Genetic Ancestry Is Associated With Measures of Subclinical Atherosclerosis in African Americans

The Jackson Heart Study


Objective—To determine whether genetic ancestry was associated with subclinical atherosclerosis measures after adjustment for traditional cardiovascular disease risk factors, inflammatory marker, socioeconomic status, and psychosocial factors in a large admixed African American population.

Approach and Results—Participants were drawn from the Jackson Heart Study. Participant’s percent of European ancestry (PEA) was estimated based on 1747 genetic markers using HAPMIX. Association of PEA with peripheral arterial disease and common carotid intima-media thickness were investigated among 2168 participants and with coronary artery calcification >0 and abdominal aortic calcification >0 among 1139 participants. The associations were evaluated using multivariable regression models. Our results showed that a 1 SD increase in PEA was associated with a lower peripheral arterial disease prevalence after adjusting for age and sex (prevalence ratio=0.90 [95% CI, 0.82–0.99]; \(P=0.036\)). Adjustments for traditional cardiovascular disease risk factors, socioeconomic status, and psychosocial factors attenuated this association (prevalence ratio=0.91 [0.82–1.00]; \(P=0.046\)). There was also a nonlinear association between PEA and coronary artery calcification and abdominal aortic calcification. The lowest PEA was associated with a lower coronary artery calcification (prevalence ratio=0.75 [0.58–0.96]; \(P=0.022\)) and a lower abdominal aortic calcification (prevalence ratio=0.80 [0.67–0.96]; \(P=0.016\)) compared with the reference group (10th–90th percentile) after adjusting for traditional cardiovascular disease risk factors, inflammatory marker, socioeconomic status, and psychosocial factors. However, we found no significant association between PEA and common carotid intima-media thickness.

Conclusions—Overall, our findings indicate that genetic ancestry was associated with subclinical atherosclerosis, suggesting unmeasured risk factors and interactions with genetic factors might contribute to the distribution of subclinical atherosclerosis among African Americans. (Arterioscler Thromb Vasc Biol. 2015;35:1271-1278. DOI: 10.1161/ATVBAHA.114.304855.)

Key Words: African Americans ■ cardiovascular disease ■ epidemiology ■ genetic ancestry ■ subclinical atherosclerosis

Subclinical atherosclerosis measures, including peripheral arterial disease (PAD), carotid intima-media thickness (CIMT), coronary artery calcification (CAC), and abdominal aortic calcification (AAC) have been shown to be strong predictors of cardiovascular disease (CVD) events in numerous studies. Several studies have reported substantial racial/ethnic differences in prevalence of subclinical atherosclerosis between African Americans and European Americans. Despite a worse CVD risk factors profile compared with European Americans, African Americans have substantially lower prevalence of CAC and AAC. In contrast, African Americans have relatively higher mean of CIMT and prevalence of PAD than European Americans.

Previous epidemiological studies have demonstrated that traditional CVD risk factors do not fully explain these differences. Other studies have also suggested that these race/ethnic differences are only partially explained by differences in socioeconomic status (SES), psychosocial factors as well as access to healthcare. These findings highlight the potentially important role of ancestry-related genetic factors in the development of subclinical atherosclerosis. However, the association of genetic ancestry with subclinical atherosclerosis is not fully understood probably because most of these studies relied on self-reported race/ethnicity or skin reflectance which are measurements that do not take into account for genetic heterogeneity within each racial/ethnic group; ignoring such heterogeneity can confound the contributions of genetic, SES, or environmental factors. This problem is particularly relevant in African Americans who are...
among the most genetic and culturally heterogeneous group in the United States, with mixed ancestry mainly from West Africa and Europe. Recent advances in genomics research have allowed to precisely estimate individual genetic ancestry using ancestry informative markers or a large set of random markers. Genetic ancestry can be a useful tool in determining whether ancestry-related genetic factors or nongenetic factors driving the disparities in subclinical atherosclerosis. Furthermore, genetic ancestry associations can be used as a precursor to admixture mapping for uncovering genes that contribute to subclinical atherosclerosis.

However, only a limited number of studies examined the association between genetic ancestry and subclinical atherosclerosis in African Americans and they reported mixed results. One study reported significant associations of European genetic ancestry with CAC and common CIMT among self-reported African Americans. In contrast, another study which involved younger participants found no significant association of African ancestry with CAC. Reiner et al. also found that African ancestry was not significantly associated with cCIMT among elderly African Americans. Potential reasons for these mixed findings, in addition to age differences, include the use of relatively small sample sizes of African Americans and relatively small number of ancestry informative markers to accurately estimate genetic ancestry. Furthermore, only 1 study has investigated the association of genetic ancestry with PAD in African Americans and reported an inverse but nonsignificant association. and no study looked at the relationship between AAC and genetic ancestry. In addition, some of these studies have not accounted for a range of nongenetic risk factors, including SES and psychosocial factors. Therefore, this study attempts to improve our understanding whether ancestry-related genetic factors play an important role in subclinical atherosclerosis by overcoming some of the limitations of previous studies, replicating earlier analysis with a large sample of African American while controlling for a wider range of nongenetic risk factors, inflammatory marker as well as SES and psychosocial factors.

