GENE-ENVIRONMENT EFFECTS OF SLC4A5 AND SKIN COLOR ON BLOOD PRESSURE AMONG AFRICAN AMERICAN WOMEN

Objective: To determine the relationship between genetic polymorphisms and environmental factors (skin color) on blood pressure among African American women.

Method: A descriptive study, consisting of 137 African American women from a Midwestern, metropolitan area was conducted. Blood pressure was measured using a digital blood pressure monitor. Self-reporting methods were utilized to obtain information on skin color. Buccal swab saliva samples were obtained for genotyping.

Results: Of the four single nucleotide polymorphisms (SNPs) on the sodium bicarbonate co-transporter gene (SLC4A5) examined in this study, only one SNP (rs10177833) and skin color interaction was found to be associated with systolic blood pressure. The additive effect of rs10177833 on systolic blood pressure is statistically different between women with dark skin color and women with medium skin color ($P=0.0153$). No SNP and skin color interaction was found to be associated with blood pressure readings in other SNPs tested (rs8179526, rs6726450 and rs6731545).

Discussion: These findings of genetic and skin color relatedness to blood pressure is important when considering appropriate diagnostic and treatment plans for African American women with hypertension. African American women with darker skin color may require further assessment for risk factors such as discrimination related stress when being seen by health professionals for hypertension. (Ethn Dis. 2012;22(2):155–161)

Key Words: Blood Pressure, Genetic, African American, Skin Color

HYPERTENSION AND AFRICAN AMERICANS

African Americans are more likely to be diagnosed with cardiovascular-related diseases (eg, hypertension, renal disease) than any other ethnic group. African American women in particular have the highest prevalence of hypertension or high blood pressure. Hypertension increases risk for cardiovascular disease, stroke, and renal disease. According to the American Heart Association, more than 40% of adult non-Hispanic Blacks have high blood pressure. When compared to Caucasians, African Americans develop hypertension earlier in life and experience more severe outcomes. Given these statistics, research is needed that focuses on reducing hypertension-related health disparities among African Americans.

Studies have attempted to explain the phenomena of high blood pressure by investigating social, biological, and environmental determinants that may contribute to development of hypertension. Risk factors that have been studied include physical activity, salt sensitivity, body mass index (BMI), obesity, gene-environment interaction, stress, and racism and skin complexion. These factors have been researched to explain increased rates of hypertension in the African American community with the aim of reducing health disparities among this population. Although studies have examined independent effects of skin color based racism and discrimination and genetic effects on blood pressure outcomes, no studies have been found that examined the combinatorial effects of these variables on blood pressure in African Americans.

RACISM AND DISCRIMINATION

Studies have explored the physiological effects of perceived racism and discrimination among African Americans. Our study conceptualizes racism as beliefs, attitudes, or institutional arrangements that may disparage individuals or groups because of their outward appearance in terms of skin color or ethnic group affiliation. Discrimination, according to previous research, has been defined as unfair treatment and behavior based on ones’ race, ethnicity, sex, social and education status, or sexual preferences. For our study, discrimination is defined as unfair treatment based on racial or ethnic origins.

Studies have examined effects of perceived racism and discrimination in the psychological and physiological health status of African Americans. Exposure to racism and discrimination may account for some blood pressure-related health disparities among African Americans.

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Americans. In conceptualizing how racism and discrimination can influence health disparities among African Americans, we refer to the John Henryism hypothesis (ie, African Americans must cope with psychosocial stressors to overcome adversity, which in turn influences psychological and physiological well-being). Racism and discrimination have been shown to be chronic stressors among African Americans that can lead to elevated blood pressure readings. Although previous work has done illustrating a relationship between perceived racial discrimination and skin tone, there remains a gap in knowledge on genetic underpinnings that also may influence blood pressure. Our study examines the interactions of genetic mechanisms and self-rated skin color that may influence increases in blood pressure among African American women.

