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Research on the Effect of Paraoxonase Single Nucleotide Polymorphisms on the Association of Race with Cardiovascular Disease

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Public Health (Epidemiology)

by

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The Dissertation of Ericha Gretchen Franey is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

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DEDICATION

To my faithfully supportive husband, family, friends

and co-workers who made this academic journey possible

EPIGRAPH

How you do **anything** is how you do **everything**.

Zen quote

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LIST OF ACRONYMS

ABI	Ankle-Brachial Index
ARIC	Atherosclerosis Risk in Communities Study
BMI	Body Mass Index
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
dbGaP	Database of Genotypes and Phenotypes
MACE	Major Adverse Cardiac Events
NHLBI	National Heart, Lung and Blood Institute
PAD	Peripheral Artery Disease
PON	Paraoxonase
SAS	Statistical Analysis Software
SNP	Single Nucleotide Polymorphism

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ABSTRACT OF THE DISSERTATION

Research on the Effect of Paraoxonase Single Nucleotide Polymorphisms on the Association of Race with Cardiovascular Disease

by

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University of California San Diego, 2019
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Background: Cardiovascular disease (CVD) is the leading cause of death in the US accounting for the highest death rates found in Blacks and Whites. Human paraoxonase (PON1, PON2, PON3) enzymes have antioxidant properties inhibiting the formation and accumulation of cholesterol. Major adverse cardiac events (MACE) is a composite cardiovascular outcome comprised of first occurrence of pre-specified cardiac events. Ankle-brachial index (ABI) is the ratio of systolic blood pressures of the lower extremity divided by the upper extremity. Peripheral arterial disease (PAD) is diagnosed as ABI <0.9. Objectives were to evaluate the association of race with 1) MACE 2) PAD and 3) change in ABI over time and effect modification of *PON* SNPs on these associations.

Methods: Data analyzed came from the Atherosclerosis Risk in Communities (ARIC) Study, a large population-based prospective cohort conducted at 4 field-centers across the US. Participants 45-64 years, Black or White race, enrolled in 1987 with four follow-up visits. Analysis was limited to 12,711 participants with genotype data.

Results: After adjusting for age, gender, BMI, cigarette and alcohol use, educational and marital status and reported aspirin use, Blacks had 1.24 times greater hazard of MACE compared to Whites, which became non-significant after adjusting for comorbidities. After adjusting for age, BMI, and gender, Blacks had 1.27 higher odds of PAD than Whites, but differences became non-significant after adjustment for smoking, education and comorbidities. After adjusting for confounders, Whites had significantly higher (better) ABI values than Blacks at both follow-up visits. When stratified by education, ABI over time was better in both Blacks and Whites who completed high school than those with less education. Due to the lack of main effect of *PON* SNPs, there was no effect modification found on these associations.

Conclusions: Modifiable health and behavioral risk factors and comorbidities may be major determinants of MACE and PAD. ABI differences by race were statistically significant but small and may not be clinically significant. Higher education may influence health management contributing to better ABI in Blacks and Whites. No effect modification was found on these associations due to the lack of main effect of the *PON* SNPs.

CHAPTER 1

CARDIOVASCULAR DISEASE

Race

Cardiovascular disease (CVD) is the most common cause of death worldwide accounting for 31% (17.9 million) of all global deaths [1]. Eighty-five percent of global CVD deaths are due to heart attack and stroke caused by fatty deposit vessel blockage preventing flow to the heart or to the brain [1]. CVD is characterized by arterial deposits generated by the formation and accumulation of macrophage cholesterol and increased production of cholesterol [2]. Under oxidative stress, these atherogenic processes increase [3]. Ultimately, narrowing of the arteries and blood vessels manifests as cardiovascular disease including coronary heart disease, heart attack, heart failure, stroke, transient ischemic attack and peripheral vascular disease and death [4]. In the United States, cardiovascular disease (CVD) accounts for 1 in every 4 deaths, resulting in approximately 610,000 deaths per year, and is the leading cause of death for both men and women [5]. It is also the leading cause of death across most racial and ethnic groups in the United States including African Americans, Whites and Hispanics, and it is the second leading cause of death after cancer for American Indians/Alaska Natives and Asian/Pacific Islanders [5]. According to the Centers for Disease Control and Prevention, the following are rates of death due to heart disease by race/ethnicity in 2008: American Indians/Alaska Natives (18.4%), Asians or Pacific Islanders (22.2%), Non-Hispanic Blacks (23.8%) and Non-Hispanic Whites (23.8%) [5]. In 1992, 24% of Latino deaths were due to cardiovascular disease [6].

Traditional Risk Factors

The American College of Cardiology Foundation (ACCF) and the American Heart Association (AHA) 2010 guidelines, state that the strongest predictors of 10-year CVD risk include age, race, gender, total and high-density lipoprotein cholesterol (HDL), blood pressure, blood pressure treatment status, diabetes and current smoking [5]. Improved understanding of

these risk factors is essential to equipping the clinician to provide patient-specific preventive care for CVD [7].

Several CVD risk factors cannot be controlled such as age, family history, race and gender [7,8]. Men older than 45 years of age and women older than 55 years of age have greater risk for developing CVD [7]. Family history of early CVD increases the risk of developing CVD, specifically having a father or brother diagnosed before age 55 years, and a mother or sister diagnosed before age 65 years [7]. A recent large international prospective study of CVD (REduction of Atherothrombosis for Continued Health [REACH]), reported that the prevalence of CVD risk factors and mortality varied across different racial and ethnic groups; death from CVD was higher in blacks (6.1%) compared to other racial groups (3.9%); death from CVD was lower in Asians (2.1%) compared to other racial groups (4.5%) [9].

CVD risk factors that can be controlled include lack of physical activity, unhealthy diet, smoking, being overweight, high blood pressure, high cholesterol, diabetes, stress and depression [7,8]. Consuming more than 20 cigarettes per day increased the risk of CVD by 2-3 times [10]. Results from the Framingham Heart Study found that high-normal blood pressure doubled the risk for CVD [11]. A diagnosis of diabetes increased risk of CVD by 2-8 fold compared to age and ethnicity/race matched individuals without diabetes [12]. High levels of small dense low-density lipoprotein cholesterol (LDL) [13] is a risk factor for development of CVD [7]. Both the number and severity of risk factors compound the risk for CVD [8]. In 2003, approximately 37% of Americans reported having two or more risk factors leading to the development of CVD, and at least one risk factor was reported by 90% of those diagnosed with CVD [14].

In the large international, prospective REACH study of men and women aged 45 years and older, rates of CVD risk factors varied by ethnicity and race [9]. Rates of hypertension were highest in Blacks (93.0%), followed by East Asians (84.1%) and South Asians (83.7%) [9]. Diabetes was highest in South Asians (57.9%), Blacks (55.0%) and Hispanics (53.3%) [9].

Blacks had the lowest rates of high cholesterol (51.4%) and Whites had the lowest rates of diabetes (38.8%) and hypertension (79.1%) [9]. Across all ethnic groups, most study participants met the BMI criterion for being overweight and most were former or current smokers [9]. A recent meta-analysis reported that low levels of HDL are more prevalent among Asians populations, increasing the risk for CVD among this minority group [15].

Major Adverse Cardiac Events (MACE)

Creating a composite cardiovascular outcome results in an endpoint which includes first occurrence of pre-specified cardiac events, commonly referred to as MACE (major adverse cardiac events), increases statistical power and improves detectability of clinically meaningful differences [16]. MACE is most commonly used in clinical interventional studies in hospitalized or outpatient populations. Prior studies have found racial and ethnic differences in MACE. For example, in a cohort of 864 renal transplant patients where MACE was defined as a composite of nonfatal myocardial infarction, coronary intervention and cardiac death, Prasad *et al.* found a higher rate of MACE after transplant for South Asians compared to East Asians, Whites and Blacks (4.4/100 patient-years vs. 1.61, 1.31 and 1.16/100 patient-years, respectively) [17]. Napan *et al.* in a cohort of 1,438 patients undergoing percutaneous coronary intervention, found 52% greater risk (HR=1.52; 95%CI=1.18, 1.96) of MACE (composite of death, myocardial infarction and revascularization) among Blacks compared to non-Blacks after adjusting for age, gender, CVD risk factors, socioeconomic status and other potential confounders [18].

Peripheral Artery Disease (PAD) and Ankle-Brachial Index (ABI)

It is estimated that 12-20% of persons older than 60 years of age, or 8.5 million people in the US have peripheral arterial disease (PAD) [19]. PAD is a predictor of future CVD events, is prevalent worldwide, and found equally in men and women [19]. It occurs most often in the lower extremities and is the third leading cause of atherosclerotic cardiovascular death after coronary artery disease and stroke [14]. PAD is defined as >50% arterial stenosis as indicated by an ankle-brachial index (ABI) value <0.9 [14]. The ankle-brachial index (ABI) is a

reproducible and valid measure for diagnosing PAD; and has been the primary screening tool for PAD during the past few decades [20] because it is a low cost, non-invasive, office-based test [21]. ABI is calculated as the ratio of systolic blood pressure measurement of the lower extremity divided by that of the upper extremity [2]. By convention, ABI <0.9 indicates >50% arterial stenosis whereas normal ABI ranges from ≥0.90 to <1.40 [4,14, 22]. Risk factors for PAD include older age, high cholesterol, hypertension, diabetes and smoking [5].

Prior studies of the association between race and PAD have been inconsistent [21, 23, 24] with some reporting higher rates of PAD in Blacks but others failing to find racial differences. For instance, in 2,343 participants from the San Diego Population study, Criqui *et al.* found that Blacks had higher odds of PAD (OR=2.30, p<0.024) than Whites after adjusting for covariates [25]. In contrast, there were no significant differences between Blacks and Whites in odds of PAD diagnosis (OR=1.89; 95%CI=0.89, 3.99) in a cohort of 403 patients from Houston, TX [26].

Previous studies report that ABI is a subclinical predictor of cardiovascular events [27, 28, 29, 30]. In 13,150 participants from the ARIC cohort, Gupta *el al.* found a 40% (95%CI=1.12, 1.74) increased risk of heart failure in those with low ABI (<0.90) compared to those with normal ABI (1.01-1.40) [27]. Yeboah *et. al.,* in 1,330 participants from the MESA cohort, found that ABI was an independent predictor of incident CHD/CVD beyond traditional risk factors for individuals of intermediate risk [28]. A meta-analysis of 16 population studies found that both low (≤0.9) and high ABI (>1.4) were significant independent predictors of CVD events and recommended inclusion of ABI to enhance the Framingham Risk Score for CVD risk prediction [29]. In the ARIC cohort, each 0.10 decline in ABI was associated with greater increase CHD risk in Blacks than Whites [24].

PARAOXONASE GENE FAMILY

Genetics of Cardiovascular Disease

Approximately 30% of the human genome is comprised of protein coding genes while the remainder regulate gene expression, including the regulatory regions located downstream (3') and upstream (5') of the coding sequence of the gene [30]. Single nucleotide polymorphisms (SNPs) are the most common type of variation found in just one base pair of the nucleotides forming double helix strands of DNA [30]. Regardless of whether these variations occur in the coding region or the regulatory region, they often influence gene expression and protein coding affecting phenotypic outcomes [30]. Complex diseases, such as CVD, can be influenced by single genetic defects caused by rare, deleterious mutations alone, or in combination with common polymorphisms that have weak effects [30]. However, the direct risk of genetic expression on phenotype outcome is influenced by a host of interactions including age, race, gender, comorbidities, diet, physical activity, environment and concomitant medications [30].

The discovery of novel disease mechanisms is the primary objective of genetic research in humans [30]. During the past 20 years, researchers have searched for genetic predictors of cardiovascular disease to add to the current risk model using known current clinical and subclinical risk factors [30]. Cardiovascular disease, including coronary artery disease, myocardial infarction, ischemic stroke and atrial fibrillation, all have known genetic contributors [31]. Much research has been conducted supporting the association of genetic polymorphisms and CVD; however, few of the results have been replicated, most studies have yielded weak associations (OR < 1.3) in small sample sizes which lack statistical power [30]. Because SNPs only confer a weak increase in an individual's risk of disease, they are not typically used as unique predictors for complex diseases such as CVD [30]. The genetics of cardiovascular disease may prove to ultimately have more indirect value for diagnostic purposes and in selection of therapy in personalized medicine [30].

Paraoxonase Genes (*PON1, PON2, PON3*)

In humans, the *PON1*, *PON2* and *PON3* genes are adjacent to one another on chromosome 7q21-q22 [32]. Paraoxonase enzymes exhibit antioxidant properties specifically by inhibiting the formation and accumulation of macrophage cholesterol conferring cellular and humoral protection against the development of atherosclerosis [33]. Because these proteins prevent oxidative stress and inflammation, there is much interest in understanding the role they play in human diseases such as atherosclerosis, diabetes and inflammatory bowel disease [34].

PON1*:* Five polymorphisms have been identified on the *PON1* promotor region [34], with the greatest interest focused on the polymorphisms located at positions 55 and 192 [3]. Synthesized in the liver, the PON1 enzyme is found predominantly in blood serum [34]. Higher PON1 protein concentrations and serum activity levels were higher in homozygous [RR] than homozygous [QQ] persons, while heterozygous [QR] persons had intermediate levels [33]. Interest in examining the association of PON1 and coronary heart disease is primarily due to recent research showing the protective effects of PON1 against HDL and LDL oxidation, as well as the role this enzyme plays in destroying oxidized lipids that are biologically active in arterial cells and lipoproteins [33]. Oxidative stress was found to inactivate *PON1* expression, lowering PON1 activity [33].

PON2*:* Two polymorphisms of interest have been located on the *PON2* coding region, the cysteine-serine [CS] amino acid substitution found at position 311 and the alanine-glycine [AG] amino acid substitution at position 148 [35]. *PON2* is expressed in nearly all tissues at the cellular level including kidney, testis, brain and liver; however, unlike PON1 and PON3, this enzyme is not found in blood serum [3]. PON2 expresses uniquely in human macrophages and increases under oxidative stress [2].

PON3*:* Two polymorphisms of interest have been found on the *PON3* coding region include the serine-threonine [ST] amino acid substitution located at position 311 and the glycine-aspartic acid [GD] amino acid substitution located at position 324 [35]. *PON3* is expressed

predominantly in the liver and kidney, with the enzyme found in blood serum associated with HDL [2]. Oxidative stress inactivates *PON3* expression and lowers PON3 activity [33].