**Materials and Methods**

Materials and Methods are available in the online-only Data Supplement.

Table 1 shows comparisons of baseline demographic and clinical characteristics of JHS participants across the percent of European ancestry (PEA) levels. Among the 2168 self-identified African American participants, the average age was 54 and 39% were men. On average, the participants had a median of 16% European ancestry (interquartile range=12%–22%). Across the levels of PEA, participants were similar in sex; prevalence of diabetes mellitus; smoking; and levels of low-density lipoprotein, high-density lipoprotein, and triglycerides (all P>0.05). However, those in the highest levels of PEA (>90% PEA) had lower prevalence of hypertension, lower mean body mass index, mean systolic blood pressure, mean high-sensitivity C-reactive protein, mean cCIMT, and mean age (all P<0.05). Furthermore, those in the highest levels of PEA had higher income and higher education compared with those in the lowest level of PEA (both P<0.001), but none of the psychosocial variables were significant.

### Association Between Percent of European Ancestry and PAD

Table 2 presents the prevalence ratio (PR) and 95% confidence interval for PAD per SD increase in PEA. The restricted cubic spline analysis indicated that the association between PEA and PAD was approximately linear (Figure [A]). PEA was significantly and inversely related with PAD as shown in Table 2. One SD (9%) increase in PEA was associated with significantly lower PAD after adjusting for age and sex (model 1: PR=0.90 [95% confidence interval, 0.82–0.99]; P=0.036). This association remained significant after adjusting for traditional CVD risk factors and inflammatory markers (model 2: PR=0.90 [0.82–0.99]; P=0.024), but adjustment for SES and psychosocial factors alone attenuated the association which became nonsignificant (model 3: PR=0.92 [0.83–1.01]; P=0.082). However, the association remained marginally significant in the fully adjusted model (model 4: PR=0.91 [0.82–1.00]; P=0.046). In sensitivity analyses, changing the ankle-brachial index cut point to <0.9, PAD was inversely associated with PEA in a similar pattern, but the associations were not statistically significant (Table I in the online-only Data Supplement).