**Review of Literature**

**Genetics and Hypertension**

Genetic susceptibility and gene-environment interaction research is increasing among researchers who seek to identify precursors to the development of hypertension. Studies have shown that investigating traditional risk factors (e.g., obesity, sex, race and other lifestyle habits) are not sufficient to identify causative agents that can increase the likelihood of hypertension development. Large cohort studies, such as the Jackson Heart Study, the Atherosclerosis Risk in Communities Study (ARIC), and others included in the Family Blood Pressure Program (FBPP), investigated the important role of genetics and hypertension development. Genome-wide linkage testing was conducted to identify specific genes associated with hypertension development. Studies have found relationships between single nucleotide polymorphisms (SNPs) located on chromosome 2 and high blood pressure among African Americans. The SLC4A5 gene is found on chromosome 2 and has been linked with hypertension susceptibility in African Americans. SLC4A5 encodes a protein that transports sodium and bicarbonate across cell membranes while regulating Cellular pH. The gene is thought to contain several SNPs linked to elevated blood pressure. The SNPs investigated in our study are those that have been found most consistently with high blood pressure in previous studies among African Americans. Meanwhile, other researchers found evidence suggesting that SNPs on chromosome 11 also are related to hypertension among African Americans. Although genetic markers on chromosomes 2 and 11 have been identified and studied, no single gene or SNP has been identified that can consistently predict hypertension development.

**Health Disparities Associated with Racism and Discrimination among African Americans**

Research studies have examined racism and discrimination as risk factors in the development of hypertension and cardiovascular disease. Studies have found varied strengths of association between perceived discrimination and increased blood pressure and hypertension. Some researchers have found positive correlations between increased blood pressure and perceived racism and discrimination, while others concluded that no significant relationships existed among these variables. Mixed results from previous research made it difficult to determine the degree to which racism and discrimination play roles in the development of hypertension. Other researchers suggested another factor to consider (ie, an individual’s skin color may influence the perception of discrimination among African Americans).

**Skin Color**

Fair, light, medium, olive, and dark skin complexions/tones result from an admixture of African, Native American, European Hispanic, and Asian genes. Darker skin colors are believed to have stronger ancestral linkage to the African continent. At one time, skin color was the single most important phenotypic factor in ascribing social status in the United States and dictated where one could eat, sit, and live. Skin color long has been a social construct in the United States, placing those with diverse skin color gradients in different social and economic statuses. Discrepancies in studies have been found when biological skin pigmentation was measured by two different methods: reflectance spectrophotometry or socially.
defined skin color measured on a self-perceived scale. The use of reflective spectrophotometry supported the genetic explanation of the relationship between skin color and high blood pressure. The genetic explanation argued that darker skin had a significantly greater linkage to African lineage, while lighter skin was linked to European ancestry because of racial admixture. Meanwhile, self-perceived skin color accounted for the cultural significance explanation, which suggested that darker skin color was associated with greater exposure to racism/discrimination and chronic stress. These researchers used self-perceived skin color measurements and found that darker skin was associated with increased blood pressure. However, several studies used reflective spectrophotometry to measure skin color and reported no association between darker skin and increased blood pressure. Another study using the same procedure to measure skin color found a positive correlation between darker skin and increased blood pressure when controlling for low socioeconomic status. One study used both reflective spectrophotometry and self-perceived skin color measurements and concluded that measurements taken with reflective spectrophotometry provided no association with high blood pressure while measurements taken with self-perceived skin color ratings produced positive associations between darker-skinned individuals and higher blood pressure. Therefore, our study will use the method of self-perceived skin color in an examination of the gene environment interaction for high blood pressure in African American women.

METHODS

Participants

Our descriptive study included 137 African American women from the Detroit metropolitan area. Recruitment strategies commenced after approval from the Institutional Review Boards of University of Michigan and Wayne State University. To meet the inclusion criteria for the parent study, participants were required to self-identify as African American, with a living family of at least three generations constituting the triad of grandmother-mother-granddaughter. Although the women self-identified as African American, the recruiters were aware of the heterogeneity of Blacks in America that included, but was not limited to Blacks of mixed heritage. More detailed information on inclusion and exclusion criteria can be found in a published article outlining these procedures in depth. After agreeing to participate in the study, informed consent was obtained during home visits that served as the site for data collection. Research assistants were trained by the principal investigator regarding all data collection methods, home visitations, and coordinating visitations. Study participants were compensated with $20.00 gift certificates each time they participated in data collection. Findings of a power analysis indicated that a multiple regression with five variables requires 91 participants to generate a power of .80 at an alpha level of .05 with a moderate effect size.