Paraoxonase and Cardiovascular Disease

PON1 activity is highest in African American and Asian ethnicities due to the higher prevalence of the 192R substitution polymorphism [36, 37]. Consistent with prior studies, a recent study by Davis *et al*. assessing racial differences of the *PON1* polymorphism and found that whites were four times more likely to have the *PON1* 192QQ genotype than African-Americans study participants; the allelic frequencies were QQ 15%, QR 34%, RR 44% in African-Americans compared to QQ 60%, QR 31%, RR 7% in whites [38]. The *PON2* 311 polymorphism was associated with angiogram diagnosed coronary artery disease (CAD) in Asian Indians, but not in Europeans and Chinese [36, 37]. Several studies have reported that the distribution and frequency of the *PON2* 311 genotype varies by geographical location and race: Han Chinese in Guang Dong (SS 72.6%, CS 25.6%, CC 1.7%); Han Chinese in Tianjin (SS 72.18%, CS 25.02%, CC 2.82%); Japanese, (SS 65%, CS 30%, CC 5%) and Indians (SS 40.2%, CS 42.3%, CC 17.5%) [39]. Results from the National Center for Biotechnology Information dbSNP database support evidence of variation in both paraoxonase gene distribution and allelic frequency across race and geographic location [40]. Previous studies report that PON1 serum activity levels vary by as much as 40-fold most likely due to differences in allelic frequencies [33,41].

Previous research with varying types of study designs have demonstrated the difference in cardiovascular disease outcomes by *PON* genotype.

Case Control Studies: A case-control study of 876 middle-aged Japanese men and women reported no significant association of either the *PON1* 55 polymorphism or the *PON2* 311 polymorphism and CVD [42]. However, the *PON1* 192 genotype was significantly associated with both CAD and ischemic stroke (p's < 0.001), with higher allelic frequencies found in patients (~75%) vs. controls (65%) [42]. The *PON1* 192R alleles vs. the *PON1* 192Q

alleles were significantly associated with higher odds of both CAD 1.60 [95%CI: 1.21, 2.12] and ischemic stroke 1.68 [95%CI: 1.28, 2.21] [42]. Similar results were found in a case-control study of 108 Turkish men and women aged 20-83 years in which the *PON1* 192RR genotype was a significant predictor of stroke in the overall study population with increased odds (OR = 5.140) in the elderly subset; prevalence of this genotype also decreased with age (20% in younger patients compared to 15.4% in elderly patients) [43]. Another case-control study of 318 Asian-Indian men and women aged 32 years and older, reported that the *PON2* 311S allelic frequency was higher in cases (71%) than controls (61%), and there was an association between increased CHD risk and *PON1* 192R (OR = 3.6 [95%CI: 2.6-4.6]) and *PON2* 311S (OR = 2.9 [95%CI: 2.4-3.5]) polymorphisms [44]. Finally, a case-control study of 701 Taiwanese participants evaluating the *PON2* polymorphisms showed 4.6 [95%CI: 1.6-15.3] greater odds of CAD in *PON2* 311SS allelic carriers compared to the other genotypes; in controls, the *PON2* 311CC and CS genotypes had statistically significant higher HDL than cases [45].

Prospective Cohort Studies: A prospective study of 538 Han Chinese men and women aged 26-80 years followed for up to 1 year for major adverse cardiac events, found no significant associations for either the *PON1* 55 or *PON1* 192 polymorphisms [46]. Similarly, a cohort study of 1527 middle-aged Dutch women found no association between *PON1* 192 genotypes and CHD risk [47]. Additionally, a large prospective cohort study of 10,593 middle-aged men in Northern Ireland and France found no association between the *PON1* 55 polymorphism and CHD risk and no significant difference in allelic frequency between cases and controls [48]. However, a prospective US cohort study in 1399 men and women 50 years and older found that participants with the *PON1* 192QQ genotype had 1.48 [95% CI: 1.09-2.03] greater risk of major adverse cardiac events compared to those with the 192RR and 192QR genotypes [49].

Paraoxonase and Major Adverse Cardiac Events (MACE)

In a prospective study of 1399 men and women aged 55 years and older in the US undergoing coronary angiography, Bhattacharyya *et al*. found that participants with the lowest *PON1* activity had 3.4 times greater risk of MACE (myocardial infarction, stroke or death) compared to those with the highest levels (HR=3.4; 95%CI=2.0, 5.9) [49]. Kang *et al.* reported a marginal effect of the *PON1* Q192R SNP on risk of MACE ($p<0.05$) in a case-control study of 538 Han Chinese men and women aged 26-80 years who were undergoing percutaneous coronary intervention and followed for up to 1 year for MACE [50]. While these studies evaluated the association between PON SNPs and MACE, to our knowledge, no prior research has examined the potential effect of the *PON* gene on this association.

Paraoxonase and Peripheral Artery Disease (PAD) and Ankle-Brachial Index (ABI)

In a recent case control study conducted by Hernandez-Aguilera *et al*., PON1 concentrations and activities were lower in 66 patients diagnosed with peripheral artery disease as compared to 8 controls [51]. In a case control study of 37 older participants (mean age 69.9$\pm$9 years) diagnosed with PAD, Pasqualini *et al*. found that *PON1* genotype and PON1 activity were associated with brachial flow-mediated vasodilation ($p=0.0004$) [52]. However, to our knowledge, no previous studies examined the effect of *PON* genes on the association between race and PAD or on the association between race and ABI.

ATHEROSCLEROSIS RISK IN COMMUNITIES Study (ARIC)

The Atherosclerosis Risk in Communities Study (ARIC) [53] is a prospective cohort study conducted in 4 communities in the United States (Washington County, MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN) each enrolling approximately 4000 participants. A total of 15972 study participants were examined at a screening baseline visit (1987-1989),and re-examined every three years for a total of four follow-up visits (see Table 1.1). Participants who completed Visit #4 (2011-2013) had the best average health profiles and lowest average age at the baseline visit (1987-1989).

Table 1.1. ARIC: Number of Study Participants by Study Visit

Study Visit	Participants Examined
Baseline (1987-1989)	15972
#1 (1990-1992)	14201
#2 (1993-1995)	12106
#3 (1995-1999)	11343
#4 (2011-2013)	6515

Since 2012, study participants have been contacted annually or semi-annually by telephone to assess health status. Enrolled study participants were Black (27%) and White (73%), male and female, aged 45-64 years. Study objectives were to investigate the etiology and natural history of atherosclerosis and to examine the risk factors and progression of subclinical to clinical cardiovascular disease events including revascularization, myocardial infarction, peripheral artery disease and death. In addition, the ARIC study examined genetic and environmental risk factors leading to ventricular dysfunction and vascular stiffness.
The Affymetrix 6.0 array platform was used to perform whole genome genotyping. For this dissertation research, using a (±) 20 kb window around each gene region, 82 *PON* SNPs (43 *PON1*, 32 *PON2*, 7 *PON3*) were identified in the ARIC cohort. All SNPs had ancestry-specific allele frequencies similar to those reported in publicly available databases

(https://www.ncbi.nlm.nih.gov/projects/gapsolr/facets.html) and were in Hardy-Weinberg equilibrium. SNPs with minor allelic frequencies (MAF) less than 5% were excluded, leaving 62 SNPs available for screening analysis. Using the Nyholt method (https://neurogenetics.qimrberghofer.edu.au/matSpDlite/), 45 SNPs were independent and not in linkage disequilibrium. Bonferroni correction for multiple comparisons yielded a significance of $p<0.001$ (0.05/45) when evaluating the univariate association of each SNP with the outcome of interest. Five principal component analysis covariates obtained from the PLINK routine [54] were used to adjust for residual population stratification. SNPs were coded as continuous variables for analysis using an additive genetic model.

Research Objectives

Prior studies report racial and ethnic differences in MACE, which may be modified by genetics. Previous reports of the association between race and PAD have been inconsistent; genetics may modify this association. ABI has previously been shown to be a subclinical predictor of cardiovascular events; however, to our knowledge, the association between race and change in ABI over multiple timepoints has not been previously reported. These associations may be modified by genetics, but to our knowledge no previous study has examined the potential effect of the *PON* gene on the association between race and MACE, with peripheral artery disease and with change in ABI over time.

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CHAPTER 2

Association of Race and Major Adverse Cardiac Events (MACE): the Atherosclerosis Risk in Communities (ARIC) Cohort

Abstract

Background and Aims: To evaluate the association of self-reported race with major adverse cardiac events (MACE) and modification of this association by paraoxonase gene (*PON*1, *PON*2 and *PON*3) single nucleotide polymorphisms (SNPs).

Methods: Included in this longitudinal study were 12,770 Black or White participants from the Atherosclerosis Risk in Communities (ARIC) cohort who completed a baseline visit (1987-1989) with *PON* genotyping. Demographic, behavioral and health information was obtained at baseline. MACE was defined as first occurrence of myocardial infarction, stroke or CHD-related death through 2004. Cox proportional hazards regression was used to evaluate the association between race and MACE after adjustment for age, gender and other demographic and cardiovascular risk factors such as diabetes and hypertension. Modification of the association between *PON* SNPs and MACE was also assessed.

Results: Blacks comprised 24.6% of the ARIC cohort; overall, 14.0% of participants developed MACE. Compared to Whites, Blacks had 1.24 times greater hazard of MACE (OR=1.24,95%CI=1.10,1.39) than Whites after adjusting for age, gender, BMI, cigarette and alcohol use, educational and marital status, and aspirin use. This association became non-significant after further adjustment for high cholesterol, diabetes and hypertension. None of the evaluated SNPs met the significance level ($p<0.001$) after Bonferroni correction for multiple comparisons.

Conclusions: No association between race and MACE was identified after adjusting for high cholesterol, diabetes and hypertension, suggesting that comorbidities are major determinants of MACE; medical intervention with focus on lifestyle and health management could ameliorate the development of MACE. Further studies are needed to confirm this observation.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in most racial and ethnic groups in the United States including Blacks and Whites, accounting for 1 in 4 deaths.[1] Composite cardiovascular outcomes create an endpoint which includes first occurrence of pre-specified cardiac events, commonly referred to as MACE (major adverse cardiac events), increasing statistical power and improving detectability of clinically meaningful differences.[2]

Prior studies have found racial and ethnic differences in MACE. For example, in a cohort of 864 renal transplant patients where MACE was defined as a composite of nonfatal myocardial infarction, coronary intervention and cardiac death, Prasad *et al.* found a higher rate of MACE after transplant for South Asians compared to East Asians, Whites and Blacks (4.4/100 patient-years vs. 1.61, 1.31 and 1.16/100 patient-years, respectively).[3] Napan *et al.* in a cohort of 1,438 patients undergoing percutaneous coronary intervention, found 52% greater hazard (HR=1.52; 95%CI=1.18, 1.96) of MACE (composite of death, myocardial infarction and revascularization) among Blacks compared to non-Blacks after adjusting for age, gender, CVD risk factors, socioeconomic status and other potential confounders.[4]

The association between race and MACE may be modified by genetics. Paraoxonase (*PON*) genes play an important role in the translation of enzymes that inhibit the arterial formation of cholesterol causing atherosclerosis leading to development of CVD.[5] However, results of the few studies examining the association between *PON* single nucleotide polymorphisms (SNPs) and MACE have been inconsistent. For instance, Kang *et al.* reported a marginal effect of the *PON1* Q192R SNP on risk of MACE ($p<0.05$) in a case-control study of 538 Han Chinese men and women aged 26-80 years who were undergoing percutaneous coronary intervention and followed up to 1 year for MACE.[6] A prospective US cohort study of 1399 men and women aged 55 years and older who were undergoing coronary angiography found that participants with the lowest *PON1* activity had 3.4 times greater hazard of MACE (myocardial infarction, stroke or death) compared to those with the highest levels (HR=3.4;

95%CI=2.0, 5.9).[7] To our knowledge, no prior study has examined the *PON* gene effect on the association between race and MACE.

The purpose of this study was to evaluate the association of Black and White race with MACE, and to evaluate the effect of paraoxonase single nucleotide polymorphisms (SNPs) on this association using data from a large cohort of older men and women (ARIC).

Material and Methods

This study was approved by the University of California San Diego Human Research Protections Program (#160359X); data was collected through authorized access from dbGaP. The ARIC[8] research study was supported by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health; each site obtained institutional review board approval and written informed consent from study participants. Data analyses performed using SAS® University Edition (SAS Institute, Cary, NC).

ARIC: The Atherosclerosis Risk in Communities Study (ARIC)[8] is a prospective cohort study enrolling approximately 4,000 participants selected by probability sampling at each of four communities in the United States (Washington County, MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN). A total of 15,972 study participants were examined at baseline (1987-1989) and at four follow-up visits conducted at three-year intervals through 2013. Since 2012, participants have been contacted annually by telephone to assess health status. Enrolled study participants were Black (27%) and White (73%) men and women, aged 45-64 years. Study objectives were to investigate the etiology and natural history of atherosclerosis including the risk factors and progression of subclinical to clinical cardiovascular disease events.

Participants: This study was limited to the 12,770 ARIC participants (24.6% Black, 75.4% White) for whom *PON* genotyping data was available who also experienced a first occurrence of myocardial infarction, stroke or CHD-related death (study definition of MACE).

Variables

Race: Study participants were self-categorized as Black or White in the ARIC[8] study.

Major Adverse Cardiac Events (MACE): MACE was defined as first occurrence of myocardial infarction, stroke or CHD-related death from the baseline visit through 2004. This information was collected from study participants via annual phone interviews.[9] Self-reported diagnoses were verified by medical records for hospitalizations and outpatient cardiovascular events; next-of-kin interviews provided information on out-of-hospitals deaths which were subsequently reviewed and assigned a diagnosis.[9]

Covariates: Demographic characteristics (e.g., age, education, marital status), health history, body mass index (BMI) kg/m^2 and results of fasting laboratory assays were obtained from the baseline visit.[8] Family history included maternal and paternal CHD events. Current marital status (yes/no), high school graduate or more education (yes/no), current cigarette smoking (yes/no) and alcohol use (yes/no) were assessed at baseline. Measures of systolic and diastolic blood pressure were obtained and 12-hour fasting glucose and cholesterol levels were assayed from blood samples at baseline. Participants taking anti-diabetic medication or having fasting glucose $\geq$126 mg/dl were categorized as having diabetes mellitus.[10] Those taking cholesterol-lowering medication or having laboratory assessed cholesterol >240 mmol/L were categorized as having high cholesterol.[11] Participants taking antihypertensive medications or having systolic pressure >140 mmHg or diastolic pressure >90 mmHg were categorized as hypertensive.[12] Intake of current medications, including aspirin, anti-diabetic medication, antihypertensive medication and lipid lowering medication were determined by review of labelled containers[8] participants brought to the clinic visit.

Genotyping: Whole genome genotyping was performed using the Affymetrix 6.0 array platform in this study population;[8] there were 82 *PON* SNPs (43 *PON1*, 32 *PON2*, 7 *PON3*) available in the ARIC cohort which included ($\pm$) 20 kb window around each gene region. After excluding SNPs with minor allelic frequencies (MAF) less than 5%, 62 SNPs remained available for screening analysis. All SNPs were in Hardy-Weinberg equilibrium and had ancestry-specific allele frequencies similar to those reported in publicly available databases

(https://www.ncbi.nlm.nih.gov/projects/gapsolr/facets.html). The 62 SNPs were screened for significant association with PAD in Blacks and Whites combined using a significance level of $p<0.001$ (0.05/45) after Bonferroni correction for multiple comparisons confirmed 45 independent SNPs using the Nyholt method (https://neurogenetics.qimrberghofer.edu.au/matSpDlite/). Five principal component analysis covariates, obtained from the PLINK routine[13] were used to adjust for residual population stratification. SNPs were coded as continuous variables for analysis using an additive model.