### Association Between Percent of European Ancestry and cCIMT

Table 3 summarizes the mean differences and SE for cCIMT by categories of PEA. The restricted cubic spline analysis indicated that the relationship between PEA and cCIMT was approximately nonlinear as shown in the Figure (B). PEA was not significantly associated with cCIMT. In the model where we adjusted for age and sex (model 1), participants with the lowest (<10th percentile) and the highest PEA (>90th percentile) had a lower mean cCIMT (mean differences=−0.021 [SE=0.014]; P=0.149 and −0.026 [SE=0.014]; P=0.118) compared with participants within the reference group (10th–90th percentile). However, the association remained nonsignificant after adjusting for traditional CVD risk factors, inflammatory marker, SES, and psychosocial factors.
Table 1. Characteristics of Participants by Levels of PEA: The Jackson Heart Study (2000–2004)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>&lt;10th Percentile (n=202)</th>
<th>10th–90th Percentile (n=1749)</th>
<th>&gt;90th Percentile (n=217)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA, %, median (IQR)</td>
<td>6.7 (5.7–7.8)</td>
<td>15.6 (12.3–20.0)</td>
<td>35.1 (31.5–41.6)</td>
<td>…</td>
</tr>
<tr>
<td>Age, y (mean±SD)</td>
<td>58±7.13.0</td>
<td>53.0±12.4</td>
<td>57.9±12.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>75 (37.1)</td>
<td>665 (38.0)</td>
<td>91 (41.9)</td>
<td>0.500</td>
</tr>
<tr>
<td>CVD risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>24 (11.9)</td>
<td>240 (13.7)</td>
<td>22 (10.1)</td>
<td>0.287</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>146 (72.28)</td>
<td>1049 (60.0)</td>
<td>130 (59.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>28 (13.9)</td>
<td>263 (15.0)</td>
<td>32 (14.8)</td>
<td>0.904</td>
</tr>
<tr>
<td>Systolic BP, mmHg (mean±SD)</td>
<td>130.4±18.6</td>
<td>125.9±17.7</td>
<td>126.3±16.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic BP, mmHg (mean±SD)</td>
<td>79.1±10.3</td>
<td>79.6±10.5</td>
<td>77.8±9.5</td>
<td>0.056</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL, median (IQR)</td>
<td>92.0 (13.0)</td>
<td>91.0 (15.0)</td>
<td>92.0 (13.0)</td>
<td>0.637</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL (mean±SD)</td>
<td>52.2±14.2</td>
<td>51.5±14.3</td>
<td>51.4±15.4</td>
<td>0.590</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL (mean±SD)</td>
<td>124.6±38.8</td>
<td>125.6±36.3</td>
<td>125.5±34.3</td>
<td>0.904</td>
</tr>
<tr>
<td>Triglycerides, mg/dL, median (IQR)</td>
<td>85.0 (61.0)</td>
<td>89.0 (59.0)</td>
<td>95.0 (69.0)</td>
<td>0.117</td>
</tr>
<tr>
<td>Body mass index, kg/m² (mean±SD)</td>
<td>31.6±7.1</td>
<td>31.7±7.1</td>
<td>30.1±6.4</td>
<td>0.005</td>
</tr>
<tr>
<td>hsCRP, mg/dL, median (IQR)</td>
<td>0.24 (0.39)</td>
<td>0.26 (0.46)</td>
<td>0.21 (0.35)</td>
<td>0.019</td>
</tr>
<tr>
<td>Antihypertension med use, n (%)</td>
<td>118 (60.2)</td>
<td>819 (49.3)</td>
<td>104 (50.7)</td>
<td>0.016</td>
</tr>
<tr>
<td>SES and psychosocial factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education: ≥bachelor degree, n (%)</td>
<td>73 (36.1)</td>
<td>717 (41.0)</td>
<td>119 (54.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Income: ≥$40,000/y, n (%)</td>
<td>83 (41.1)</td>
<td>886 (50.7)</td>
<td>138 (63.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Perceived global stress (mean±SD)</td>
<td>1.1±0.98</td>
<td>1.2±0.99</td>
<td>1.2±1.03</td>
<td>0.522</td>
</tr>
<tr>
<td>Every day discrimination (mean±SD)</td>
<td>2.0±1.1</td>
<td>2.0±0.97</td>
<td>2.0±0.91</td>
<td>0.785</td>
</tr>
<tr>
<td>Lifetime discrimination (mean±SD)</td>
<td>1.4±1.0</td>
<td>1.4±1.0</td>
<td>1.5±1.0</td>
<td>0.564</td>
</tr>
<tr>
<td>Subclinical atherosclerosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAD, n (%)</td>
<td>40 (19.8)</td>
<td>258 (14.8)</td>
<td>28 (12.9)</td>
<td>0.107</td>
</tr>
<tr>
<td>cCIMT, mm, median (IQR)</td>
<td>0.73 (0.21)</td>
<td>0.69 (0.22)</td>
<td>0.71 (0.21)</td>
<td>0.009</td>
</tr>
<tr>
<td>CAC, n (%)</td>
<td>35 (38.9)</td>
<td>390 (42.3)</td>
<td>63 (60.0)</td>
<td>0.188</td>
</tr>
<tr>
<td>AAC, n (%)</td>
<td>49 (54.4)</td>
<td>550 (59.5)</td>
<td>75 (59.5)</td>
<td>0.636</td>
</tr>
</tbody>
</table>

AAC indicates abdominal aortic calcification; BP, blood pressure; CAC, coronary artery calcium; cCIMT, common carotid intima-media thickness; CVD, cardiovascular disease; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; PAD, peripheral arterial disease; PEA, percent of European ancestry; and SES, socioeconomic status.

*P value by ANOVA, χ², or Kruskal–Wallis as appropriate.

Association Between Percent of European Ancestry and CAC

Table 3 summarizes the PR and 95% confidence interval for CAC and AAC prevalence by categories of PEA. The restricted cubic spline analysis indicated that the associations of PEA with CAC and AAC were nonlinear and fitted strongly at the lowest levels of PEA (Figure [C and D]). We found a significant association between CAC and PEA; participants with the lowest level of PEA (<10th percentile) had significantly lower CAC prevalence (PR=0.78 [0.61–0.99]; P=0.041) compared with participants within the reference group (10th–90th percentile) after adjusting for age and sex (model 1). Adjustment for traditional CVD risk factors and inflammation marker further strengthened the association of PEA with CAC (model 2: PR=0.77 [0.61–0.98]; P=0.036). This association was also significant when we adjusted for SES and psychosocial risk factors (model 3: PR=0.76 [0.59–0.98]; P=0.033). In the fully adjusted model, the association remained strong and significant (model 4: PR=0.76 [0.58–0.96]; P=0.022). A similar pattern of association was observed with AAC (Table 3).