Measures

Demographic Survey

The demographic survey obtained information from participants regarding age, educational level, household income, employment status, and extensive family history of hypertension (age at diagnosis, medications, and other treatments).

Skin Color

Skin color was measured using the skin color and discrimination questionnaire-DAS ‘95. The question and possible responses for skin color was, “Compared to most Black people, what shade of skin color do you have? Would you say: very dark brown, dark brown, medium brown, light brown or very light brown?” To assure adequate representation in the categories, women who answered very dark brown were combined with people who answered dark brown and women who answered very light brown were combined with people who answered light brown.

Blood Pressure

Blood pressure was measured using a digital blood pressure monitor with a size-appropriate upper arm cuff (model # A&D UA 767PC). Procedures for participant preparation for blood pressure measurement were in accordance with JNC-7 recommendations. Cutpoints for blood pressure readings were classified as: normotensive (≤120/80), pre-hypertensive (120/80 to 139/89), stage 1 hypertensive (140/90 to 159/99), and stage 2 hypertensive (≥160/100). For the purposes of this study, if participant blood pressure readings were in the stage 1 or stage 2 hypertensive range, they were considered hypertensive.

Body Mass Index

Weight was measured by an electronic scale (BWB/807 Tanita Tokyo, Japan). Height was measured by a portable stadiometer (Model 214 Road Rod, Seca Corporation, Hanover, Md.). Body mass index (BMI) of 25 to 29 was considered overweight, while BMI ≥30 was indicative of obesity.

Buccal Swab and Genotyping

Buccal swab saliva samples were collected at baseline from all participants by rubbing the swab on the inside of the cheek. DNA was isolated using the PureGene DNA Isolation Kit from Gentra Systems (Minneapolis, Minn.). Genotyping, based on polymerase chain reaction (PCR) amplification techniques, was conducted at the University of Michigan Molecular and Behavioral Sciences laboratory using the TaqMan assay and ABI Prism® Sequence Detection System (Applied Biosystems, Foster City, Calif.). Quality control measures for genotyping assays included robotic liquid handling, separate pre- and post-PCR areas, standard protocols, and quality control analyses, including 5%
duplicates, positive and negative controls, computerized sample tracking, and data validity checks. Genes were selected to represent biological pathways or positional candidate genes from systems known to be associated with hypertension and previously linked to hypertension.

**DATA ANALYSIS**

Data management, demographic statistics, linear regression model, and linear mixed model analyses were conducted by using R statistical language. To examine the main effects of each variable (skin color, and SNPs) on the multivariate-adjusted, residual phenotypes, we first performed linear regression analysis to obtain the residuals for systolic blood pressure and diastolic blood pressures. For each SNP, we conducted linear regression analysis to test the association between each SNP and the residuals of SBP and DBP. For rs6726450, G allele was associated with 10.29 mm Hg higher average SBP. For rs6731545, G allele was associated with 7.11 mm Hg lower DBP. The number of women diagnosed with hypertension was higher for those with dark skin color (n=21, 72.41%) than for women with either medium skin color (n=45, 62.50%) or light skin color (n=22, 61.11%). Among women who were being treated for hypertension, women with dark skin color had the lowest medication treatment percentage (n=11, 52.38%) compared to women with light skin color (n=12, 54.54%) and medium skin color (n=31, 68.89%).

Table 1 summarizes the main effects of skin color on SBP and DBP. Compared to women with dark skin color, women with medium skin color had 7.11 mm Hg lower DBP (P=.007). Women with light skin color had 5.6 mm Hg lower DBP compared to women with dark skin color. Although the P was at the borderline (P=.055), a larger sample is needed to examine this association in the future. While a 5.6 mm Hg may not be statistically significant, the influence of this difference may be clinically important. No associations were found between skin color and SBP.