Statistical Analysis: Descriptive statistics were calculated and reported as percentages for categorical variables and means (± standard deviations [SD]) for continuous data. Differences by race and MACE were analyzed using independent t-tests for continuous variables and chi-square tests for categorical variables. All covariates were noncollinear based on correlation coefficients of $r<0.30$. Covariates with at least marginally significant differences by race and development of MACE and known confounders were retained for further analysis. Statistical significance was defined as $p<0.05$.

Forward stepwise Cox proportional hazards regression analysis was used to estimate the hazard ratio (HR) of having first occurrence MACE (myocardial infarction, stroke or CHD-related death) by race, after adjustment for covariates. Kaplan Meier analysis generated a survival curve depicting time to experience a first major adverse cardiac event for Blacks and for Whites. Covariates with a p-value less than or equal to 0.05 were retained in the multivariable model. Assessment of the proportional hazards assumption ($p\leq0.05$) was performed for all variables. Confounders, identified as variables yielding a 10% change in the estimated coefficient between full and reduced models, were retained in the final model. Model 1 examined the unadjusted association between race and MACE. Model 2 added age, gender and BMI. Model 3 added cigarette and alcohol use. Model 4 added marital and educational status. Model 5 added aspirin use. Model 6 added high cholesterol, hypertension and diabetes. None of the 62 *PON* SNPs screened met the significance criteria of $p<0.001$ and were therefore

not retained for further analysis. Interactions between race and covariates were evaluated with possible effect modification considered when $p<0.05$.

Results

Table 2.1 shows baseline differences for Blacks and Whites in the ARIC cohort. Major adverse cardiac events (MACE) was found in 1,790 (14.0%) of the participants overall, with a higher proportion in Blacks (17.7%) compared to Whites (12.8%) ($p<0.0001$). Blacks were younger (53.3$\pm$5.8 vs. 54.3$\pm$5.7 years respectively, $p<0.0001$), and had higher mean BMI (29.7$\pm$6.1 vs. 27.0$\pm$4.9 kg/m^2, respectively, $p<0.0001$) compared to Whites. Compared to Whites, Black study participants were less likely to be men ($p<0.0001$) and have paternal ($p<0.0001$) or maternal ($p=0.0002$) family history of CVD. Blacks also had a lower proportion of current alcohol use ($p<0.0001$), and fewer had attained a high school education ($p<0.0001$), were married ($p<0.0001$) or reported aspirin use ($p<0.0001$) than Whites. Rates of current smoking, and diagnosed diabetes and hypertension were higher in Blacks than Whites (p's<0.0001). There was no difference in rates of diagnosed high cholesterol ($p=0.11$) between the races.

Comparisons of baseline characteristics by MACE in the ARIC cohort are shown in Table 2.2. Those with MACE were older (56.1$\pm$5.5 vs. 53.8$\pm$5.7 years, respectively, $p<0.0001$) and had a higher average BMI (28.6$\pm$5.3 vs. 27.5$\pm$5.3 kg/m^2 respectively, $p<0.0001$). Compared to those without MACE, a greater proportion of participants with MACE reported being Black, male (p's<0.0001), more likely to have paternal ($p=0.015$) and maternal family history of CVD ($p<0.0001$), more likely to be current cigarette users ($p<0.0001$) and more likely to be diagnosed with hypertension, high cholesterol, and diabetes (p's<0.0001). Additionally, fewer participants with MACE reported completing a high school education ($p<0.0001$), being currently married ($p=0.0005$) and currently using alcohol ($p<0.0001$) compared to those without MACE. There was no difference between those with and without MACE for current aspirin use ($p=0.21$). None of the 62 *PON* SNPs in the ARIC population met the screening criterion for statistical

significance (p<0.001) and were therefore, not retained for further analysis (Supplemental Table 2.A).

The Kaplan-Meier curve in Figure 2.1 graphically depicts the time to experience a first major adverse cardiac event is longer for Whites compared to Blacks. The association between race and MACE after forward step-wise adjustment for covariates in ARIC is shown in Table 2.3. Diabetes and hypertension were confounders and retained in the final model. The unadjusted hazard of MACE for Blacks in comparison to Whites was 1.46 (CI=1.32,1.61, p<0.0001, Model 1). After adjusting for age, gender, BMI, cigarette use, alcohol use, educational status and marital status and current aspirin use, Blacks had 1.24 times greater hazard of MACE than Whites (OR=1.24, 95% CI=1.10, 1.39, Model 5). This association became non-significant in the subsequent Model 6 that further adjusted for high cholesterol, diabetes and hypertension.

Main effects for all variables in Model 6 are shown in Table 2.4. Age, male gender, cigarette use, high cholesterol, hypertension, diabetes (p's<0.0001), BMI and aspirin use (p's<0.01) were all significantly and independently associated with higher hazard of MACE. High school education attainment and alcohol use were significantly associated with lower hazard of MACE (p<0.001). None of the SNPs met the initial screening significance criteria (p<0.001) and therefore were not included in the model to assess effect modification on this association. When effect modification between race and each covariate was tested, the only significant interaction was between race*gender (p=0.03). When stratified by gender, race was not significant in either model; therefore, this interaction may be spurious and will be left out of the final model.

Discussion

Major adverse cardiac events (MACE) are the most commonly used composite end points in cardiovascular research to assess the safety and efficacy of treatment interventions for cardiovascular clinical events.[14] In this longitudinal analysis of 12,770 ARIC participants,

Blacks had 1.24 times greater hazard of MACE compared to Whites after adjusting for age, gender, BMI, cigarette use, alcohol use, educational and marital status and reported aspirin use. However, this association became non-significant after further adjustment for hypertension, diabetes and high cholesterol. While this study did not find significant effect modification of *PON* SNPs on the association between race and MACE; to our knowledge this is the first study to report results of such an evaluation.

In this study of men and women aged 45 years and older from the ARIC cohort, there was a higher prevalence of MACE in Blacks than Whites (17.7% vs. 12.8%). Previous studies have observed racial disparities in cardiovascular disease outcomes.[15,16] A recent comprehensive narrative literature review summarized the well-established fact that Blacks have higher rates of cardiovascular disease, including myocardial infarction and CHD-related death.[15] A comparative study by Feinstein *et al.*, assessing racial differences in risk of first cardiovascular events, found that Black men were more likely than White men to be diagnosed with CVD (HR 1.06; 95% CI 0.90, 1.26); however, Black men were less likely than White men to have a first CVD event (HR 0.77; 95% CI 0.60, 1.00).[16] In contrast, our results suggested that race was not independently associated with MACE after adjustment for morbidities such as high cholesterol, hypertension and diabetes, which are known CVD risk factors.

The lack of a significant association between race and MACE after adjustment for comorbidities in the present study suggests that differences in MACE may be influenced by racial disparities in risk factors and medical management rather than biological differences in the development of atherosclerosis. Attempting to understand racial disparities in CVD outcomes, Cram *et al.* reported that for coronary revascularization rates, Black and Hispanics with similar insurance were significantly less likely to receive these procedures compared to Whites.[17] Prior research reports that Blacks present at a later clinical stage in the development of PAD than Whites. Furthermore, diabetes and neuropathy, both more prevalent in Blacks, affect the distal arteries and contribute to a PAD diagnosis.[18] Finally, it has been previously

demonstrated that racial disparities exist in health care, with most Blacks receiving lower quality than the majority of Whites in the United States.[19,20,21,22,23]

The most important consideration when selecting events to create a composite outcome is choosing events that participants perceive as being of equal importance and with similar impacts on health.[4] Here, we selected the first occurrence of myocardial infarction, stroke, CHD-related death as the events to create the composite outcome. This was based on the composite scores most often used in prior studies and the individual outcomes available in the ARIC dataset. Few studies have been conducted using MACE as an endpoint for non-treatment or non-interventional studies in populations that are generalizable. For instance, a study conducted by Belonge, *et al.* assessed the association of endurance exercise (which can exacerbate underlying cardiac issues) and major adverse cardiac events (death, hospitalization to coronary or myocardial infarction) and found the risk to be very low, occurring in 4 of 62,862 athletes.[24]

The influence of genetic differences on the risk of MACE is relatively unknown. PON1 enzymes have anti-inflammatory and antioxidant properties and may protect against atherosclerosis.[25] The enzyme is expressed in the liver and delivered to multiple tissues not expressing the enzyme.[25] Decreased PON1 enzyme activity has been shown to increase inflammation in animal studies, and increase oxidative stress among patients with atherosclerosis, diabetes and/or hypercholesterolemia.[25] Numerous studies have been conducted assessing the association of the *PON* gene cluster and CVD; however, results have been inconclusive, suggesting more comprehensive multi-centered studies are needed.[26] As discussed previously, the few previous studies assessing the effect of *PON* SNPs on the association between race and MACE composite outcome were among clinical (rather than population-based) samples undergoing cardiovascular treatment intervention.[7, 27]

It is biologically plausible that there is an association between race and MACE after adjustment for biological risk factors. We may have failed to find this association in adjusted

models because of the small sample of Blacks relative to Whites in this cohort. Future studies assessing the genetic differences and gene-environment interactions with respect to MACE need to be evaluated across diverse ethnic study populations with adequate sample sizes.

Several limitations and strengths of this study were considered. While MACE composite outcomes have been used since the mid-1990s, the research community continues to assess the validity and define the utility of this measure. Therefore, inherent weaknesses previously identified during application of this composite measure must be acknowledged here. Misclassification due to self-identified race and ascertainment of other risk factors may contribute to residual confounding affecting the estimation of the association between race and MACE. This study also has several strengths including the use of data from a relatively large cohort of both Black and White men and women who were enrolled using a standardized protocol. This study also adjusted for multiple behavioral and lifestyle covariates including educational status, which could contribute to differences in diagnosis, access to care and treatment. Finally, the effects of genetics as well as the interaction between race and MACE risk factors were examined.

Conclusions: While this study found an overall higher prevalence of MACE among Black participants, race was not significantly associated with MACE after adjusting for comorbidities. This suggests the risk of MACE may be modified through medical management affecting disease outcomes. Additionally, racial disparities in health care may be the most significant contributing influence on the results observed here. No effect modification was found on this association since none of the SNPs met the initial screening significance criteria ($p<0.001$) and therefore were not included in this assessment. Further research is warranted to better qualify the components included in the MACE composite outcome and to continue to assess the validity of this outcome measure representing cardiovascular disease risk.

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Table 2.1. Baseline characteristics by race*; ARIC, 1987-1989 (n=12770)

	Black (n=3138)	White (n=9632)	
	Mean (SD)	Mean (SD)	p-value[a]
Age (yr)	53.3 (5.8)	54.3 (5.7)	<0.0001
BMI (kg/m^2)	29.7 (6.1)	27.0 (4.9)	<0.0001
	N (%)	N (%)	
MACE	555 (17.7)	1235 (12.8)	<0.0001
Male	1175 (37.4)	4529 (47.0)	<0.0001
Family CVD History			
Paternal	561 (22.0)	3161 (35.5)	<0.0001
Maternal	428 (15.1)	1663 (18.0)	0.0002
Marital Status	1852 (59.8)	8267 (87.1)	<0.0001
High School Education	1873 (59.8)	8018 (83.4)	<0.0001
Current Smoking Status	919 (29.3)	2372 (24.6)	<0.0001
Current Alcohol Use	988 (31.8)	6269 (65.2)	<0.0001
Hypertension	1739 (55.7)	2587 (27.0)	<0.0001
High Cholesterol	812 (27.2)	2475 (25.8)	0.1148
Diabetes	602 (19.7)	834 (8.7)	<0.0001
Aspirin	893 (28.9)	5033 (52.6)	<0.0001

[a]Race differences: comparisons performed with t-tests for continuous variables, chi-square tests for categorical variables

Table 2.2. Baseline characteristics by major adverse cardiac events (MACE); ARIC (n=12770)

	MACE[a] (n=1790)	No MACE (n=10980)	
	Mean (SD)	Mean (SD)	p-value[b]
Age (yr)	56.1 (5.5)	53.8 (5.7)	<0.0001
BMI (kg/m^2)	28.6 (5.3)	27.5 (5.3)	<0.0001
	N (%)	N (%)	
Black Race	555 (31.0)	2583 (23.5)	<0.0001
Male	1059 (59.2)	4645 (42.3)	<0.0001
Family CVD History			
Paternal	553 (35.1)	3169 (32.0)	0.0150
Maternal	355 (21.2)	1736 (16.7)	<0.0001
Marital Status	1362 (77.3)	8757 (80.9)	0.0005
High School Education	1186 (66.3)	8705 (79.4)	<0.0001
Current Smoking Status	640 (35.8)	2651 (24.2)	<0.0001
Current Alcohol Use	895 (50.3)	6362 (58.1)	<0.0001
Hypertension	958 (53.8)	3368 (30.8)	<0.0001
High Cholesterol	596 (33.9)	2691 (24.8)	<0.0001
Diabetes	458 (25.8)	978 (9.0)	<0.0001
Aspirin	854 (48.2)	5072 (46.6)	0.2140

[a]MACE was defined as first occurrence of myocardial infarction, stroke or CHD-related death;
[b]Comparisons performed with t-tests for continuous variables, chi-square tests for categorical variables

Table 2.3. Association between race and major adverse cardiac events (MACE), adjusting for traditional risk factors for MACE and SNPs; results of Cox proportional hazard models; ARIC, 1987-1989

	N	HR (95% CI)	Variable(s) in model
Model 1	12770	1.46 (1.32,1.61)[a]	Race
Model 2	12751	1.55 (1.40,1.72)[a]	Model 1 + age, gender, BMI
Model 3	12704	1.32 (1.18,1.47)[a]	Model 2 + alcohol use and cigarette use
Model 4	12512	1.19 (1.06,1.33)[b]	Model 3 + marital status and educational status
Model 5	12409	1.24 (1.10,1.39) [a]	Model 4 + current aspirin use
Model 6	12189	0.98 (0.87,1.11)	Model 5 + high cholesterol, diabetes and hypertension

Reference is White race; [*]p<0.001; [b]p<0.05

Table 2.4. Adjusted independent associations of race, and each covariate with major adverse cardiac events (MACE); results of Cox proportional hazard Model 6, ARIC, 1987-1989 (n=12189)

