Published in final edited form as:

Biol Res Nurs. 2011 July ; 13(3): 306–319. doi:10.1177/1099800411403469.

The Association Between Variants on Chromosome 9p21 and Inflammatory Biomarkers in Ethnically Diverse Women With Coronary Heart Disease: A Pilot Study

Theresa M. Beckie, PhD¹, Jason W. Beckstead, PhD¹, and Maureen W. Groer, PhD¹

¹University of South Florida, Tampa, FL, USA

Abstract

Background—The most consistently replicated genetic variants associated with coronary heart disease (CHD) in populations of European descent have been found on chromosome 9p21. Yet there is little known about these associations in ethnic groups of African ancestry. These disease-associated variants are located in a genomic region of unknown function. The purpose of this exploratory study was to examine the allelic frequencies and haplotype structure of single nucleotide polymorphisms (SNPs) for Black and White women with CHD. The authors also sought to explore the relationship between these genetic variants and biomarkers of inflammation.

Methods—Using polymerase chain reaction amplification, the authors genotyped 8 SNPs in a 58-kilobase region of chromosome 9p21 in a cohort of women with CHD ($n = 91$). The authors examined the interethnic relationship between the SNPs and four inflammatory biomarkers (C-reactive protein, intercellular adhesion molecule-1, interleukin-6, and tumor necrosis factor-alpha) using analysis of variance (ANOVA).

Results—We found considerable interethnic allelic and haplotype diversity across the 9p21 locus, with only two SNPs in perfect linkage disequilibrium (LD) in both races. A pair of high- and low-risk haplotypes was most common in White women, while about 41% of Blacks carried the risk alleles for three of the eight SNPs the authors examined. The interethnic associations between the SNP genotypes and inflammatory markers were divergent in both direction and magnitude.

Conclusions—Our results lend support for the importance of ancestry-specific allelic context when examining variants on chromosome 9p21. Additional work is needed to elucidate the genetic contribution to inflammatory biomarkers for diverse racial groups.

Keywords

coronary heart disease; single nucleotide polymorphism; genetics; women

Recognized as the leading cause of death in women, coronary heart disease (CHD) is a multifactorial phenotype resulting from a complex interaction of multiple genes and environmental factors (Arnett et al., 2007; Roberts, Wells, Stewart, Dandona, & Chen, 2010). The widely accepted view of atherosclerosis points to inflammation as a critical regulator from the disease's inception through its progression to the ultimate complication of thrombosis (Libby, Okamoto, Rocha, & Folco, 2010). Fueled by traditional risk factors including tobacco consumption, hypertension, obesity, and insulin resistance, inflammatory processes drive many aspects of the biology of atherosclerotic plaque. Dysregulated metabolic status including atherogenic dyslipidemia, hyperglycemia, insulin resistance, and proinflammatory states (Grundy et al., 2005) confer increased risk for CHD and poor long-term prognosis (Espinola-Klein et al., 2007; Gami et al., 2007; Ridker, Buring, Cook, & Rifai, 2003). Obesity is associated with a proinflammatory state (Despres & Lemieux, 2006) mediated by dysregulated adipose tissue releasing inflammatory cytokines (Yan et al., 2008). Inflammation is more prevalent in women (Feinberg, Schwartz, & Behar, 2008) and is more predictive of CHD in women than in men (Cook, Buring, & Ridker, 2006; Pischon et al., 2008).

Inflammatory biomarkers commonly involved in the initiation and progression of atherosclerotic lesions as well as in the development of atheroma complications (Libby, 2002) include C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and intercellular adhesion molecule-1 (ICAM-1; Ridker, Hennekens, Buring, & Rifai, 2000). Higher levels of these biomarkers are predictive of cardiovascular events in postmenopausal women. Although inflammatory biomarker concentrations are heritable phenotypes (Schnabel et al., 2009), the variability of and genetic contribution to these concentrations for most inflammatory markers remains incompletely understood for diverse racial groups.