Discussion

In this study of a large sample of African Americans, we found evidence that genetic ancestry is associated with measures of subclinical atherosclerosis. We also found European ancestry was inversely associated with prevalence of PAD in African Americans. This association was attenuated, but remained marginally significant when we fully adjusted for traditional CVD risk factors, inflammatory marker, SES, and psychosocial factors. We also identified significant nonlinear associations of PEA with CAC and AAC, which shows that participants with the lowest European ancestry had a lower prevalence of CAC and AAC. Adjustment for traditional CVD risk factors, inflammatory marker, SES, and psychosocial factors did not explain the observed associations. The apparent nonlinear associations of PEA with CAC and AAC suggest that unmeasured risk factors and interaction with genetic factors could play a more important role than genetic effects in this sample of African Americans. However, we did not find significant association between genetic ancestry and cCIMT. Overall, our findings suggest that unmeasured risk factors and...
ancestry-related genetic differences may contribute to the distribution of subclinical atherosclerosis in African Americans.

Few prior studies have examined the associations between genetic ancestry and subclinical atherosclerosis in African Americans. Although nonsignificant, Allison et al.29 observed inverse association between European ancestry and PAD among 712 African Americans of the Multi-Ethnic Study of Atherosclerosis (MESA); in contrast, in our study, this association was marginally significant after adjusting for traditional CVD risk factors, inflammatory marker, SES, and psychosocial factors. Our findings are also in line with previous studies that reported a significant association between European ancestry and CAC.26,30 For example, Wassel et al reported that PEA was linearly, positively associated with the prevalence of CAC among 712 African Americans in the MESA study, suggesting genetic components to CAC in African Americans. Unlike their findings, however, our results showed a dose–response relationship between PEA and CAC such that only participants with the lowest level of PEA had significantly lower CAC prevalence, indicating unknown risk factors and gene–environment interactions could play a more important role than genetic factors in this cohort. We did not observe a significant association between cCIMT and European ancestry in our study, which is similar to 1 study that found no association between African ancestry and cCIMT among elderly African Americans of the Cardiovascular Health Study (CHS).27 However, another study reported a linear, negative association between PEA and cCIMT independent of CVD risk factors and SES in African Americans of the MESA study, suggesting genetic influence on cCIMT.26 It is possible that differences in regional origin, sample size, estimation of ancestry, and methodological approaches in the measurement of cCIMT could have attributed to the discrepancies between these studies.26,27,31 Our study was also the first to look at PEA and AAC and displayed a similar pattern of association with those of CAC. There is no literature on genetic ancestry and AAC with which to compare our results, but our findings are in line with 1 study that reported a lower prevalence of AAC in African Americans than that in whites using self-reported race/ethnicity.7 In aggregate, the current and these previous studies provide evidence in support to the ancestry-related differences in the distribution of subclinical atherosclerosis within African Americans.

Explanations for the differences in associations between PEA and the different subclinical atherosclerosis measures remain unclear. However, these findings indicate that distinct pathophysiological processes or risk factors might be involved in different ethnic groups for the different atherosclerosis measures, which might explain some of the observed differences by genetic ancestry.5,12,18,32,33 Evidence suggests that hypertension, diabetes mellitus, and obesity are more prevalent in African Americans or in those with low PEA and that they are strong and independent risk factors for PAD and cCIMT.12,32,33

Our results showed that association between PEA and PAD remained significant after adjusting for these CVD risk factors and inflammatory marker. This association attenuated, but persisted after additional adjustment for SES and psychosocial factors. This suggests that unmeasured risk factors may play a role in the pathogenesis of PAD. Several studies have found higher level of novel risk factors, such as interleukin-6, fibrinogen, d-dimer, and homocysteine, were associated with increased odds of PAD among African Americans.12,33–35 The same studies have also indicated that traditional or novel CVD risk factors do not entirely explain the high prevalence of PAD in African Americans, suggesting genetic components to the development of PAD.12,32–35 In fact, a recent study found a novel region in chromosome 11 that was associated with high PAD in African Americans.36 Thus, it is probably the interplay of traditional CVD and novel risk factors, SES, and genetic factors could explain the differences in PAD among African Americans.

Although African Americans have worse CVD risk factor profiles, we observed significantly lower prevalence of CAC and AAC in African Americans with the lowest PEA even after adjustment for traditional CVD risk factors, inflammatory marker, SES, and psychosocial factors. This finding is consistent with a large body of evidence reporting that standard CVD risk factors do not fully explain the ancestry-related differences in CAC and AAC.5,26,35 It is highly possible that the observed differences in CAC and AAC could be because of nongenetic factors that were not accounted for in our study. Several previous studies have indicated that unmeasured risk factors, such as bone density, vitamin D, calcium metabolism, and other environmental factors, might play an important role in the development of CAC and AAC.5,26,37–40 Indeed, bone density has been found to be inversely related with CAC; African Americans have higher levels of bone density than whites.38 Other studies have also highlighted the association of lower vitamin D with CAC.5,36,40 Alternatively, it is also possible that genetic factors may contribute to CAC in African Americans.26,30,41 For example, recent admixture mapping of CAC in African Americans identified seven genomic regions in which excess European ancestry was associated with higher levels of CAC.41 Previous studies have also identified that the 9p21 region was associated with CAC in predominantly European-derived populations.42,43 Taken together, the apparent lower level of CAC and AAC in participants with the lowest PEA in our study indicates the importance of other unmeasured risk factors and gene by environment interactions than genetic effects.26,37,41