	HR (95% CI)
Black Race	0.98 (0.87,1.11)
Age (per 1 yr)	1.06 (1.05,1.07)[a]
Gender (male)	1.92 (1.68, 2.19)[a]
BMI (kg/m^2)	1.02 (1.01,1.03)[b]
Marital Status (yes)	0.89 (0.78, 1.00)
Educational Status (yes)	0.78 (0.70,0.87)[a]
Cigarette Use (yes)	1.91 (1.67,2.19)[a]
Alcohol Use (yes)	0.79 (0.71,0.87)[a]
Aspirin Use (yes)	1.11 (1.00,1.23)[b]
High Cholesterol (yes)	1.36 (1.22,1.50)[a]
Diabetes (yes)	2.28 (2.03,2.56)[a]
Hypertension (yes)	1.15 (1.05,1.27)[a]

Reference is White race; Model adjusted for race, age, gender, BMI, cigarette use, alcohol use, marital status, educational status, aspirin use, high cholesterol, diabetes, hypertension;
[a]$p<0.001$; [b]$p<0.01$

Supplemental Table 2.A. Association of SNP with major adverse cardiac events (MACE) by race; screening results of univariate logistic regression, ARIC, 1987-1989

	Black	White
	p-value	p-value
PON1 SNPs		
rs2057681	0.5912	0.0446‡
rs3917527	0.1525	0.0039‡
rs2301711	0.9103	0.0158‡
rs2299260	0.4784	0.6721
rs2299261	0.6599	0.5739
rs854568	0.6431	0.4192
rs13223537	0.3682	0.6726
rs705378	0.7088	0.5253
rs854569	0.1142	0.4523
rs17166829	0.0330‡	0.9589
rs3917538	0.2786	0.5578
rs3917521	0.3223	0.6179
rs854565	0.9087	0.0240‡
rs854566	0.9492	0.2523
rs2237583	0.3767	0.1864
rs854572	0.1852	0.9575
rs3917541	0.3658	0.0049‡
rs3917551	0.2052	0.0041‡
rs3917550	0.9750	0.4234
rs2074354	0.2849	0.8526
rs3917490	0.6963	0.7713
rs2299262	0.2017	0.4169
rs854571	0.0245‡	0.6734
rs13236941	0.0370‡	0.7080
rs2272365	0.4625	0.8396
rs705382	0.2693	0.9339
rs2269829	0.5676	0.0483‡
rs2299257	0.8453	0.0799

References: *p<0.001; ‡p<0.05

Supplemental Table 2.A. Association of SNP with major adverse cardiac events (MACE) by race; screening results of univariate logistic regression, ARIC, 1987-1989, continued

	Black	White
	p-value	p-value
PON2 SNPs		
rs2299267	0.9991	0.9154
rs43037	0.0424‡	0.7042
rs7778623	0.9760	0.6162
rs43052	0.4252	0.6123
rs4729190	0.8554	0.3005
rs1557782	0.9967	0.5502
rs43063	0.6183	0.6388
rs6958904	0.8288	0.7846
rs2299263	0.6084	0.7650
rs7785039	0.4878	0.9684
rs3757707	0.1712	0.7622
rs43061	0.6193	0.4339
rs43065	0.1491	0.5700
rs2374993	0.8612	0.7983
rs10241004	0.8500	0.8825
rs10261470	0.6424	0.6136
rs10953151	0.4355	0.8897
rs6973380	0.8062	0.7005
rs10487133	0.0163‡	0.9451
rs7493	0.8390	0.7165
rs12534203	0.4505	0.9360
rs10953149	0.4896	0.9430
rs12535571	0.8993	0.9899
rs1639	0.3253	0.5626
rs43044	0.6536	0.2165
rs6950550	0.6212	0.4517
rs12530498	0.5058	0.5507
rs43048	0.8311	0.1943
rs7802018	0.6417	0.7300
PON3 SNPs		
rs468	0.2708	0.7405
rs1053275	0.7518	0.7637
rs11768074	0.8650	0.8064
rs9641162	0.0935	0.6589
rs10953143	0.1210	0.8951

References: *p<0.001; ‡p<0.05

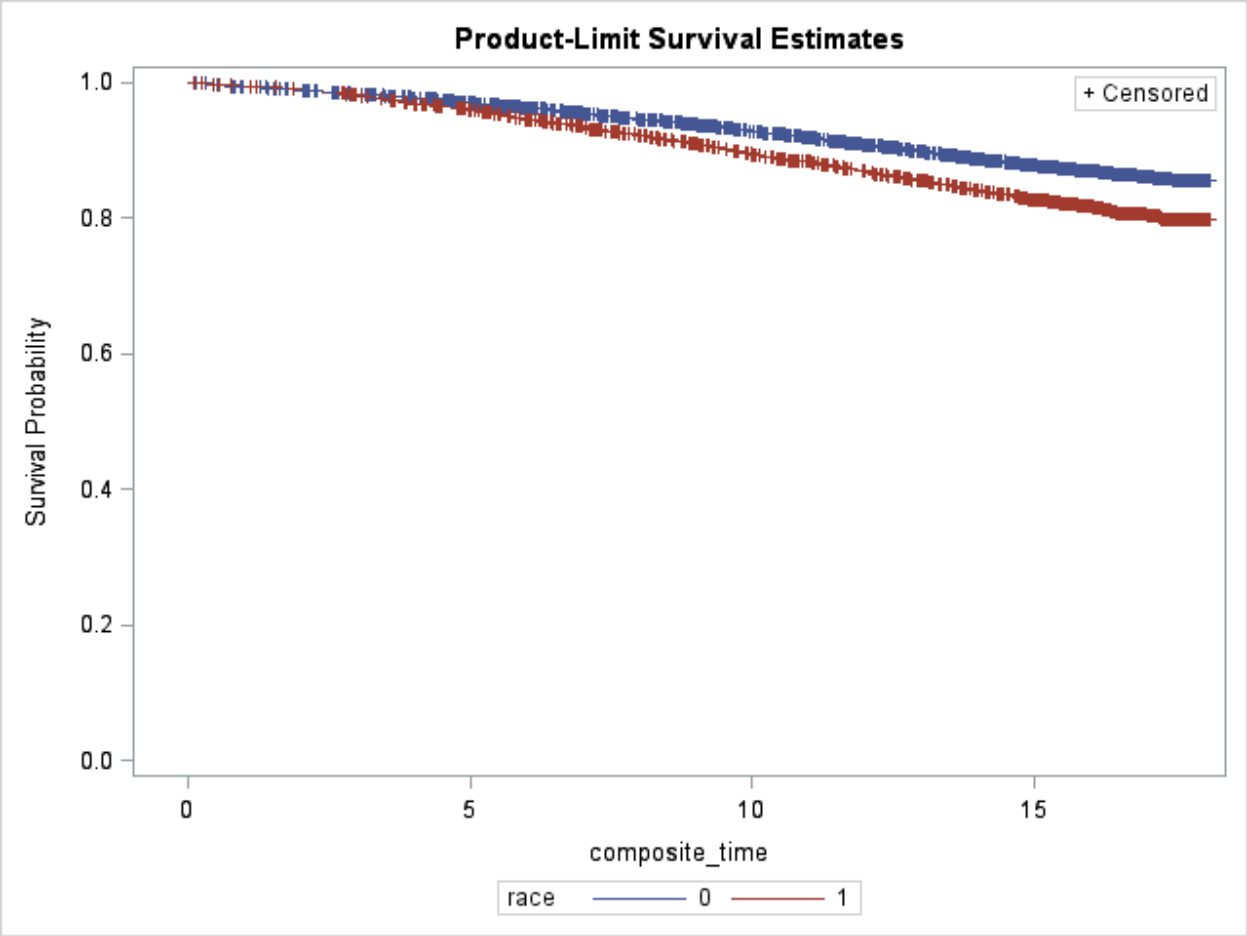

Figure 2.1. Kaplan-Meier curve for participants to reach first major adverse cardiac event (MACE); ARIC, 1987-1989 (N=12770)

Reference: White Race (0), Black Race (1); Test of Equality over Strata (p<0.0001)

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CHAPTER 3

Association of Race and Peripheral Artery Disease: the Atherosclerosis Risk in Communities (ARIC) Cohort

Abstract

Objective: To evaluate the association of self-reported race with peripheral artery disease (PAD) and modification of this association by paraoxonase gene (*PON*1, *PON*2 and *PON*3) single nucleotide polymorphisms (SNPs).

Methods: This cross-sectional study included 11,992 Black or White participants from the Atherosclerosis Risk in Communities (ARIC) cohort with *PON* genotyping. Ankle brachial index (ABI) was measured at baseline (1987-1989); PAD was defined as ABI<0.90. Data also included demographic, health and behavioral information. Logistic regression was used to evaluate the association between race and PAD after adjustment for age, gender, BMI, education, smoking, high cholesterol, hypertension and diabetes. The associations between *PON* SNPs and PAD were also assessed.

Results: Blacks comprised 24.6% of the ARIC cohort; overall, 4.0% of participants had PAD. After adjustment for age, gender and BMI, Blacks had 1.27 times greater odds of PAD than Whites (95%CI=1.04,1.57), but this association became non-significant after adjustment for smoking, education, cholesterol, hypertension and diabetes. None of the SNPs evaluated met significance after Bonferroni correction for multiple comparisons ($p<0.001$).

Conclusions: No association between race and PAD was identified after adjusting for health and behavioral covariates, suggesting that modifiable risk factors are major determinants of PAD. Further studies are needed to confirm this observation.

Introduction

It is estimated that 12-20% of persons older than 60 years of age, or 8.5 million people in the US have peripheral arterial disease (PAD) [1]. PAD is a predictor of future CVD events, is prevalent worldwide, and found equally in men and women [1]. PAD occurs most often in the lower extremities and is defined as >50% arterial stenosis as indicated by an ankle-brachial index (ABI) value <0.9 [2]. ABI is a low cost, office-based assessment that accurately diagnoses PAD [2]. Normal ABI values range from 0.9 to 1.4 [2,3].

Risk factors for PAD include older age, high cholesterol, hypertension, diabetes and smoking [1]. Prior studies of the association between race and PAD have been inconsistent [4,5,6] with some reporting higher rates of PAD in Blacks but others failing to find racial differences. For instance, in 2,343 participants from the San Diego Population study, Criqui *et al.* found that Blacks had higher odds of PAD (OR=2.30, p<0.024) than Whites after adjusting for covariates [7]. In contrast, there were no significant differences between Blacks and Whites in odds of PAD diagnosis (OR=1.89; 95%CI=0.89, 3.99) in a cohort of 403 patients from Houston, TX [8].

Additionally, genetics may modify the association between race and PAD. The development of atherosclerotic vascular diseases such as PAD are caused by oxidative damage from lipids and lipoproteins; this process may be mitigated by paraoxonase (PON) antioxidant enzymes [9,10,11] by reducing oxidative damage to low-density lipoproteins [12]. A recent case control study reported that PON1 concentrations and activities were lower in 66 PAD patients as compared to 8 controls [13]. Another case control study of 37 older participants (mean age 69.9$\pm$9 years) with PAD found that *PON1* genotype and PON1 activity had direct relations to brachial flow-mediated vasodilation (p=0.0004) [12]. To our knowledge, no previous study has examined the possibility that *PON* genes moderate the association between race and PAD.

The purpose of this study was to examine the association of Black and White race with peripheral artery disease, and to evaluate the effect of paraoxonase single nucleotide polymorphisms (SNPs) on this association using data from the Atherosclerosis Risk in Communities (ARIC) [14] Study cohort.

Methods

This study was approved by the University of California San Diego Human Research Protections Program (#160359X) and data was collected through authorized access from dbGaP. The ARIC [14] study collected data at four sites and was supported by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health; each site obtained institutional review board approval and written informed consent from participants. All data analyses were performed using SAS® University Edition (SAS Institute, Cary, NC).

ARIC: The Atherosclerosis Risk in Communities (ARIC) Study [14] is a prospective cohort study conducted in 4 communities in the United States (Washington County, MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN) each enrolling approximately 4,000 participants selected by probability sampling. A total of 15,972 study participants aged 45-64 years were examined at baseline (1987-1989) and re-examined every three years during four follow-up visits, with the last follow-up occurring in 2013. Since 2012, participants have been contacted annually by telephone to assess health status. Enrolled participants were Black (27%) and White (73%) men and women. Objectives were to investigate the etiology and natural history of atherosclerosis and to examine the risk factors and progression of subclinical to clinical cardiovascular disease events including coronary heart disease, heart failure, stroke and atrial fibrillation. In addition, the ARIC study examined genetic and environmental risk factors leading to ventricular dysfunction and vascular stiffness.

Participants: This study was limited to the 11,992 ARIC participants (24.6% Black, 75.4% White) with ABI values <1.4 for whom *PON* genotyping data was available [Figure 3.1].

Variables

Race: In the ARIC [14] study, race was dichotomized as self-identified Black or White.

Peripheral Artery Disease: PAD was defined based on ABI values from the baseline visit. Single systolic blood pressure measures were taken in one upper extremity and one lower extremity to generate ABI values calculated as the ratio of lower to upper extremity [15]. Based on convention [1], participants with ABI values <0.9 were categorized with PAD and participants with ABI values ≥0.9 and <1.4 were categorized as normal and free of PAD. Participants with ABI values ≥1.4 (n=779) were excluded from this analysis [Figure 3.1].

Covariates: Demographic characteristics (e.g., age, education, marital status), health history, body mass index (BMI) kg/m^2 and results of fasting laboratory assays were obtained from the baseline visit 14]. Family history included maternal and paternal CHD events. Current marital status (yes/no), high school graduate or more education (yes/no), current cigarette smoking (yes/no) and alcohol use (yes/no) were assessed at baseline. Measures of systolic and diastolic blood pressure were obtained and 12-hour fasting glucose and cholesterol levels were assayed from blood samples at baseline. Participants taking anti-diabetic medication or having fasting glucose ≥126 mg/dl were categorized as having diabetes mellitus [16]. Those taking cholesterol-lowering medication or having laboratory assessed cholesterol >240 mmol/L were categorized as having high cholesterol [17]. Participants taking antihypertensive medications or having systolic pressure >140 mmHg or diastolic pressure >90 mmHg were categorized as hypertensive [18]. Intake of current medications, including aspirin, anti-diabetic medication, antihypertensive medication and lipid lowering medication were determined by review of labelled containers [14] participants brought to the clinic visit.

Genotyping: Whole genome genotyping was performed using the Affymetrix 6.0 array platform [14]; there were 82 *PON* SNPs (43 *PON1*, 32 *PON2*, 7 *PON3*) available in the ARIC cohort which included (±) 20 kb window around each gene region. After excluding SNPs with minor allelic frequencies (MAF) <5%, 62 SNPs remained available for screening analysis. All SNPs were in Hardy-Weinberg equilibrium and had ancestry-specific allele frequencies similar to

those reported in publicly available databases (https://www.ncbi.nlm.nih.gov/projects/gapsolr/facets.html). The 62 SNPs were screened for significant association with PAD in Blacks and Whites combined and separately using a significance level of $p<0.001$ (0.05/45) after Bonferroni correction for multiple comparisons confirmed 45 independent SNPs using the Nyholt method (https://neurogenetics.qimrberghofer.edu.au/matSpDlite/). Five principal component analysis covariates (PCs), obtained from the PLINK routine [19] were used to adjust for residual population stratification. SNPs were coded as continuous variables for analysis using an additive genetic model.