Four SNPs in gene SLC4A5 on chromosome 2 were examined for their association with SBP and DBP. Two SNPs (rs6726450 and rs6731545) were found to be associated with both SBP and DBP. For rs6726450, G allele was associated with 10.29 mm Hg higher SBP (P=.001) and 3.77 mm Hg higher DBP (P=.037) compared to A allele. Also, for rs6731545, G allele was associated with 9.13 mm Hg lower SBP (P=.001) and 4.03 mm Hg lower DBP (P=.037) compared to A allele. No associations were found between two other SNPs (rs10177833 and rs8179526) and blood pressures.

The results of SNP and skin color interaction on systolic and diastolic blood pressure are presented in Table 2. Only one SNP (rs10177833) × skin color interaction was found to be associated with SBP. The additive effect of rs10177833 on SBP was statistically

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**RESULTS**

The mean age for the full sample was approximately 50.50 (SD=17.38) years. Women with medium skin color (53.65 [SD=18.04] years) were older than women with dark skin color (47.72 [SD=17.01] years) and light skin color (46.42 [SD=15.44] years). The mean BMI for women with light skin color (32.54 [SD=8.34] kg/m²) and medium skin color (32.50 [SD=7.70] kg/m²) were slightly higher than women with dark skin color (31.26 [SD=7.87] kg/m²). The average SBP was highest among women with dark skin color (141.01 [SD=20.96] mm Hg) followed by women with medium skin color (137.36 [SD=21.78] mm Hg) and light skin color (132.52 [SD=19.72] mm Hg). Similarly, women with dark skin color had the highest DBP (88.16 [SD=13.95] mm Hg), followed by women with light skin color (82.99 [SD=11.98] mm Hg) and medium skin color (80.83 [SD=11.24] mm Hg). The number of women diagnosed with hypertension was higher for those with dark skin color (n=21, 72.41%) than for women with either medium skin color (n=45, 62.50%) or light skin color (n=22, 61.11%). Among women who were being treated for hypertension, women with dark skin color had the lowest medication treatment percentage (n=11, 52.38%) compared to women with light skin color (n=12, 54.54%) and medium skin color (n=31, 68.89%).

Table 1. Linear mixed model analysis for skin color on predicting blood pressures after adjusting for age, BMI, and taking antihypertensive medication

<table>
<thead>
<tr>
<th>Skin color</th>
<th>n</th>
<th>β</th>
<th>SE</th>
<th>P</th>
<th>β</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>36</td>
<td>-8.21</td>
<td>4.84</td>
<td>.102</td>
<td>-5.60</td>
<td>2.87</td>
<td>.055</td>
</tr>
<tr>
<td>Medium</td>
<td>72</td>
<td>-6.54</td>
<td>4.3</td>
<td>.141</td>
<td>-7.11</td>
<td>2.53</td>
<td>.007*</td>
</tr>
<tr>
<td>Dark</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<.05.
Findings of our study supported the hypothesis that individuals with darker skin color had higher DBP readings than those with lighter skin tones.

different between women with dark skin color and women with medium skin color ($P=.015$; Table 3). This finding suggested that the C allele was the risk allele for women with dark skin color, but a protective allele among women with medium skin color. No SNP and skin color interaction was associated with blood pressure readings in other SNPs tested (rs8179526, rs6726450 and rs6731545).

## DISCUSSION

Consistent with previous research, findings of our study supported the hypothesis that individuals with darker skin color had higher DBP readings than those with lighter skin tones. Diastolic blood pressure has been shown to fluctuate in response to stress and increases in DBP were an important risk factor for development of hypertension and related cardiovascular morbidity and mortality. Stress as a result of racial discrimination based on skin color could lead to increases in blood pressure among African Americans, with results of this study supportive of those claims.9, 21

Although the expectation that women with darker skin color would have overall higher systolic and diastolic blood pressure readings was supported, other risk factors were not consistent with this hypothesis. Based on risk factors delineated by the American Heart Association for hypertension, women with the highest blood pressure readings were expected to be have other risk factors, including being older and having higher BMI readings.1 We found that women with darker skin color had the highest overall systolic and diastolic blood pressure readings, but were younger and had lower BMI readings when compared to women with medium or light skin color. These findings pointed to perceived stress discrimination based on skin color as a more potent risk factor for increased blood pressure than either age or BMI.