### Table 2. PR and 95% CI of PAD per SD Increase in PEA Among African Americans: The Jackson Heart Study (2000–2004)

<table>
<thead>
<tr>
<th>PEA</th>
<th>PR (95% CI)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: demographic</td>
<td>0.90 (0.82–0.99)</td>
<td>0.036</td>
</tr>
<tr>
<td>Model 2: traditional CVD factors</td>
<td>0.90 (0.82–0.99)</td>
<td>0.024</td>
</tr>
<tr>
<td>Model 3: SES and psychosocial factors</td>
<td>0.92 (0.83–1.01)</td>
<td>0.082</td>
</tr>
<tr>
<td>Model 4: fully adjusted</td>
<td>0.91 (0.82–1.00)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

PAD defined as ankle-brachial index ≤0.99. SD, 9.0%. Model 1, adjusted for demographic: age and sex; model 2, adjusted for model 1 and traditional CVD risk factors: body mass index, current smoking, diabetes mellitus, hypertension, fasting plasma glucose, triglycerides, low-density lipoprotein cholesterol, and high-sensitivity C-reactive protein; model 3, adjusted for model 1 and SES and psychosocial factors: household annual income, education, perceived global stress, and every day, and lifetime discrimination; and model 4, adjusted for all the covariates.

CI Indicates confidence interval; CVD, cardiovascular disease; PAD, peripheral arterial disease; PEA, percent of European ancestry; PR, prevalence ratio; and SES, socioeconomic status.
Our study has some limitations which should be taken into consideration when interpreting the results. First, although we adjusted for several known risk factors of subclinical atherosclerosis, it is possible that residual confounding may have affected our results. Future research is needed to determine whether unmeasured risk factors and gene–environment interactions can help account for the observed associations in African Americans. Second, our sample came from a single site of Jackson, MS, may not be generalizable to the general population of African Americans across the United States. Furthermore, the PEA in our study was skewed toward lower values and few participants had ≥25% European ancestry, thus, we were unable to examine the full range of PEA associations with subclinical atherosclerosis measures. Fourth, the time lag between evaluation of some of the baseline covariates (eg, SES, current smoking, and psychosocial factors) and measures of CAC and AAC in Examination 2, which could have influenced the associations of PEA with CAC and AAC. Finally, the cross-sectional design of our study limits our ability to draw conclusions whether genetic ancestry predicts subclinical atherosclerosis independent of nongenetic factors. Future studies are warranted to replicate our findings, perhaps using larger sample sizes of African Americans, with a full range of ancestry to overcome any chance findings might present in our analyses.

Despite these limitations, our study used a large well-characterized sample of African Americans and hence had greater statistical power than prior studies that examined the association of PEA with subclinical atherosclerosis. Moreover, PEA in our study was estimated based on a large number of ancestry informative markers, which minimized the errors associated with estimating genetic ancestry compared with prior studies. Our study also benefits from the state-of-the-art measures of subclinical atherosclerosis and availability of a wide range of important confounding covariates for adjustment.
Atherosclerosis processes. Furthermore, our findings provide background for pursuing disparities in subclinical atherosclerosis. Additionally, our study is also the first to show the association of AAC with genetic ancestry. Finally, our study significantly contributes to the limited body of literature on genetic ancestry and subclinical atherosclerosis among African Americans, which often used skin reflectance as a surrogate for genetic ancestry or not enough large number of ancestry informative markers to determine ancestry with precision.18,19,27

Conclusions
In conclusions, genetic ancestry was associated with subclinical atherosclerosis in a large sample of African Americans. We found significantly lower prevalence of CAC and AAC in participants with the lowest European ancestry, which seems not fully explained by traditional CVD risk factors, SES, and psychosocial factors. We also found that greater European ancestry was associated with lower prevalence of PAD although the association was attenuated after adjusting for SES and psychosocial factors. Our findings suggest that unmeasured risk factors and interacting with genetic determinants might play an important role to the distribution of subclinical atherosclerosis in African Americans. Future studies are needed to validate these findings and explore gene–environment interactions to better understand the complex biological mechanisms and to reduce ancestry-related disparities in subclinical atherosclerosis.

Acknowledgments
We thank the investigators, the staff, interns, and the participants of the Jackson Heart Study for their long-term commitment and valuable contributions to the study. We also thank Drs Jose Vargas and Amadou Gaye for their comments on the final draft of the article.