Statistical Analysis: Descriptive statistics were calculated and reported as percentages for categorical variables and means (± standard deviations [SD]) for continuous data. Differences by race and PAD were analyzed using independent t-tests for continuous variables and chi-square analyses for categorical variables. All covariates were noncollinear based on correlation coefficients of $r<0.30$. Covariates with at least marginally significant differences by race and presence of PAD as well as known confounders of the race-PAD association were retained for further analysis. Logistic regression was used to model the odds of PAD for Blacks compared to Whites. Statistical significance was defined as $p<0.05$.

Forward stepwise multivariate logistic regression analysis was used to identify confounders (defined as a 10% change in the estimated coefficient between full and reduced models) and to assess the association between race and PAD, after adjustment for covariates. Model 1 examined the unadjusted association between race and PAD. Model 2 adjusted for age, gender and BMI. Model 3 adjusted for Model 2 variables and cigarette use. Model 4 adjusted for Model 3 variables and educational status. Model 5 adjusted for Model 4 variables and high cholesterol, hypertension and diabetes. None of the 62 *PON* SNPs screened met the significance criteria of $p<0.001$) and were therefore not retained for further analysis. Interactions

between race and covariates were evaluated with possible effect modification considered when $p<0.05$.

Results

Table 3.1 shows baseline differences for Blacks and Whites in the ARIC cohort. Peripheral artery disease as defined by ABI <0.90, was found in 482 (4.0%) participants, with a higher prevalence in Blacks (5.0%) than Whites (3.7%) (p=0.003). Blacks were younger (53.3±5.8 vs. 54.3±5.7 years respectively, p<0.0001), but had a higher mean BMI (29.6±6.0 vs. 26.9±4.8 kg/m^2, respectively, p<0.0001) than Whites. There was a lower proportion of men (p<0.0001) among Black participants and they were less likely to have a paternal (p<0.0001) or maternal (p=0.0002) family history of CVD than whites. Fewer Blacks used alcohol, completed a high school education, were married or used aspirin (p's<0.0001). However, Blacks were more likely to smoke and to have a diagnosis of hypertension or diabetes (p's<0.0001); there was no difference in prevalence of high cholesterol (p=0.22).

Comparisons of baseline characteristics by PAD diagnosis showed that participants with PAD were significantly older (55.4+5.9 vs. 54.0+5.7 years, respectively (p=<0.0001) and had a higher average BMI 28.1±5.9 vs. 27.6±5.2 kg/m^2 respectively, p=0.018) (see Table 3.2). Those with PAD were more likely to be Black (p=0.003), less likely to be male (p<0.0001), and had a higher prevalence of smoking, hypertension, high cholesterol and diabetes (p's<0.0001). However, those with PAD were less likely to be married (p=0.0005), to have completed high school education (p<0.0001) and to use alcohol (p=0.018). None of the 62 *PON* SNPs in the ARIC population met the screening criterion for statistical significance (p<0.001) and were therefore not retained for further analysis (Supplemental Table 3.A).

Step-wise logistic regression models (Table 3.3) showed that after adjusting for age, gender and BMI (Model 2), Blacks had 1.27 times greater odds of PAD than Whites (OR=1.27, 95% CI=1.04, 1.57). This association became non-significant in subsequent models that adjusted for cigarette use, educational status, high cholesterol, diabetes and hypertension

(Models 3-5). None of the SNPs met the initial screening significance criteria ($p<0.001$) and therefore were not included in the model to assess effect modification on this association.

Main effects for all variables in Model 5 are shown in Table 3.4. Age, cigarette use, high cholesterol, hypertension (p's<0.001) and diabetes ($p<0.01$) were all significantly and independently associated with higher odds of PAD. Male gender was significantly associated with lower odds of PAD ($p<0.001$). There were no significant interactions between race and any of the covariates.

Discussion

Atherosclerosis is the formation of fatty deposits of mostly low-density lipoprotein (LDL) cholesterol on the interior lining of the arterial walls [15]. Atherosclerosis manifests in a variety of ways, including peripheral artery disease (PAD) [4], and accounts for 1 in every 4 US deaths [20]. In this cross-sectional study of 11,992 ARIC cohort study participants, Blacks had higher odds of PAD (OR=1.27, 95%CI=1.04,1.57) than Whites after adjustment for age, BMI, and gender, but this association was attenuated to non-significance after adjustment for comorbidities (i.e. hypertension and diabetes) and lifestyle risk factors (i.e. smoking, education). While this study did not find significant effect modification of *PON* SNPs on the association between race and PAD, to our knowledge this is the first study to report results of such an evaluation.

In this study of men and women aged 45 years and older from the ARIC cohort, there was a higher prevalence of PAD in Blacks than Whites (5.0% vs. 3.7%). These results are consistent with other [5,7], including Selvin *et al*. who reported higher rates of PAD among Blacks than Whites (7.9% vs. 4.4%) using NHANES data [21]. Also, in accord with the NHANES data [21], we found a higher prevalence of cardiovascular disease risk factors including older age, higher BMI, current smoking, hypertension, high cholesterol and diabetes, among ARIC participants with PAD. However, our results suggested that race was not independently associated with PAD as adjustment for PAD risk factors such as smoking,

hypertension and diabetes attenuated the association to non-significance. Results of the present study are in accord with Collins *et al.,* who also reported no association between race (Black or White) and PAD after adjustment for diabetes, smoking, hypertension and age [8].

In contrast, studies from selected populations have reported associations between race and PAD [5,7]. For example, the San Diego Population study [7] reported that Blacks had higher odds of PAD than Whites (OR=2.30, p<0.024). However, that study included participants with prior revascularization, which normalizes ABI, resulting in a higher prevalence of PAD than observed in ARIC. In addition, a study based on the MESA cohort reported that Blacks had significantly greater odds of PAD than Whites (OR=1.67; 95%CI=1.23, 2.26) after adjusting for multiple covariates [5]; however, MESA excluded those with known CVD, making a direct comparison with the ARIC cohort, which had no such exclusions, difficult.

The lack of a significant association between race and PAD after adjustment for health and behavioral CVD risk factors in the present study suggests that differences in PAD are influenced more by racial disparities in risk factors and their management rather than racial differences in the biological development of atherosclerosis. Prior research reports that Blacks present at a later clinical stage in the development of PAD than Whites. Furthermore, diabetes and neuropathy, both more prevalent in Blacks, affect the distal arteries and contribute to a PAD diagnosis [22]. Finally, it has been previously demonstrated that racial disparities exist in health care, with Blacks receiving lower quality than the majority of Whites in the United States [23,24,25,26,27].

The influence of genetic differences on the risk of PAD is relatively unknown. PON1 enzymes have anti-inflammatory and antioxidant properties and may protect against atherosclerosis [28]. The enzyme is expressed in the liver and delivered to multiple tissues not expressing the enzyme [28]. Decreased PON1 enzyme activity has been shown to increase inflammation in animal studies, and increase oxidative stress among patients with atherosclerosis, diabetes and/or hypercholesterolemia [28]. Recent studies report associations

between *PON* SNPs and atherosclerotic disease or related phenotypes. The *PON1* rs2299260 SNP was significantly associated with a 50% increased risk of resistant hypertension in European-Americans for each additional copy of the C allele [29]. Additionally, another *PON1* promoter region SNP, rs705379, was associated with blood pressure in middle-aged individuals [30]. However, others have failed to find an association between *PON1* polymorphisms and cardiovascular disease. In two separate meta-analyses, no association was found between a L55M *PON1* polymorphism and ischemic stroke [31], and no association was found between the 55L allele and stroke after adjusting for risk factors [32].

It is biologically plausible that there is an association between race and PAD after adjustment for risk factors. Previous studies show that Blacks have a thicker and stiffer aorta compared to Whites [33] and aortic stiffening may artificially lower ABI measures. Aboyans *et al*. found that among the healthy participants of the MESA cohort, Blacks had ABI values approximately 0.2 lower than Whites [22]. We may have failed to find this association in adjusted models because of the small sample of Blacks relative to Whites in this cohort. Future studies assessing the genetic differences and gene-environment interactions with respect to PAD need to be evaluated across diverse ethnic study populations with adequate sample sizes [34].

Several limitations and strengths of this study were considered. ABI may be underestimated due to categorization based on a single measure of systolic blood pressure from one upper and one lower extremity and rather than multiple measures in all extremities. The ARIC cohort may not be comparable to other cohorts such as MESA that have reported an association with race and PAD because of differences in selection criteria [35]. Misclassification due to self-identified race and ascertainment of other risk factors may contribute residual confounding affecting the estimation of the association between race and PAD. Here PAD was defined as ABI<0.90 without consideration of prior revascularization, which would normalize ABI, potentially underestimating the prevalence of PAD [36]. Finally, this study excluded

participants with an ABI≥1.4 because of arterial stiffening due to calcification; it is estimated that PAD prevalence affects approximately 1% of persons in this group [7], resulting in an underestimation of the prevalence of PAD. This study also has several strengths including the use of data from a relatively large cohort of both Black and White men and women who were enrolled using a standardized protocol. This study also adjusted for educational status, which could contribute to differences in diagnosis, access to care and treatment. Finally, the effects of genetics as well as the interaction between race and PAD risk factors were examined. Conclusions: While this study found an overall higher prevalence of PAD among Black participants, race was not significantly associated with PAD after adjusting for health and behavioral risk factors PAD. This suggests the risk of PAD may be modified through medical management and lifestyle changes. No effect modification was found on this association since none of the SNPs met the initial screening significance criteria ($p<0.001$) and therefore were not included in this assessment. To our knowledge, this is the first study assessing the effect of the *PON* gene cluster (*PON1, PON2 and PON2*) on the association between race and peripheral artery disease. Additional studies are needed to further assess the impact of genetics on the association between race and peripheral artery disease.

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Table 3.1. Baseline characteristics by race; ARIC, 1987-1989 (n=11992)

	Black (n=2952)	White (n=9040)	
	Mean (SD)	Mean (SD)	p-value*
Age (yr)	53.3 (5.8)	54.3 (5.7)	<0.0001
BMI (kg/m^2)	29.6 (6.0)	26.9 (4.8)	<0.0001
	N (%)	N (%)	
Peripheral Artery Disease	146 (5.0)	336 (3.7)	0.0032
Male	1106 (37.5)	4212 (46.6)	<0.0001
Family CVD History			
Paternal	531 (22.1)	2959 (35.4)	<0.0001
Maternal	397 (15.0)	1558 (18.0)	0.0002
Marital Status	1745 (59.9)	7757 (87.1)	<0.0001
High School Education	1766 (60.0)	7534 (83.4)	<0.0001
Current Smoking Status	856 (29.0)	2229 (24.7)	<0.0001
Current Alcohol Use	936 (32.0)	5907 (65.4)	<0.0001
Hypertension	1625 (55.3)	2420 (26.9)	<0.0001
High Cholesterol	753 (26.8)	2310 (25.6)	0.2231
Diabetes	565 (19.6)	776 (8.6)	<0.0001
Aspirin	846 (29.1)	4735 (52.7)	<0.0001

*Comparisons by race performed with t-tests for continuous variables and chi-square tests for categorical variables

Table 3.2. Baseline characteristics by peripheral artery disease (PAD); ARIC, 1987-1989 (n=11992)

	PAD† (n=482)	No PAD (n=11510)	
	Mean (SD)	Mean (SD)	p-value*
Age (yr)	55.4 (5.9)	54.0 (5.7)	<0.0001
BMI (kg/m^2)	28.1 (5.9)	27.6 (5.2)	0.0180
	N (%)	N (%)	
Black Race	146 (30.3)	2806 (24.4)	0.0032
Male	148 (30.7)	5170 (44.9)	<0.0001
Family CVD History			
Paternal	142 (33.7)	3348 (32.3)	0.5482
Maternal	89 (19.9)	1866 (17.1)	0.1349
Marital Status	352 (74.1)	9150 (80.6)	0.0005
High School Education	338 (70.1)	8962 (78.0)	<0.0001
Current Smoking Status	182 (37.8)	2903 (25.2)	<0.0001
Current Alcohol Use	249 (52.0)	6594 (57.5)	0.0177
Hypertension	222 (46.3)	3823 (33.4)	<0.0001
High Cholesterol	180 (37.9)	2883 (25.4)	<0.0001
Diabetes	87 (18.2)	1254 (11.0)	<0.0001
Aspirin	236 (49.3)	5345 (46.8)	0.2968

†PAD was defined as ABI values <0.9;
*Comparisons performed with t-tests for continuous variables and chi-square tests for categorical variables

Table 3.3. Association of race with peripheral artery disease (PAD) after adjustment for PAD risk factors and SNPs; results of multivariate logistic regression, ARIC, 1987-1989

	N	OR (95% CI)	Variable(s) in model
Model 1	11992	1.35 (1.11,1.65)*	Race
Model 2	11981	1.27 (1.04,1.57)‡	Model 1 + age, gender, BMI
Model 3	11973	1.22 (0.99,1.50)	Model 2 + cigarette use
Model 4	11962	1.17 (0.94,1.45)	Model 3 + educational status
Model 5	11729	1.04 (0.83,1.30)	Model 4 + high cholesterol, diabetes, and hypertension

Reference is White race; *p<0.001; ‡p<0.05

Table 3.4. Adjusted independent associations of race, each covariate with PAD; results from multivariate logistic regression Model 5, ARIC, 1987-1989 (n=11729)

	OR (95% CI)
Black Race	1.04 (0.83,1.30)
Age (per 1 yr)	1.04 (1.02,1.06)*
Gender (male)	0.54 (0.45 0.67)*
BMI (kg/m^2)	1.01 (0.99,1.03)
Cigarette Use (yes)	2.00 (1.64,2.44)*
Educational Status (yes)	0.86 (0.70,1.07)
High Cholesterol (yes)	1.53 (1.26,1.86)*
Diabetes (yes)	1.44 (1.11,1.87)†
Hypertension (yes)	1.43 (1.16,1.75)*

Reference is White race; Model adjusted for race, age, gender, BMI, cigarette use, educational status, high cholesterol, diabetes and hypertension; *p<0.001, †p<0.01

Supplemental Table 3.A. Association of SNP with peripheral artery disease (PAD) by race; screening results of univariate logistic regression, ARIC, 1987-1989

