2.9	18	9.8
Doctorate	1	2.2	0	0.0	2	2.9	3	1.6
Missing	1	2.2	0	0.0	1	1.4	2	1.1
Marital status								
Married	11	24.5	23	33.3	9	13.0	43	23.5
Single	4	8.9	20	29.1	54	78.4	78	42.6
Divorced	10	22.2	17	24.6	5	7.2	32	17.5
Separated	4	8.9	2	2.9	0	0.0	6	3.3
Widowed	15	33.3	5	7.2	0	0.0	20	10.9
Missing	1	2.2	2	2.9	1	1.4	4	2.2
Household income								
Less than 10 k	8	17.7	8	11.6	14	20.3	30	16.4
10 k to 20 k	9	20.0	7	10.1	6	8.7	22	12.0
20 k to 30 k	7	15.6	8	11.6	6	8.7	21	11.5
30 k to 40 k	7	15.6	11	15.9	7	10.1	25	13.7
40 k to 60 k	6	13.3	14	20.4	15	21.8	35	19.1
60 k to 80 k	3	6.7	10	14.5	9	13.0	22	12.0
80 k and higher	3	6.7	8	11.6	8	11.6	19	10.4
Missing	2	4.4	3	4.3	4	5.8	9	4.9
Sources of income*								
Wages from employment	16	35.6	47	68.1	29	43.9	92	51.1
Social Security	24	53.3	14	20.3	2	3.0	40	22.2
Retirement/pension	20	44.4	10	14.5	3	4.5	33	18.3
IRA/401 Ks	2	4.4	2	2.9	0	0.0	4	2.2
Welfare	1	2.2	4	5.8	4	6.1	9	5.0
Investments	3	6.7	4	5.8	0	0.0	7	3.9
Other sources of income	6	13.3	9	13.0	36	54.5	51	28.3

*Participants were encouraged to indicate more than one source of income if appropriate.

positive attitudes regarding benefits of genetic testing than participants who were 21 years and younger (M = 1.62, SD = 0.54). The participants who were between 22 and 50 years of age (M = 1.16, SD = 0.35) and those who were over 50 years of age (M = 1.18, SD = 0.45) had significantly more positive attitudes about awareness of genetic testing than those who were 21 years and younger (M = 1.60, SD = 0.97) (see Table 4).

5.7. MANOVA by Educational Level. The results of the one-way MANOVA used to compare the three subscales measuring AGT by the educational level of the participants was

Table 3: One-way MANOVA—subscales measuring attitudes toward genetic testing by triad membership.

Triad	Subscales					
	Benefits		Awareness		Outcomes	
	M	SD	M	SD	M	SD
Grandmother	1.50	0.51	1.18	0.44	2.20	0.56
Mother	1.33	0.44	1.17	0.40	2.14	0.50
Granddaughter	1.38	0.46	1.35	0.71	2.16	0.62

MANOVA F ratio: $F[6, 316] = 1.55$, $P = 0.162$, $d = 0.03$ (based on Wilk's lambda).
Between subjects: benefits: $F[2, 160] = 1.86$, $P = 0.159$, $d = 0.02$.
Awareness $F[2, 160] = 1.96$, $P = 0.145$, $d = 0.02$.
Outcomes $F[2, 160] = 0.19$, $P = 0.830$, $d =< 0.01$.
Note: Lower scores indicate more positive perceptions of attitudes toward genetic testing.
Twenty granddaughters were less than 12 years of age and did not complete the attitudes toward genetic testing survey.

Table 4: One-way MANOVA—subscales measuring attitudes toward genetic testing by age of participants.

Age	Subscales					
	Benefits		Awareness		Outcomes	
	M	SD	M	SD	M	SD
21 years and younger	1.62_{a}	0.54	$1.60_{a,b}$	0.97	2.29	0.53
22 to 50	1.28_{a}	0.40	1.16_{a}	0.35	2.05	0.53
Over 50 years	1.45_{a}	0.49	1.18_{b}	0.45	2.25	0.57

MANOVA F ratio: $F[6, 316] = 3.90$, $P = 0.001$, $d = 0.07$ (based on Wilk's lambda).
Between subjects: benefits: $F[2, 160] = 5.19$, $P = 0.007$, $d = 0.06$.
Awareness $F[2, 160] = 6.49$, $P = 0.002$, $d = 0.08$.
Outcomes $F[2, 160] = 3.01$, $P = 0.052$, $d = 0.04$.
Note: Lower scores indicate more positive perceptions of attitudes toward genetic testing.
Means in a column sharing subscripts are significantly different.
Twenty granddaughters were less than 12 years of age and did not complete the attitudes toward genetic testing survey.

statistically significant (see Table 5), $F[15, 425.53] = 3.01$, $P = 0.001$, $d = 0.09$. Statistically significant differences were obtained for benefits, $F[5, 156] = 3.66$, $P = 0.004$, $d = 0.11$, and awareness, $F[5, 156] = 5.86$, $P < 0.001$, $d = 0.16$. Participants who had a graduate degree (M = 1.15, SD = 0.24) had significantly more positive attitudes regarding the benefits of genetic testing than those who had completed high school or obtained a GED (M = 1.61, SD = 0.60). The participants who had not completed high school (M = 1.84, SD = 1.04) had significantly poorer attitudes regarding their awareness of genetic testing than participants with the other five educational levels. No statistically significant differences were found among the participants on the three subscales measuring AGT when compared by income levels or triad membership.