When examining the genetic relationship with blood pressure, our results were consistent with findings of previous studies that found SNP rs101778833 was significantly associated with increases in SBP among African American and West African women.3, 42 These results are important to note because rs101778833 was related to increases in SBP in West

### Table 2. Linear mixed model analysis for each SNP on predicting blood pressures after adjusting for age, age², BMI, and taking antihypertensive medication

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>Coded allele</th>
<th>Chromosome position</th>
<th>Systolic blood pressure</th>
<th>Diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6726450</td>
<td>.20</td>
<td>G</td>
<td>74302810</td>
<td>β: 10.29, SE: 2.81, P: .001*</td>
<td>β: 3.77, SE: 1.77, P: .037b</td>
</tr>
<tr>
<td>rs6731545</td>
<td>.17</td>
<td>G</td>
<td>74321754</td>
<td>β: -9.13, SE: 3.08, P: .004*</td>
<td>β: -4.03, SE: 1.87, P: .035b</td>
</tr>
<tr>
<td>rs8179526</td>
<td>.47</td>
<td>T</td>
<td>74323287</td>
<td>β: 1.28, SE: 2.6, P: .624</td>
<td>β: .03, SE: 1.57, P: .984</td>
</tr>
</tbody>
</table>

*P<.005.  
bP<.05.

### Table 3. Linear mixed model analysis with Interactions of SNP × skin color on predicting blood pressures after adjusting for age, age², BMI, and taking antihypertensive medication

<table>
<thead>
<tr>
<th>SNP</th>
<th>Skin color =light β (SE)</th>
<th>Skin color =medium β (SE)</th>
<th>SNP by skin color =light β (SE)</th>
<th>SNP by skin color =medium β (SE)</th>
<th>Outcome: systolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6726450</td>
<td>-13.13(18.20)</td>
<td>-1.92(16.88)</td>
<td>9.50(8.50)</td>
<td>4.83(10.28)</td>
<td>1.67(9.22)</td>
</tr>
<tr>
<td>rs6731545</td>
<td>-3.75(16.09)</td>
<td>6.57(14.80)</td>
<td>-3.30(7.41)</td>
<td>-3.16(9.35)</td>
<td>-7.83(8.44)</td>
</tr>
<tr>
<td>rs10177833</td>
<td>1.72(7.57)</td>
<td>9.03(7.18)</td>
<td>6.75(5.27)</td>
<td>-11.14(6.68)</td>
<td>-15.09(6.05)</td>
</tr>
<tr>
<td>rs8179526</td>
<td>-5.04(10.47)</td>
<td>-5.60(9.54)</td>
<td>1.85(6.47)</td>
<td>-2.83(8.18)</td>
<td>-6.07(7.17)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SNP</th>
<th>Skin color =light β (SE)</th>
<th>Skin color =medium β (SE)</th>
<th>SNP by skin color =light β (SE)</th>
<th>SNP by skin color =medium β (SE)</th>
<th>Outcome: diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6726450</td>
<td>6.96(17.46)</td>
<td>15.60(16.06)</td>
<td>9.14(5.27)</td>
<td>-3.98(6.35)</td>
<td>-8.03(5.71)</td>
</tr>
<tr>
<td>rs6731545</td>
<td>-4.98(15.03)</td>
<td>-24.08(14.08)</td>
<td>-7.35(4.46)</td>
<td>-5.75(5.59)</td>
<td>6.18(5.15)</td>
</tr>
<tr>
<td>rs10177833</td>
<td>-4.15(8.15)</td>
<td>-6.23(7.85)</td>
<td>-1.73(3.29)</td>
<td>-8.64(4.10)</td>
<td>-3.23(8.22)</td>
</tr>
<tr>
<td>rs8179526</td>
<td>.32(10.91)</td>
<td>-4.45(9.69)</td>
<td>.04(3.66)</td>
<td>-2.90(5.00)</td>
<td>-1.37(4.28)</td>
</tr>
</tbody>
</table>

*aP<.05.
African women who tended to have darker skin color and lower BMI. It is not clear if stress related to discrimination based on skin color was experienced in West Africa as in the United States, but these similarities in genetic risks for hypertension are interesting and provide impetus for further study.