	Black	White
	p-value	p-value
PON1 SNPs		
rs2057681	0.9947	0.8722
rs3917527	0.8375	0.5322
rs2301711	0.1360	0.6351
rs2299260	0.5123	0.1003
rs2299261	0.8122	0.2193
rs854568	0.2244	0.1633
rs13223537	0.1535	0.0684
rs705378	0.9282	0.4397
rs854569	0.5176	0.4767
rs17166829	0.0805	0.7485
rs3917538	0.4571	0.9580
rs3917521	0.5483	0.9439
rs854565	0.1548	0.0647
rs854566	0.3861	0.0875
rs2237583	0.0781	0.6137
rs854572	0.9750	0.1455
rs3917541	0.6250	0.4097
rs3917551	0.6899	0.5496
rs3917550	0.2443	0.9268
rs2074354	0.7320	0.7374
rs3917490	0.6549	0.8163
rs2299262	0.9118	0.4064
rs854571	0.6993	0.8011
rs13236941	0.9029	0.5255
rs2272365	0.9712	0.3879
rs705382	0.9449	0.2007
rs2269829	0.9903	0.8249
rs2299257	0.4264	0.3982

References: *p<0.001; ‡p<0.05

Supplemental Table 3.A. Association of SNP with peripheral artery disease (PAD) by race; screening results of univariate logistic regression, ARIC, 1987-1989, continue

	Black	White
	p-value	p-value
PON2 SNPs		
rs2299267	0.2237	0.6372
rs43037	0.4647	0.0695
rs7778623	0.8670	0.6447
rs43052	0.4381	0.1883
rs4729190	0.4182	0.3619
rs1557782	0.7779	0.7316
rs43063	0.1277	0.2860
rs6958904	0.3154	0.8105
rs2299263	0.0753	0.6131
rs7785039	0.7217	0.2540
rs3757707	0.5383	0.2228
rs43061	0.0432‡	0.1082
rs43065	0.8161	0.8751
rs2374993	0.5561	0.3062
rs10241004	0.0527	0.0792
rs10261470	0.4254	0.3101
rs10953151	0.9777	0.2626
rs6973380	0.0277‡	0.5168
rs10487133	0.6886	0.0118‡
rs7493	0.0280‡	0.6093
rs12534203	0.6239	0.2769
rs10953149	0.7062	0.4616
rs12535571	0.8454	0.6763
rs1639	0.0244‡	0.4959
rs43044	0.9125	0.0821
rs6950550	0.1685	0.4825
rs12530498	0.4852	0.9222
rs43048	0.3622	0.0228‡
rs7802018	0.4710	0.8903
PON3 SNPs		
rs468	0.1868	0.0344‡
rs1053275	0.7866	0.2072
rs11768074	0.6982	0.1708
rs9641162	0.0793	0.9115
rs10953143	0.4628	0.2114

References: *p<0.001; ‡p<0.05

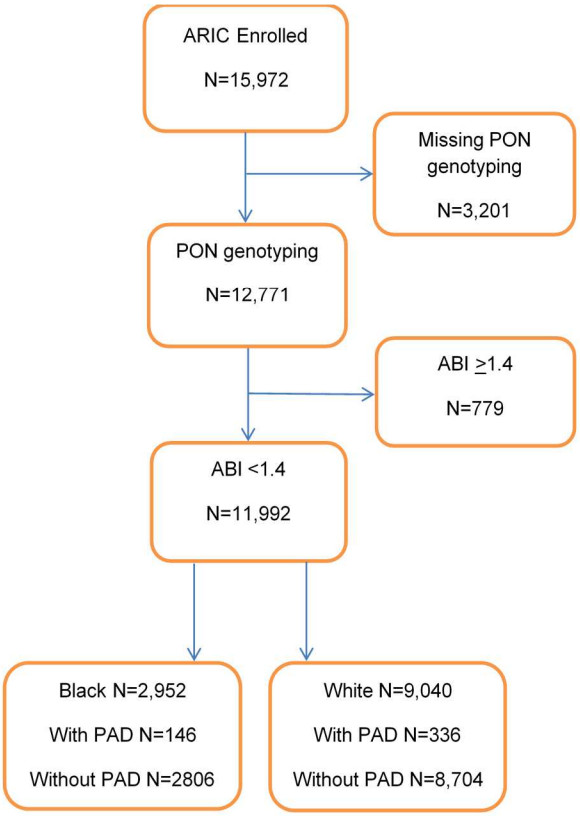

Figure 3.1. Sample Population Inclusion Chart; ARIC, 1987-1989

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CHAPTER 4

Association of Race and Change in Ankle-Brachial Index: the Atherosclerosis Risk in Communities (ARIC) Cohort

Abstract

Objective: This study evaluates the association of self-reported race with change in ankle-brachial index (ABI) over time and modification of this association by paraoxonase gene (*PON1, PON2 and PON3*) single nucleotide polymorphisms (SNPs).

Methods: This longitudinal study included 11,992 (N=2,952 Black, N=9,040 White) participants from the Atherosclerosis Risk in Communities (ARIC) cohort with *PON* genotyping. Health, demographic and behavioral information were collected, and ankle-brachial index (ABI), an indicator of peripheral artery disease (PAD) was measured at baseline (1987-1989). Mixed-effects models examined whether race was associated with change in ABI over time (between race and within race) from baseline through follow-up visit 3(1993-1995) and visit 4(1996-1998), after adjustment for known PAD risk factors such as age, smoking, hypertension, high cholesterol and diabetes.

Results: Blacks comprised 24.6% of the ARIC cohort; baseline ABI was 1.12$\pm$0.13 in Blacks and 1.13$\pm$0.13 in Whites (p=0.0003). Change in ABI over time differed between Whites and Blacks (race-time interaction, p<0.0001); ABI *increased* from baseline to visits 3 and 4 in Whites, (p's<0.0001); but *decreased* from baseline to visit 3 in Blacks, (p<0.0001). There was a significant interaction between education and race. Stratified analyses showed that ABI values were better in both Blacks and Whites who completed high school or more education compared to those who completed less education. None of the *PON* SNPs met the significance level (p<0.001) after Bonferroni correction for multiple comparisons.

Conclusions: ABI differences by race were small and although statistically significant, may not be clinically significant. Change in ABI over time varies by race and may be modified by education. Results suggest that higher education may influence the lifestyle and behavioral choices contributing to better ABI in both Blacks and Whites. Further studies are needed to confirm this observation.

Introduction

Peripheral artery disease (PAD) occurs most often in the lower extremities and is the third leading cause of atherosclerotic cardiovascular death after coronary artery disease and stroke [1]. Risk factors for PAD include older age, high cholesterol, hypertension, diabetes and smoking [2]. The ankle-brachial index (ABI) is a reproducible and valid measure for diagnosing PAD; and has been the primary screening tool for PAD during the past few decades [2] because it is a low cost, non-invasive, office-based test [3]. By convention, ABI <0.9 indicates >50% arterial stenosis whereas normal ABI ranges from $\geq$0.90 to <1.40 [4,5].

Previous studies report that ABI is a subclinical predictor of cardiovascular events [6,7,8,9]. In 13,150 participants from the ARIC cohort, Gupta *el al.* found a 40% (95%CI=1.12, 1.74) increased risk of heart failure in those with low ABI (<0.90) compared to those with normal ABI (1.01-1.40) [6]. Yeboah *et. al.,* in 1,330 participants from the MESA cohort, found that ABI was an independent predictor of incident CHD/CVD beyond traditional risk factors for individuals of intermediate risk [7]. A meta-analysis of 16 population studies found that both low ($\leq$0.9) and high ABI (>1.4) were significant independent predictors of CVD events and recommended inclusion of ABI to enhance the Framingham Risk Score for CVD risk prediction [8]. In the ARIC cohort, each 0.10 decline in ABI was associated with greater increase CHD hazard in Blacks than Whites [9].

PAD is prevalent worldwide and found in all U.S. ethnic groups [3]. Some, but not all, studies report that Blacks have a higher prevalence of PAD compared to Whites [2,3,10], independent of traditional cardiovascular risk factors [4,11]. However, to our knowledge, the association between race and change in ABI over multiple timepoints has not been previously reported.

Genetics may modify the association between race and ABI. Oxidative damage to lipids and lipoproteins contributes to the development of atherosclerotic vascular diseases such as PAD. Previous studies report that this progression may be mitigated by paraoxonase (PON)

antioxidant enzymes [12,13,14] through reduction in low-density lipoprotein oxidation [15]. For example, in a case study of 37 older people (mean age 69.9±9 years) with PAD, *PON1* genotype and PON1 activity were directly related to brachial flow-medicated vasodilation (p=0.0004) [15]. Furthermore, among 66 PAD patients and 8 controls, PON1 concentrations and activities were decreased in individuals with PAD [16]. However, the potential modification of any race-ABI association by genetic factors has not been reported.

The purpose of this study was to evaluate the association of Black and White race with change in ankle-brachial index over time, and to evaluate the effect of paraoxonase single nucleotide polymorphisms (SNPs) on this association using data from a large well-characterized sample of older men and women.

Materials and Methods

This study used data from the ARIC [17] cohort with genetic data collected through authorized access from dbGaP. The multi-site ARIC study was supported by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health; each site obtained institutional review board approval and written informed consent from study participants prior to participation. This study was approved by the University of California San Diego Human Research Protections Program (#160359X); all analyses were performed using SAS® University Edition (SAS Institute, Cary, NC).

The Atherosclerosis Risk in Communities Study (ARIC) [17]: is a prospective cohort study investigating the etiology of atherosclerosis, examining the risk factors and progression of subclinical to clinical cardiovascular disease events conducted in 4 communities in the United States (Washington County, MD; Forsyth County, NC; Jackson, MS; and Minneapolis, MN) with each enrolling approximately 4,000 participants selected by probability sampling. Secondary study objectives examined environmental and genetic risk factors leading to vascular stiffness. A total of 15,972 study participants aged 45-64 years consisting of Black (27%) and White (73%) men and women were examined at baseline and re-examined during four follow-up visits

through 2013. Data for this analysis was collected at baseline (1987-1989), visit 3 (1993-1995) and visit 4 (1996-1998).

Participants: There were 11,992 ARIC participants (24.6% Black, 75.4% White) with ABI values <1.4 for whom *PON* genotyping data was available [Figure 4.1]. Of these, the 7,672 participants (5,925 Whites and 1,747 Blacks) who completed a baseline and at least one follow-up visit where ABI was measured, were included in the mixed effects repeated measures analysis.

Variables

Race: In the ARIC [17] study, race was categorized based on self-identification as Black or White.

Ankle-Brachial Index: Single systolic blood pressure measures were taken in one upper extremity and one lower extremity at baseline, visit 3 and visit 4 [17]; ABI values were calculated as the ratio of lower to upper extremity blood pressure [6] with ABI <0.9 [23] considered diagnostic of PAD and participants with ABI ≥1.4 (n=779) excluded from this analysis [Figure 4.1].

Covariates: Health history, demographic characteristics (e.g., age, education, marital status), body mass index (BMI) kg/m^2 and results of 12-hour fasting laboratory assays as well as measures of systolic and diastolic blood pressure were obtained at the baseline visit [17]. ARIC family history included maternal and paternal CHD events. Current marital status (yes/no), high school graduate or more education (yes/no), current cigarette smoking (yes/no) and alcohol use (yes/no) were assessed at baseline. Participants taking cholesterol-lowering medication or having cholesterol >240 mmol/L were categorized as having high cholesterol [18]; those taking anti-diabetic medication or having fasting glucose ≥126 mg/dl were categorized as having diabetes mellitus [19] and those taking antihypertensive medications or having systolic pressure >140 mmHg or diastolic pressure >90 mmHg were categorized as hypertensive [20]. Current medication use including aspirin, anti-diabetic and antihypertensive medication and lipid

lowering medication was determined by review of labelled containers [17] brought by participants to the baseline clinic visit.

Genotyping: There were 82 *PON* SNPs (43 *PON1*, 32 *PON2*, 7 *PON3*) available in the ARIC cohort which included (±) 20 kb window around each gene region. Whole genome genotyping was performed using the Affymetrix 6.0 array platform [14]. SNPs with minor allelic frequencies (MAF) less than 5% were excluded from the analysis, leaving 62 SNPs available for screening analysis. All SNPs were in Hardy-Weinberg equilibrium and had ancestry-specific allele frequencies similar to those reported in publicly available databases (https://www.ncbi.nlm.nih.gov/projects/gapsolr/facets.html). The 62 SNPs were screened for significant association with PAD in Blacks and Whites combined using a significance level of $p<0.001$ (0.05/45) after Bonferroni correction for multiple comparisons confirmed 45 independent SNPs using the Nyholt method (https://neurogenetics.qimrberghofer.edu.au/matSpDlite/). Five principal component analysis covariates obtained from the PLINK routine [21] were used to adjust for residual population stratification.

Statistical Analysis: ABI values were analyzed as a continuous variable. Baseline descriptive statistics were calculated and reported as rates for categorical data and means (± standard deviations [SD]) for continuous data. Differences by race and baseline ABI were examined using independent t-tests for continuous variables and chi-square analysis for categorical variables. All covariates were noncollinear as determined by a correlation coefficient of ($r<0.30$). Covariates as well as known confounders with at least marginally significant differences by race and baseline ABI were retained for further analysis. Mixed-effects models were used to assess the association between race and change in ABI over time as well as change in ABI over time within race. Results were reported as least squares (LS) means for participants with a baseline ABI and at least one follow-up (visit 3 or visit 4) ABI (mixed

modelling adjusts for follow-up visit missing data). Statistical significance was defined as $p<0.05$.

Covariates significantly associated with ABI ($p<0.05$) in the repeated measures model were retained for multivariable analysis. Non-significant covariates were removed using a backward step-wise model selection removing the covariate with the largest p-value first and comparing full and reduced models. Where the likelihood ratio test was $p<0.05$ and the covariates were $p<0.05$, the covariate was retained in the final model. Interactions between race and covariates in the final model with $p<0.05$ were considered potential effect modifiers and stratified in the final adjusted mixed-effects repeated measures model.

Results

Baseline differences between Blacks and Whites are shown in Table 4.1. Average baseline ABI was lower in Blacks than Whites (1.12 ± 0.13 vs. 1.13 ± 0.13, respectively; $p=0.0003$). Compared to Whites, Black participants were younger (54.3 ± 5.7 vs. 53.3 ± 5.8 years respectively; $p<0.0001$) and had higher mean BMI (26.9 ± 4.8 vs.29.6 ± 6.0 kg/m^2, respectively; $p<0.0001$). There was a lower proportion of men among Black participants ($p<0.0001$) and they were less likely to have a paternal ($p<0.0001$) or maternal ($p=0.0002$) family history of CVD than White participants. Blacks were also less likely to use alcohol, to have completed high school education or more, to be married and to be taking aspirin (p's<0.0001); Blacks were more likely to have hypertension, diabetes and be current smokers (p's<0.0001), but there was no significant difference by race in prevalence of high cholesterol ($p=0.22$) between Black and White participants. Of the 62 *PON* SNPs screened, none met the criterion for statistical significance ($p<0.001$) and were therefore, not retained for further analysis (Supplemental Table 4.A).

