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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.
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 HapMap3 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 95th) 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 (<10th percentile), medium (10th - 90th percentile), and highest (>90th 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).

References