The SNP rs101778833 is part of the SLC4A5 gene located on chromosome 2. This particular gene had been implicated in risk for hypertension among African Americans in previous studies, and our findings also are supportive of previous work. However, results of our study are novel in that they present the interaction of genetic effects and skin color on changes in blood pressure. Our study revealed that rs101778833 and darker skin color together were associated with higher SBP readings. Although women with darker skin color were younger and had lower BMI than the other women in the study, their BMI readings were still in the obese category, which is a major risk factor for hypertension development. These findings of an association between genetics, skin color, and blood pressure is important when considering appropriate diagnostic and treatment plans for African American women with hypertension. African American women with darker skin color may require further assessment for risk factors, such as discrimination-related stress (if identified by a patient as an issue) when being seen by health professionals for hypertension. Health providers should be aware that women with darker skin color also may need to be assessed for hypertension at earlier ages than other patients due to their particular genetic and environmental (skin color) risks for this chronic disease, particularly when they indicate experiences of racism or discrimination related stress related to their skin color. Additional studies on genetic and skin color interactions for hypertension with larger samples are needed before conclusions can be made on the causative nature of these results.

Implications for Practice

Although the results indicated that the 5.6 mm Hg difference in DBP was not statistically different, it is important to understand that it may be clinically significant. For instance, a patient who presents with a repeated DBP reading of 90 would be considered hypertensive, while a patient with a DBP reading of 5.6 mm Hg less (84.4) would not. The differences between these two diastolic blood pressure readings in patients would prompt quite different diagnoses and treatment regimens in the clinical setting. Referral for follow-up genetic testing for hypertension related risk alleles would also differ based on these clinical presentations.

Another area of concern clinically would be the impression that patients are being racially profiled for certain diseases based on their skin color. Practitioners who are aware that certain types of disorders (eg, sickle cell, cystic fibrosis) can be race related are not racial profiling patients when suggesting tests for these disorders. The same logic would apply when testing for high blood pressure in African Americans with darker skin tones that express stress related to perceived discrimination based on their color. Health care providers, regardless of color, should not deny testing and diagnosis for anyone for fear of being accused of racial profiling. They should suggest any testing that could increase the likelihood of appropriate diagnoses.

Limitations

Several limitations need to be considered for this study. Data used for this study had been collected as part of a larger parent study. The study was limited to 137 parent study participants who had participated in a larger hypertension genetics study. The study was limited to African American women who were living in a large metropolitan area in the Midwest. African American women who live in suburban or rural areas may have different outcomes and experiences than those who live in urban areas. African American men may have different outcomes than the women included in this study. Due to budgetary constraints we did not perform ancestry informative markers on the participants in the sample. We are aware that population admixture exists in the African American population and can be a confounder of the genetic effects. The use of subjective measures (eg, self reported skin color) may have areas of bias that could affect study outcomes. Participants in the study may have given socially acceptable responses (rated themselves as lighter or darker skin color) instead of providing accurate accounts of this variable. It is possible that multiple rare polymorphisms in the biological and positional candidate genes that were not studied could have influenced blood pressure. Despite these limitations, the approach employed in our study with an adequate sample size illustrated the use of SNPs in candidate genes to construct a more complete picture of gene-environment (skin color) interaction for hypertension.

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8. Williams DR, Sun YV, Darling, Sun, Kardia, Jackson


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**Acquisition of funding:** Taylor, Sun, Kardia, Jackson

**Administrative:** Taylor, Darling, Sun, Kardia, Jackson

**Supervision:** Taylor, Sun, Kardia

**G E N E R A L V I S I O N S , S K I N C O L O R , A N D B L O O D P R E S S U R E - T a y l o r e t a l**

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