A mixed-effects model assessing the association between race and change in ABI over follow-up showed a significant race-time interaction (see Table 4.2). Additionally, among the covariates, adjusted analyses showed that older age ($p<0.05$), as well as cigarette use, high

cholesterol, diabetes and hypertension (p's<0.0001) were each independently associated with lower ABI overall (worse), while male gender and a high school or more education were each independently associated with higher ABI overall (better) (p's<0.0001). None of the SNPs met the initial screening significance criteria (p<0.001) and therefore were not included in the model to assess effect modification on this association.

Figure 4.2 shows mean ABI by race at each visit. After adjustment for age, gender, educational status, cigarette use, high cholesterol, hypertension and diabetes, there was a difference in ABI of 0.018 where Whites had significantly lower ABI than Blacks at baseline (p<0.0001), but significantly higher (p's<0.0001) ABI at visits 3 and 4 (ABI difference of 0.054 and 0.030, respectively). Among Whites within race analysis showed that ABI levels significantly (p's<0.0001) increased from baseline to visit 3 and visit 4 (ABI difference of 0.042 and 0.038, respectively). In contrast, among Blacks ABI significantly decreased by a difference of 0.012 between baseline and visit 3 (p<0.0001); the ABI difference of 0.0084 was not significantly lower at visit 4 (p=0.08).

When effect modification between race and each covariate was tested, the only significant interaction was between race and education (p=0.02). Stratification by education indicated that regardless of race, participants who had completed a high school education or more (Figure 4.3.A) had higher ABI than participants *with less than a* high school education (Figure 4.3.B) at follow-up visits 3 and 4; however, baseline ABI values were similar regardless of education level. Among participants *with* a high school education or more, ABI was significantly (p's<0.0001) higher among Whites than Blacks at both follow-up visits 3 and 4 (ABI difference of 0.060 and 0.033, respectively). Among those *without* a high school education, Whites had a significantly higher ABI value of 0.039 than Blacks at the visit 3 follow-up only (p=0.002) but there was no significant difference at visit 4 (ABI difference=0.001).

Discussion

Low ABI has been associated with increased 10-year cardiovascular mortality rate in men and women suggesting that ABI screening may improve cardiovascular risk prediction [8]. In this longitudinal study, Whites had significantly higher (better) ABI values at both follow-up visits compared to baseline than Blacks after adjustment for potentially confounding covariates. However, while these racial differences were statistically significant, the ABI differences were small (<0.15) [22,23] and not likely to be clinically meaningful. Race, age, education, cigarette smoking, cholesterol, diabetes and hypertension were all significantly and independently associated with change in ABI over time. ABI over time was better in both Blacks and Whites who completed a high school education compared to those with less education. Among Whites without a high school education, ABI decreased and was similar to Blacks without a high school education by visit 4 of the follow-up period. While statistically significant, these ABI differences were small (<0.15) [22,23] and therefore may not be clinically significant. Although this study did not find significant effect modification of *PON* SNPs on the association between race and ABI over time; to our knowledge this is the first study to report results of such an evaluation. Results from this study suggest that change over time in ABI by race may be modified by education or other lifestyle factors but not by genetic factors evaluated here.

Although differences may not be clinically significant, in this sample of men and women aged 45 and older, ABI was significantly lower in Blacks (1.12) than Whites (1.13) at baseline and both follow-up visits. These results are consistent with previous studies [2,24] of 1,775 healthy participants from the MESA cohort which reported that ABI values were on average, 0.02 lower in Blacks compared to non-Hispanic Whites. Furthermore, our results are also consistent with those of Singh *et al. who* reported mean ABI values of 1.11 in non-Hispanic Blacks compared to 1.13 in non-Hispanic Whites in 3,348 NHANES participants [24].

The association of higher educational level and better ABI has been previously reported. Aboyans *et al*. reported a correlation between higher education and increased ABI [2]. Additionally, a separate study of the MESA cohort reported that higher education was protective

against PAD [25]. Previous studies also reported an association between education and increased risk of cardiovascular disease and hypertension. In 13,948 ARIC cohort participants [26], Kubota *et al.* reported that over 50% of men and women with less than high school education had a CVD event in their lifetime as compared to 42.2% of men and 28.0% of women with some college or more. These results suggest the importance of education, associated with socioeconomic status), influencing access to quality health care information and its impact on decision-making towards healthier lifestyle and better health outcomes.

The effect of education on change in ABI over time in this study suggests that ABI is influenced more by racial disparities in risk factors and their management than racial differences in the biological development of atherosclerosis. Prior studies demonstrate that racial disparities exist with Blacks receiving lower quality health care than the majority of Whites in the United States [27,28,29,30,31]. Blacks present at a later clinical stage in the development of PAD than Whites. Furthermore, diabetes and neuropathy, both more prevalent in Blacks, affect the distal arteries and contribute to diagnosis of PAD [2]. In accord with this, the present study found Black race, increasing age, cigarette smoking, high cholesterol, diabetes and hypertension were associated with decreasing ABI, trending towards PAD.

The influence of genetic differences on ABI is relatively unknown. In this analysis, there was no significant effect modification of *PON* SNPs on the association between race and change in ABI over time, and to our knowledge this is the first study to examine this issue. Paraoxonase enzymes exhibit antioxidant properties and inhibit the formation and accumulation of macrophage cholesterol, thereby ameliorating the development of atherosclerosis [32]. Some studies reported positive associations between *PON* SNPs and PAD [33], cardiovascular disease [34] and blood pressure [35,36]. However, other studies failed to find an association between *PON* polymorphisms and stroke [37,38] or CAD [39]. This study did not find an association between *PON* SNPs and change in ABI over time. However, because these enzyme proteins prevent oxidative stress and inflammation, improved understanding of their influence on

change over time in inflammatory diseases such as atherosclerosis, merits additional research [40].

It is plausible that there is an association between race and change in ABI over time due to differences in biological risk factors. Aortic stiffening may artificially lower ABI measurements and previous studies show that Blacks have a thicker and stiffer aorta compared to Whites [5]. Among the healthy participants of the MESA cohort, Aboyans *et al*. found that Blacks had ABI values approximately 0.2 lower than Whites [2]. However, previous research by Nicoloff *et al.* and Cronenwett *et al.* reported that an ABI decrease of >0.15 over time can effectively detect significant PAD progression [22,23]. Thus, while statistically significant, the ABI differences reported in our study were less than 0.15 and may not be clinically relevant. Future studies assessing genetic differences and gene-environment interactions with respect to change in ABI over time need to be evaluated across diverse ethnic study populations with adequate sample size [41].

Several limitations of this study were considered. Misclassification due to self-identified race and ascertainment of other risk factors may contribute residual confounding affecting the estimation of the association between race and ABI. ABI in this study may have been underestimated due to categorization based on a single measure of systolic blood pressure from one upper and one lower extremity rather than multiple measures of all extremities. Potential bias may exist due to differences between participants included and not included in the mixed-effects model analysis (data not shown). Sensitivity analyses showed that participants included in the longitudinal analyses had higher baseline ABI, lower BMI and were younger, and more likely to be married, to have completed a high school education or more and to currently use alcohol (p's<0.0001) than those excluded from the analyses. Those included in the longitudinal analyses were also less likely to be Black (p=0.0008), a current smoker (p<0.0001), or have a diagnosis of hypertension or diabetes (p's<0.0001) than those excluded from the analyses potentially biasing these results toward the null hypothesis.

This study also has several strengths including the use of data from a relatively large cohort of Black and White men and women who were enrolled using a standardized protocol. It also adjusted for educational level, which has been shown to contribute to differences in diagnosis and treatment. Finally, unlike previous studies, this study examined the effects of genetics as well as the interaction between race and PAD risk factors.

Conclusions: In this study, racial differences were small and while statistically significant, may not be clinically significant. Change in ABI over time differed significantly between Blacks and Whites but was modified by education. Results suggest that compared to those without a high school diploma, ABI over time is better in both Blacks and Whites who complete a high school education or more. No effect modification was found on this association since none of the SNPs met the initial screening significance criteria ($p<0.001$) and therefore were not included in this assessment, suggesting lifestyle factors rather than genetics may modify this association. Further studies are needed to confirm these observed associations and the lack of an effect of genetics on the association between race and change in ABI over time.

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Table 4.1. Baseline characteristics by race; ARIC, 1987-1989 (n=11992)

	Black (n=2952)	White (n=9040)	
	Mean (SD)	Mean (SD)	p-value*
Baseline ABI	1.115 (0.131)	1.125 (0.128)	0.0003
Age (yr)	53.3 (5.8)	54.3 (5.7)	<0.0001
BMI (kg/m^2)	29.6 (6.0)	26.9 (4.8)	<0.0001
	N (%)	N (%)	
Male	1106 (37.5)	4212 (46.6)	<0.0001
Family CVD History			
Paternal	531 (22.1)	2959 (35.4)	<0.0001
Maternal	397 (15.0)	1558 (18.0)	0.0002
Marital Status	1745 (59.9)	7757 (87.1)	<0.0001
High School Education	1766 (60.0)	7534 (83.4)	<0.0001
Current Smoking Status	856 (29.0)	2229 (24.7)	<0.0001
Current Alcohol Use	936 (32.0)	5907 (65.4)	<0.0001
Hypertension	1625 (55.3)	2420 (26.9)	<0.0001
High Cholesterol	753 (26.8)	2310 (25.6)	0.2231
Diabetes	565 (19.6)	776 (8.6)	<0.0001
Aspirin	846 (29.1)	4735 (52.7)	<0.0001

*race differences: comparisons performed with t-tests for continuous variables, chi-square tests for categorical variables

Table 4.2. Association of race with change in ankle-brachial index; estimated coefficients from fixed effects; mixed-effect model repeated measures; ARIC, 1987-1989 (n=7672)

	Coefficient	Standard Error (SE)	p-value
Intercept	1.17	0.013	<0.0001
Time (Baseline)	-0.038	0.0027	<0.0001
Race (Black) *Time (Baseline)	0.048	0.0062	<0.0001
Race (Black)	-0.030	0.0059	<0.0001
Age (per 1 yr)	-0.00046	0.00023	0.0485
Gender (male)	0.054	0.0025	<0.0001
High School Education (yes)	0.024	0.0034	<0.0001
Cigarette Use (yes)	-0.041	0.0032	<0.0001
High Cholesterol (yes)	-0.018	0.0031	<0.0001
Diabetes (yes)	-0.031	0.0049	<0.0001
Hypertension (yes)	-0.024	0.0029	<0.0001

Reference is White race

Supplemental Table 4.A. Association of SNP with ankle-brachial index (ABI) by race; screening results of univariate linear regression, ARIC, 1987-1989

	Black	White
	p-value	p-value
PON1 SNPs		
rs2057681	0.7894	0.6310
rs3917527	0.4994	0.6782
rs2301711	0.2530	0.7073
rs2299260	0.1074	0.3923
rs2299261	0.6232	0.6295
rs854568	0.3073	0.9730
rs13223537	0.0896	0.3096
rs705378	0.4722	0.4831
rs854569	0.5197	0.3072
rs17166829	0.2556	0.3584
rs3917538	0.1846	0.3160
rs3917521	0.3939	0.3671
rs854565	0.1920	0.8139
rs854566	0.2266	0.6749
rs2237583	0.3296	0.4893
rs854572	0.8074	0.1119
rs3917541	0.3908	0.9052
rs3917551	0.4133	0.7229
rs3917550	0.6240	0.9619
rs2074354	0.6937	0.1549
rs3917490	0.6534	0.7458
rs2299262	0.5749	0.6456
rs854571	0.2367	0.6101
rs13236941	0.8623	0.1334
rs2272365	0.5640	0.1252
rs705382	0.7958	0.5989
rs2269829	0.9813	0.4547
rs2299257	0.3248	0.6933

References: *p<0.001; ‡p<0.05

Supplemental Table 4.A. Association of SNP with ankle-brachial index (ABI) by race; screening results of univariate linear regression, ARIC, 1987-1989, continued

	Black	White
	p-value	p-value
PON2 SNPs		
rs2299267	0.3821	0.2442
rs43037	0.8061	0.6057
rs7778623	0.3718	0.3284
rs43052	0.3273	0.4041
rs4729190	0.7394	0.7811
rs1557782	0.2672	0.8412
rs43063	0.4227	0.2230
rs6958904	0.2450	0.6738
rs2299263	0.2276	0.4817
rs7785039	0.8731	0.5165
rs3757707	0.7453	0.6976
rs43061	0.3288	0.3019
rs43065	0.1645	0.7556
rs2374993	0.9450	0.8512
rs10241004	0.1286	0.2341
rs10261470	0.7171	0.7718
rs10953151	0.2403	0.4992
rs6973380	0.0591	0.5017
rs10487133	0.3630	0.6179
rs7493	0.1684	0.4528
rs12534203	0.6885	0.6980
rs10953149	0.3882	0.7788
rs12535571	0.4492	0.3212
rs1639	0.4128	0.2933
rs43044	0.7834	0.7149
rs6950550	0.2243	0.0971
rs12530498	0.5771	0.1484
rs43048	0.9265	0.8929
rs7802018	0.8691	0.9285
PON3 SNPs		
rs468	0.8689	0.0911
rs1053275	0.3222	0.5131
rs11768074	0.6575	0.7083
rs9641162	0.4896	0.5634
rs10953143	0.1363	0.7493