Sources of Funding
This research is supported by Intramural Program of National Human Genomics Institute, National Institutes of Health. The Jackson Heart Study is supported by contracts HHSN268201300046C, HHSN268201300047C, HHSN268201300048C, HHSN268201300049C, and HHSN268201300050C from the National Heart, Lung, and Blood Institute and the National Institute on Minority Health and Health Disparities.

Disclosures
None.

References


African Americans are at greater risk for developing peripheral arterial disease and common carotid intima-media thickness, but at lower risk for developing coronary artery calcification and abdominal aortic calcification relative to whites. It is unclear whether genetic or nongenetic factors are driving these differences. African Americans are the most genetically heterogeneous group in the United States, with varying proportion of African and European ancestry. We evaluated the associations between percent of European ancestry and subclinical atherosclerosis in a large sample of admixed African Americans. Our results demonstrated that a higher percent of European ancestry was associated with a lower peripheral arterial disease prevalence whereas the lowest European ancestry was associated with a lower coronary artery calcification and abdominal aortic calcification prevalence. Adjustment for the traditional and social CVD risk factors reduced the percent of European ancestry–peripheral arterial disease associations whereas it further strengthened the associations of percent of European ancestry with coronary artery calcification and abdominal aortic calcification. These findings suggest that unmeasured risk factors and their interactions with genetics might play an important role in the distribution of subclinical atherosclerosis in African Americans.
Genetic Ancestry Is Associated With Measures of Subclinical Atherosclerosis in African Americans: The Jackson Heart Study
Samson Y. Gebreab, Pia Riestra, Rumana J. Khan, Ruihua Xu, Solomon K. Musani, Fasil Tekola-Ayele, Adolfo Correa, James G. Wilson, Charles N. Rotimi and Sharon K. Davis

Arterioscler Thromb Vasc Biol. 2015;35:1271-1278; originally published online March 5, 2015; doi: 10.1161/ATVBAHA.114.304855

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/35/5/1271

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2015/03/05/ATVBAHA.114.304855.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Materials and Methods

The JHS is a population-based study that was developed to understand the causes of cardiovascular disease (CVD) in African Americans. Between September 2000 and March 2004, 5,301 non-institutionalized African-American adults between the ages of 21-94 were recruited from the Jackson, Mississippi tri-county (Hinds, Madison, and Rankin counties) metropolitan area (Jackson MSA) using four recruitment pools: random sampling (17%), volunteer enrollees (30%), enrollees from the Atherosclerosis Risk in Communities (ARIC) Study (30%), and from family members (23%). Details of the study design and data collection methods are described elsewhere.1-3 A total of 2946 JHS participants gave informed consent for DNA extraction and had information on proportion of European genetic ancestry through the Candidate-Gene Association Resource (CARe) Study.4 After excluding missing information on subclinical atherosclerosis measures and covariates, the analysis samples included 2168 for PAD and CIMT outcomes, and 1039 for CAC and AAC outcomes.

Subclinical atherosclerosis measures

In this study, the primary outcome is subclinical atherosclerosis measures, including peripheral arterial disease (PAD), carotid intima media thickness (CIMT), coronary artery calcification (CAC) and abdominal aortic calcification (AAC). PAD was defined using baseline Exam 1 measures of Ankle Brachial Index (ABI). ABI, the ratio of the blood pressure in the lower legs to the blood pressure in the arms, was measured by trained technicians using a sphygmomanometer along with an Ultrasonic Doppler Flow Detector, Model 811-B by Parks Medical Electronic-Inc., Aloha, Oregon U.S.A.5 Two ABIs (one for the right leg and one for the left leg) were calculated as the average of the two ankles systolic BP measurements divided by the average of the two brachial readings. The lowest of the two ABIs was considered the ABI for the participant for the current study. In our analysis, the presence of PAD was defined as ABI equal to or below 0.99. We used this ABI-cut point because an ABI <=0.99 has been shown to be significantly associated with higher levels of CVD risk factors, as well as subclinical atherosclerosis and incident CVD.6-7 Sensitive analysis was also performed with PAD defined as ABI below < 0.90.

CIMT was measured at baseline Exam 1 from ultrasound examination of the left and right carotid arteries at the common, bifurcation, and internal sites using a Hewlett Packard Sonos 4500 ultrasound imaging device.5 Three circumferential views were assessed at the common and bifurcation segments (anterior, lateral, and posterior). A single view was obtained at the internal segment. For the analysis, we used the estimate based on the maximum likelihood of the average right and left common carotid far wall and referred to it as cCIMT. We examined cCIMT as continuous outcomes. Since the distribution of cCIMT was right skewed, the values were log-transformed.