6. Discussion

The present study found that urban African American women and girls across multiple generations were aware of the benefits and outcomes of genetic testing correlating with increased age and education level. These findings were similar to previous research by Murphy and Thompson (2009) who found African Americans to believe that genetic testing is beneficial but lacked understanding of the process itself [30]. In the present study, positive attitudes toward awareness and perceived benefits of genetic testing increased with age and level of education, possibly due to increased exposure through life experiences and/or education. These findings contradict Donovan and Tucker's research (2000) in which education levels were unrelated to the degree of knowledge regarding the genetics of heritable disease [34]. The Health Belief Model asserts that knowledge and understanding of perceived benefits must outweigh the risks if preventative action (such as genetic testing) is to be employed. Specific education to reach a less informed younger generation is indicated to expand knowledge in benefits of genetic testing.

A unique component of the present study's research design was the recruitment of three generations of women in African American families. The African American family structure has been described as matriarchal one in which the eldest woman often makes health care decisions for the family [39]. Based on the results of the present study, the most likely family member to have the least amount of knowledge and, thus, decline the testing would be the daughter. However, because of the strong maternal hierarchy of the family, the younger generation typically conforms to the grandmother's wishes. Respect for the eldest female family member could be a contributing factor for African American women and girls to participate in genetic testing for hypertension. African Americans over 50 years of age need to be apprised of the guidelines and policy recommendations for ethical use of genetic testing on families and children [40, 41].

Although research has shown that while some African Americans have lower incomes, higher unemployment, and less access to health insurance and medical care compared

Table 5: One-way MANOVA—subscales measuring attitudes toward genetic testing by educational level of participants.

Educational level	Subscales					
	Benefits		Awareness		Outcomes	
	M	SD	M	SD	M	SD
Less than high school	1.60	0.53	$1.84_{a,b,c,d,e}$	1.04	2.40	0.51
High school/GED	1.61_{a}	0.60	1.21_{a}	0.60	2.18	0.54
Some college	1.35	0.42	1.15_{b}	0.31	2.11	0.49
Associate degree	1.40	0.48	1.18_{c}	0.32	2.00	0.55
Bachelor degree	1.29	0.38	1.17_{d}	0.32	2.10	0.61
Graduate degree	1.15_{a}	0.24	1.07_{e}	0.24	2.25	0.63

MANOVA F ratio: $F\,[15, 425.53] = 3.01$, $P = 0.001$, $d = 0.09$ (based on Wilk's lambda).
Between subjects: benefits: $F\,[5, 156] = 3.66$, $P = 0.004$, $d = 0.11$.
Awareness $F\,[5, 156] = 5.86$, $P < 0.001$, $d = 0.16$.
Outcomes $F\,[5, 156] = 1.12$, $P = 0.353$, $d = 0.04$.
Note: Lower scores indicate more positive perceptions of attitudes toward genetic testing.
Means in a column sharing subscripts are significantly different.
Twenty granddaughters were less than 12 years of age and did not complete the attitudes toward genetic testing survey.

to Caucasians, income level was not associated with attitudes towards genetic testing in the present study [42]. Forman and Hall (2009) suggest that cost, availability, lower socioeconomic status, and limited access to health care and preventive services are a significant barrier to genetic testing [16]. Focusing though solely on economic issues ignores the multifactorial nature of barriers to genetic services among African Americans such as age, education, and family structure which were significant in the present study [43].

7. Limitations

The women in the present study gave prior consent to being genetically tested and, therefore, their perceptions may be substantially biased towards positive acceptance. Additionally, the attitudes expressed in this paper are reflections by African American women and girls only and cannot be generalized to women of other ethnic groups or men. Findings may also not be generalizable to those who live in other geographic areas, as the study population was recruited from a large urban midwestern city. As the survey incorporated only ten items, the AGT questionnaire may not address the entire gamut of psychosocial barriers and perceptions held by participants. Because the overall Cronbach alpha for the AGT instrument was 0.66 and the commonly acceptable level is 0.70, we recognize this as a possible limitation in the study. However, after conducting factor analysis for the three subscales, we determined that each of the factor loadings was greater than 0.40 and did not load on more than one subscale. Three subscales (benefits, awareness, and outcomes) emerged from the factor analysis explaining 58.91% of the variance in AGT. The three subscales had eigenvalues greater than 1.00, indicating that they were each explaining statistically significant amounts of variance and were adequate.

In addition, genetic testing is not commonly used clinically for essential hypertension and, therefore, based on the HBM, responses may be impacted by the fact that this testing is not commonly used in practice. However, based on this and future studies, this trend in lack of testing for chronic disease such as hypertension for the use in the health care setting for health care-related decision making could be changing.

8. Conclusions

Diverse barriers to genetic risk assessment exist for African American women and girls. By elucidating perceived barriers to genetic testing by African American women and girls, health care providers can design gender-specific, culturally relevant services for outreach, genetic counseling and testing to promote early and appropriate intervention for an at-risk population. Genetic testing has the potential to reveal specific markers that may identify risk for, or protection against, the development of hypertension. By identifying such markers prior to the condition's onset, more meaningful genetic counseling can be delivered to family members. Likewise, a genetic test has the potential to provide information to an individual diagnosed with hypertension on how to best manage the condition. Greater participation by African American women and girls in genetic testing can provide a better foundation for knowledge regarding the etiology of hypertension in this population as well as its appropriate management.

The present study highlights the complex nature of an individual's decision to pursue genetic testing. For these women, openness to undergo hypertension risk assessment may have been influenced by familial, educational, and age-related factors. Further investigation is needed in each of these subcategories to understand how they contribute to African American women and girls' perceptions towards genetic testing. This information could shape specific outreach to address corresponding gaps in knowledge and understanding of genetic testing. As the present study represented the beliefs of African American women and girls from an urban metropolitan area, additional research is indicated to clarify motivations for pursuing genetic testing in hypertension across other settings and groups, so that healthcare providers can best guide prevention and intervention efforts.

Conflict of Interests

The authors have no conflict of interests.

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