References: *p<0.001; ‡p<0.05

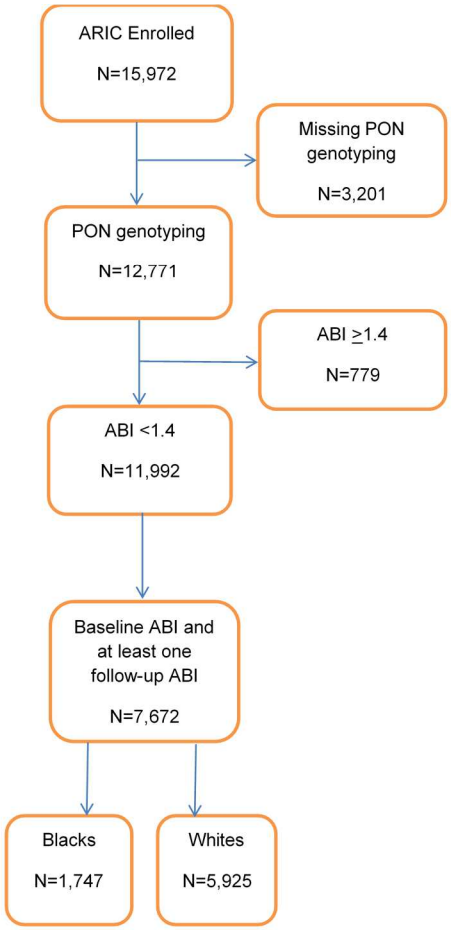

Figure 4.1. Flow chart sample derivation; ARIC, 1987-1989

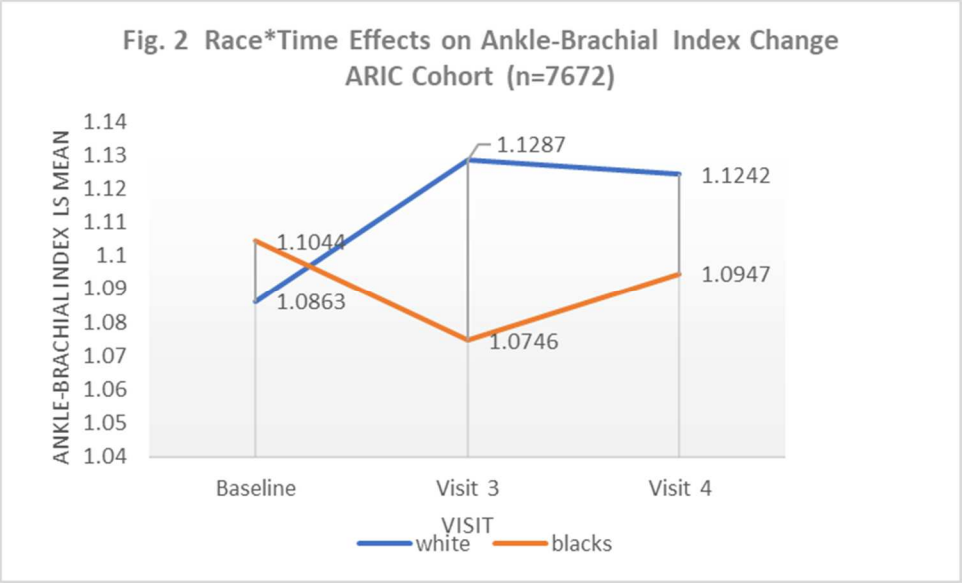

Figure 4.2. Race*time effects on change in ankle-brachial index; results of mixed-effect model repeated measures, ARIC baseline visit (1987-1989), visit 3 (1993-1995), visit 4 (1996-1998)

Reference: LS Means = Least Squares Means; Model adjusted for race, age, gender, educational status, cigarette use, high cholesterol, diabetes and hypertension; time ($p<0.0001$), race*time ($p<0.0001$); Between race ABI change difference: Baseline ($p<0.0001$), Visit 3 ($p<0.0001$), Visit 4 ($p<0.0001$); Within race ABI change for Whites: Visit 3 ($p<0.0001$), Visit 4 ($p<0.0001$); Within race ABI change for Blacks: Visit 3 ($p<0.0001$), Visit 4 ($p=0.0819$); Sample size: Baseline (Whites=5925, Blacks=1747), Visit 3 (Whites=2231, Blacks=1236), Visit 4 (Whites=4107, Blacks=932)

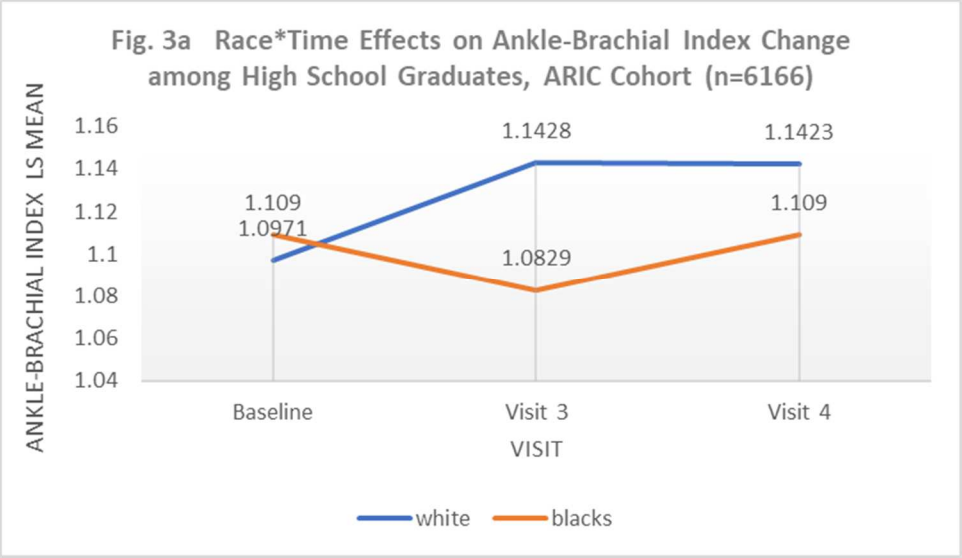

Figure 4.3.A. Race*time effects on change in ankle-brachial index stratified by high school graduate status; results of mixed-effect model for repeated measures, ARIC baseline visit (1987-1989), visit 3 (1993-1995), visit 4 (1996-1998)

Reference: Model adjusted for race, age, gender, cigarette use, high cholesterol, diabetes and hypertension; time ($p<0.0001$), race*time ($p<0.0001$); Between race ABI change difference: Baseline ($p=0.0042$), Visit 3 ($p<0.0001$), Visit 4 ($p<0.0001$); Within race ABI change for Whites: Visit 3 ($p<0.0001$), Visit 4 ($p<0.0001$); Within race ABI change for Blacks: Visit 3 ($p<0.0001$), Visit 4 ($p=0.9960$); Sample size: Baseline (Whites=5053, Blacks=1113), Visit 3 (Whites=1901, Blacks=787), Visit 4 (Whites=3507, Blacks=619)

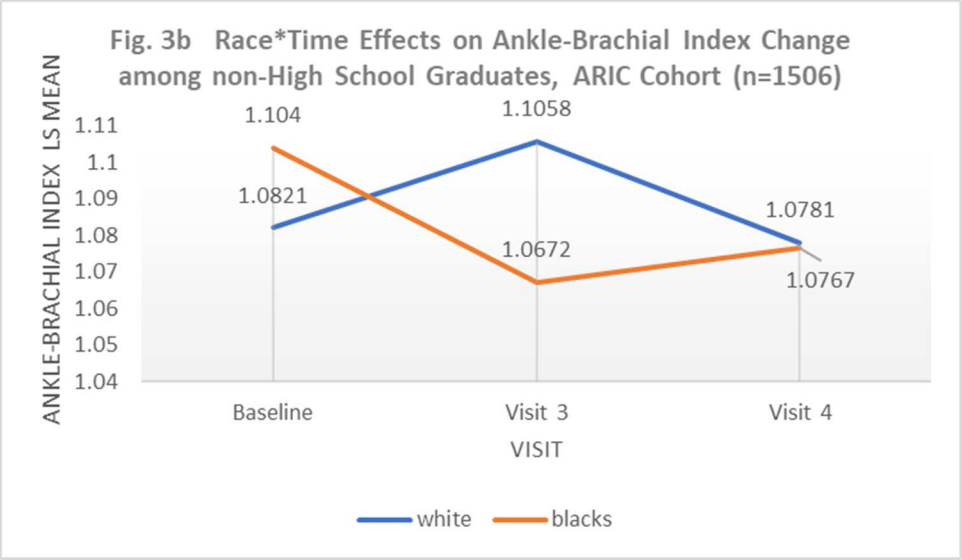

Figure 4.3.B. Race*time effects on change in ankle-brachial index stratified by high school graduate status; results of mixed-effect model for repeated measures, ARIC baseline visit (1987-1989), visit 3 (1993-1995), visit 4 (1996-1998)

Reference: Model adjusted for race, age, gender, cigarette use, high cholesterol, diabetes and hypertension; time (p=0.0346), race*time (p<0.0001); Between race ABI change difference: Baseline (p=0.0018), Visit 3 (p=0.0003), Visit 4 (p=0.9000); Within race ABI change for Whites: Visit 3 (p=0.0062), Visit 4 (p=0.6106); Within race ABI change for Blacks: Visit 3 (p<0.0001), Visit 4 (p=0.0047); Sample size: Baseline (Whites=872, Blacks=634), Visit 3 (Whites=330, Blacks=449), Visit 4 (Whites=600, Blacks=313)

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CHAPTER 5

Discussion and Conclusions

Overview

This dissertation research was conducted using data from the Atherosclerosis Risk in Communities (ARIC) cohort and evaluates the association of race with outcomes indicative of cardiovascular disease (CVD) and the effect of paraoxonase single nucleotide polymorphisms on these associations. Three separate (CVD) outcomes were analyzed: 1) major adverse cardiac events (MACE), 2) peripheral artery disease (PAD) and 3) ankle-brachial index (ABI). Findings from this research may be used to better understand factors that contribute to and modify each of these CVD measures. Results may provide improved understanding of how lifestyle and health management intervention strategies can ameliorate the development of these three CVD outcomes.

Major Adverse Cardiac Events (MACE)

Results from this longitudinal analysis indicate that major adverse cardiac events (MACE) were more prevalent among Blacks than Whites in the ARIC cohort (17.7% vs. 12.8%). After adjusting for age, gender, BMI, cigarette use, alcohol use, educational and marital status and reported aspirin use, Blacks had 1.24 times greater hazard of MACE compared to Whites. However, in contrast with previous studies [1,2], after further adjustment for known CVD risk factors, hypertension, diabetes and high cholesterol, this association became non-significant, suggesting race was not independently associated with MACE. These results suggest that the development of cardiovascular disease may be influenced less by biological differences and more by racial disparities in risk factors and lifestyle and medical management, consistent with other studies [3,4,5,6,7,8,9].

None of the SNPs met the initial screening significance criteria ($p<0.001$) and were not included in this assessment; therefore, this study found no significant effect modification of *PON* SNPs on the association between race and MACE. The influence of genetic differences on the risk of MACE is relatively unknown and to our knowledge, this is the first study to examine this issue. Prior studies were conducted in clinical patients [10, 11], rather than a population-based

cohort; thus, previous reports are less generalizable than the results of this study. Although the association between race and MACE may exist after adjustment for biological risk factors, the small sample of Blacks relative to Whites included in this analysis may have limited the ability to find an association in adjusted models.

Additional studies are needed to better understand the complex factors influencing the association of race with MACE; in addition, future studies evaluating genetic interactions should be conducted in ethnically diverse population-based cohorts with large sample sizes.

Peripheral Artery Disease (PAD)

Results from this cross-sectional study found a higher prevalence of PAD in Blacks than Whites (5.0% vs. 3.7%). After adjusting for age, BMI, and gender, Blacks had higher odds of PAD (OR=1.27, 95%CI=1.04,1.57) than Whites. However, this association was attenuated to non-significance after adjustment for smoking and education, hypertension, high cholesterol and diabetes. Consistent with a prior study [12], these results suggest that race was not independently associated with PAD after adjustment for lifestyle and health risk factors; differences in PAD may be influenced more by racial disparities in health management of risk factors rather than racial differences in the biological development of atherosclerosis similar to previous studies [4,5,6,7,8,9]. In contrast, previous studies conducted in selected populations reported an association between race and PAD [13,14], making it difficult to draw a direct comparison with the ARIC cohort.

Recent studies report associations between *PON1* SNPs and resistant hypertension [15] and blood pressure [16]. None of the SNPs met the initial screening significance criteria ($p<0.001$) and were not included in this assessment; therefore, this study found no significant effect modification of *PON* SNPs on the association between race and PAD. The effect of genetics on the association between race and PAD is relatively unexplored and to our knowledge this is the first study that attempted to report on this issue. It is biologically plausible that there is an association between race and PAD after adjustment for risk factors. Prior

research [17] found that increased aortic thickening and stiffening in Blacks may artificially lower ABI measures and a recent study found lower ABI values among Blacks compared to Whites [4].

While this study evaluated data from a relatively large cohort of both Black and White participants, the lack of association between race and PAD in adjusted models could be due to the small sample of Blacks relative to Whites in the final analysis or due to a true lack of association. Future studies assessing genetic interactions with respect to PAD need to be evaluated across diverse ethnic populations with adequate sample sizes.

Ankle-Brachial Index (ABI)

In this longitudinal study, after adjustment for confounders, Whites compared to Blacks had significantly higher (better) ABI values at both follow-up visits compared to baseline. Consistent with previous studies [4,18], average ABI was significantly lower in Blacks (1.12) than Whites (1.13) at baseline. Previous research reported that an ABI decrease of >0.15 over time is indicative of significant PAD progression [19,20]. While statistically significant, the ABI differences reported in this study were less than 0.15, and thus, although statistically significant, may not be clinically relevant. In addition, race, age, education, cigarette smoking, cholesterol, diabetes and hypertension were all significantly and independently associated with change in ABI over time. When stratified by educational status, ABI over time was better in both Blacks and Whites who completed a high school education compared to those with less education, consistent with previously reported studies [4,13]. Among Whites without a high school education, ABI decreased and was similar to Blacks without a high school education by visit 4 of the follow-up period. This suggests the influence education has on access to health care information affecting lifestyle and health management decisions [5,6,7,8,9].

Prior research demonstrated the antioxidant characteristics of paraoxonase enzymes to inhibit the formation and accumulation of macrophage cholesterol, slowing the development of atherosclerosis [16]. However, this study did not find significant effect modification of *PON*

SNPs on the association between race and ABI over time since none of the SNPs met the initial screening significance criteria ($p<0.001$) and therefore were not included in this assessment; to our knowledge this is the first study to evaluating the potential modification by *PON SNP*s. Results of previous studies were inconsistent with some reporting positive associations between *PON* SNPs and PAD [21], cardiovascular disease [22] and blood pressure [23,16], but others failing to find an association between *PON* polymorphisms and stroke [24,25] or CAD [26].

While it is plausible that there is an association between race and change in ABI over time due to differences in biological risk factors, the effect of education on change in ABI over time observed in this study suggests that ABI is influenced more by racial disparities in health management than racial differences in the biological development of atherosclerosis.

Conclusions

Although Blacks had a higher prevalence of MACE, race was not significantly associated with MACE after adjusting for comorbidities. Racial disparities in health care may influence the observed results. Additionally, MACE may be modified through medical management affecting disease outcomes. This study found race was not significantly associated with PAD, after adjusting for health and behavioral risk factors, suggesting risk of PAD may be modified through medical management and lifestyle changes. Finally, change in ABI over time differed significantly by race, but although statistically significant, these differences were small and not likely to be of clinical significance. Furthermore, differences were modified by education suggesting that ABI over time is better in both Blacks and Whites who complete a high school education or more.

To our knowledge, this is the first study assessing the effect of *PON* gene clusters (*PON1, PON2 and PON2*) on the associations between race and 1) MACE, 2) PAD, and 3) change in ABI over time. Our results suggest that *PON* SNPs did not modify the associations between race and cardiovascular disease studied here indicating that lifestyle factors rather than genetics may modify these associations.

Higher education may influence better health management through improved lifestyle and behavioral choices, thereby leading to a greater impact on the development of cardiovascular disease and associated comorbidities. Further studies are needed to confirm the associations observed in this dissertation research and the lack of an effect of genetics on the association between race and cardiovascular disease outcomes.

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