CAC and AAC were measured at Exam 2 using non-enhanced cardiac computed tomography scans. The complete methodology for cardiac gated CT Scans of the coronary arteries has been reported elsewhere.5 Briefly, CT images were read by experienced and trained technologists at the JHSCT Data Acquisition center (Jackson, Mississippi, US). Quality control and image analysis were performed at a core reading center (Wake Forest University school of Medicine, Winston-Salem, NC, USA). CAC and AAC were quantified utilizing Agatston scoring and modified to account for slice
thickness. The total CAC score is the sum of the score of the left main, left circumflex, left anterior descending and right coronary artery, and the total AAC score is the sum of the infrarenal abdominal aorta, left common iliac and right common iliac arteries. The reproducibility for CAC and AAC was 0.99. The presence of CAC and AAC were defined as Agatston score > 0.

**Covariates**

Risk factors and covariates were measured at Exam 1 (2000-2004) and Exam 2 (2005-2008). Demographic factors (age [years] and sex [male/female]) and risk factors (body mass index (BMI), hypertension, type 2 diabetes, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglyceride, high sensitivity C-reactive Protein (hsCRP) were measured at Exams 1 and 2 while cigarette smoking, SES (annual family income and education), and psychosocial factors (global perceived stress scale, every day and lifetime discrimination) were only measured at baseline Exam 1. BMI was calculated as weight (kg)/height (m)^2. Fasting serum HDL cholesterol [mg/dl] and triglyceride [mg/dl] were assayed using standard techniques and LDL cholesterol [mg/dl] was calculated by the Friedewald equation as previously described. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg, or taking antihypertensive medications within two weeks prior to the visit, or self-reported history of hypertension. Type II diabetes was defined as fasting glucose ≥126 mg/dl, or taking anti-diabetic medications, or self-reported diabetes diagnosis. Cigarette smoking status was classified as current smoker or non-current smoker.

SES was defined based on self-reported annual family income and education of the participants. Income was categorized as high income (≥$40,000) or low income (<$40,000). Education was categorized as less than bachelor degree or higher. Psychosocial factors were assessed using three standardized questionnaires. The Global Perceived Stress Scale is an 8-item scale (α= 0.72) that assessed the severity of chronic stressors over the past year in domains such as employment, health, and relationships. Perceived everyday discrimination is a 9-item scale (α=.88) based on the William’s Scale that assessed how often participants experience day to day discrimination. Lifetime discrimination was adapted from a scale by Nancy Krieger. Participants were asked whether they had experienced various types of discrimination over their lifetime across 9 domains (α=.78) at school, getting a job, at work, getting housing, getting resources/money, getting medical care, on the street or public place, getting services or other ways. The average score of the items for GPSS, every day and lifetime discrimination was used and transformed into standard deviation units.

**Global European Ancestry Estimates**

Global European ancestry estimates for our sample were calculated using HAPMIX in analyses supported by the Candidate Gene Association Resource (CARE) consortium. Briefly, two datasets were used as reference populations: 1,178 European Americans samples and 756 samples of African American ancestry, represented here by Yoruba (YRI) from west-Africa, were used as parental samples to select AIMs from the Affymetrix 6.0 genome-wide genotyping platform. After removing
related pairs and outlier samples determined by quality control procedures using EIGENSOFT\textsuperscript{13}, a total of 3,192 unlinked AIMs were obtained with allele frequency between parental samples of at least 30 percent. The HAPMIX program was used to infer local ancestry (0, 1 or 2 European chromosomes) at each location in the genome, using phased CEU and YRI haplotypes from HapMap\textsuperscript{3} as the reference.\textsuperscript{13} HAPMIX was run in a mode that assigns European or African ancestry to each allele, thus resolving the local ancestry of each allele when both genotype and local ancestry are heterozygous. Finally, a global European ancestry for each sample was computed as the average of local ancestry estimates across the genome (scaled to 0.0, 0.5 or 1.0). The percent of global European ancestry estimates for this sample study had a median of 16.0\% and interquartile ranges (IQR=15\%).

Statistical Analysis

Descriptive analysis was conducted to examine the distribution of baseline characteristics of the participants across the percent of European ancestry (PEA) levels. Analysis of variance (ANOVA) was used to compare normally distributed continuous variables and presented as means ± standard deviation (SD). Comparisons for variables with a non-normal distribution were performed using the Kruskal-Wallis test and presented as median [IQR]. Chi-square was used to compare categorical variables and presented as proportions.

Multivariable Poisson regression analysis with a robust error variance was used to estimate prevalence ratios (PRs) for the associations of PEA with PAD, CAC and AAC. We investigated the association of PEA with naturally log transformed cCIMT using multivariable linear regression analysis. Previous studies have found non-linear relationships between genetic ancestry and CVD-related measures.\textsuperscript{15,16} To detect any non-linear relationships and to graphically visualize the dose-response relationships between PEA and subclinical atherosclerosis measures, we first fitted Restricted Cubic Spline (RCS) using a SAS macro created by Desquilbet and Mariotti.\textsuperscript{17} The number of knots was selected using the Akaike Information Criterion (AIC) to avoid over-fitting. Five knots placed at equally-spaced percentiles of PEA distribution (5th, 25th, 50th, 75th and 95\textsuperscript{th}) was found to give a smaller AIC, thus, adequately capture the relationships. Our RCS analysis indicated that PAD-PEA relationship was linear, so PEA was used as a continuous variable per standard deviation (SD) to model PAD. However, the relationships of PEA with cCIMT, CAC and AAC were non-linear. Thus, we categorized PEA into three groups based on the cohort’s distribution of percent European admixture to facilitate interpretation, as done previously\textsuperscript{15,16}: lowest (<10\% percentile), medium (10\% - 90\% percentile), and highest (>90\% percentile) levels of PEA. Four models were estimated for each outcome. The first model adjusted for demographic variables, age and sex (Model 1). We then adjusted for CVD risk factors, including BMI, current smoker, hypertension, type II diabetes, HDL, LDL, triglyceride, and hsCRP (Model 2). Next, we adjusted for SES and psychosocial risk factors, including income, education, GPSS, and every day and lifetime discrimination (Model 3). Finally, we adjusted for all risk factors and covariates (Model 4). We also tested for two-way interactions of PEA with SES and psychosocial risk factors.
All tests were two-sided; p-values <0.05 were considered to be statistically significant. All analyses were conducted with Statistical Analysis Systems (SAS) release 9.2 (SAS Institute, Cary, NC).18

References


**Supplementary Table I:** Odds Ratio (OR) and 95% Confidence Interval (CI) of Peripheral Arterial Disease (PAD) per Standard Deviation Increase in Percent of European Ancestry (PEA) among African Americans: The Jackson Heart Study (2000–2004)

<table>
<thead>
<tr>
<th>PEA</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model1: Demographic</td>
<td>0.86 (0.73, 1.03)</td>
<td>0.094</td>
</tr>
<tr>
<td>Model2: Traditional CVD factors</td>
<td>0.86 (0.72, 1.02)</td>
<td>0.090</td>
</tr>
<tr>
<td>Model3: SES &amp; Psychosocial factors</td>
<td>0.90 (0.76, 1.07)</td>
<td>0.245</td>
</tr>
<tr>
<td>Model4: Fully adjusted</td>
<td>0.88 (0.72, 1.06)</td>
<td>0.172</td>
</tr>
</tbody>
</table>

PAD defined as ABI < 0.90
Standard deviation, 9.0%
Model1: Adjusted for demographic: age and gender;
Model2: Adjusted for model 1 and traditional CVD risk factors: BMI, current smoking, diabetes, hypertension, fasting plasma glucose, triglycerides, LDL cholesterol, HDL cholesterol, and hsCRP;
Model3: Adjusted for model 1 and SES & Psychosocial factors: household annual income, education, perceived global stress, and every day and lifetime discrimination;
Model4: Adjusted for all the covariates
PEA: percent of European ancestry; PAD: peripheral artery disease; ABI: ankle-brachial index; hsCRP: high sensitivity C - reactive protein.

**Supplementary Table II:** Mean Differences (Standard Error (SE)) of Common Carotid Intima Media Thickness (cCIMT), per standard deviation increase in European Ancestry (PEA) among African Americans: The Jackson Heart Study (2000–2004)

<table>
<thead>
<tr>
<th>PEA</th>
<th>Mean differences (SE)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model1: Demographic</td>
<td>-0.004(0.04)</td>
<td>0.342</td>
</tr>
<tr>
<td>Model2: Traditional CVD factors</td>
<td>-0.002(0.04)</td>
<td>0.630</td>
</tr>
<tr>
<td>Model3: SES &amp; Psychosocial factors</td>
<td>-0.002(0.04)</td>
<td>0.639</td>
</tr>
<tr>
<td>Model4: Fully adjusted</td>
<td>-0.001(0.04)</td>
<td>0.839</td>
</tr>
</tbody>
</table>

Standard deviation, 9.0%
Model1: Adjusted for demographic: age and gender;
Model2: Adjusted for model 1 and traditional CVD risk factors: BMI, current smoking, diabetes, hypertension, fasting plasma glucose, triglycerides, LDL cholesterol, HDL cholesterol, and hsCRP;
Model3: Adjusted for model 1 and SES & Psychosocial factors: household annual income, education, perceived global stress, and every day and lifetime discrimination;
Model4: Adjusted for all the covariates
PEA: percent of European ancestry; hsCRP: high sensitivity C - reactive protein.