Several genome-wide association studies (GWAS) have identified susceptibility genes linked to CHD (Helgadottir et al., 2007; McPherson et al., 2007; Samani et al., 2007; Samani et al., 2008; Wellcome Trust Case Control Consortium, 2007). One of the most robust susceptibility loci is located in a 58-kilobase (kb) region on chromosome 9p21 (Schunkert et al., 2008). A meta-analysis of the association between chromosome 9p21 single nucleotide polymorphisms (SNPs) and CHD provided convincing evidence in seven additional case-control studies of northern European individuals combined with the four populations of the previous GWAS (Schunkert et al., 2008). Investigators have found associations between 9p21 SNP genotypes and CHD with similar effect sizes and allele frequencies in Japanese and Korean (Hinohara et al., 2008), Irish (Meng et al., 2008), East Indian (Maitra et al., 2009), and South Asian populations (Saleheen et al., 2010), and in cohorts from Finland, Sweden, and France (Karvanen et al., 2008). We know little, however, about possible associations between 9p21 SNP genotypes and CHD in Black racial groups (Assimes et al., 2008). Assimes et al. (2008), examining three 9p21 SNPs (rs10757274, rs2383206, and rs10757278), found they were associated with CHD in Whites but not in Blacks. Moreover, the disease-associated variants are located in a genomic region of unknown function (Silander et al., 2009). Comparisons across studies can be challenging when investigators have genotyped different SNPs on chromosome 9p21 associated with CHD. Haplotype (a set of SNPs found on the same chromosome that tend to be inherited

together) analyses offer more genetic information than analyses of a single SNP. Examining additional SNPs at this locus may refine and improve the identification of the risk haplotype (Schunkert et al., 2008).

Despite convincing evidence of the relationship between 9p21 SNPs and CHD, most investigators have found no relationship between these SNPs and disease severity (S. N. Chen, Ballantyne, Gotto, & Marian, 2009; Z. Chen et al., 2009; Horne, Carlquist, Muhlestein, Bair, & Anderson, 2008), mortality (Ellis et al., 2010), or traditional risk factors (Assimes et al., 2008; Brautbar et al., 2009; Karvanen et al., 2008). Likewise, in the Women's Genome Health Study, genetic variation in 9p21 in White women was associated with incident cardiovascular disease but not traditional risk factors or CRP concentrations (Paynter et al., 2009). Contrary to reports of no association between 9p21 polymorphisms and lipid profiles (Broadbent et al., 2008; Schunkert et al., 2008), Ellis and colleagues (2010) found that participants of the Coronary Disease Cohort Study ($N = 251$; 27.5% women) with the rs1333049-C risk genotype had significantly higher levels of total cholesterol and triglycerides than those who did not carry that genotype.

As evidence of the involvement of gene variants in CHD accumulates, the study of interactions between genetic polymorphisms and environmental exposures in CHD becomes ever more important. Definitive evidence of the extent to which inflammatory biomarkers such as CRP are causal risk factors in CHD, markers of established risk factors such as smoking to which they are correlated, markers of multiple genetic influences, or some combination of these possibilities remains tentative (Elliott et al., 2009). The significant genetic determination of CHD has been poorly characterized in women and ethnic minorities, and there remains a paucity of sex-specific biomarker data (Novack, Cutlip, Jotkowitz, Lieberman, & Porath, 2008). Moreover, investigators have not extensively studied whether ethnicity affects the production of pro-inflammatory cytokines or the association of inflammation with CHD risk in women. More substantial data are needed regarding the interethnic risk-allele prevalence of a set of common SNPs at loci associated with CHD risk and whether the relationship is modified by gender, by the presence of other genes, or by environmental factors (Humphries, Drenos, Ken-Dror, & Talmud, 2010). The role of chromosome 9p21 in CHD remains unclear, particularly for understudied phenotypes including women of ethnic groups other than those of European origin.

Thus, the purpose of this descriptive pilot study was to examine the allelic frequencies and haplotype structure of genetic variants on chromosome 9p21 for Black and White women with CHD. We also sought to explore the relationships between these genetic variants and biomarkers of inflammation linked to CHD. To this end, we conducted polymerase chain reaction (PCR) amplification using commercially available TaqMan genotyping assays to examine the genotype frequencies of eight variants on chromosome 9p21. The amplification of genomic DNA sequences using PCR technology has become a standard approach to genotyping. The capacity of PCR to amplify a specific segment of DNA in exponential progression makes it a powerful tool in the study of polymorphisms. We analyzed inflammatory biomarkers (CRP, IL-6, TNF- α , and ICAM-1) using the Luminex 200 IS system, a laser-based fluorescent analytical system, in conjunction with LINCplex bioassays.

Method

Participants and Setting

Data for this study are from a sub-study of a larger randomized clinical trial that was already underway. Supplemental funding permitted the opportunity to analyze blood samples from women subsequently enrolled between 2006 and 2008. Details of the study design, recruitment, cardiac rehabilitation interventions as well as depression and inflammatory biomarker outcomes of the parent trial have been published previously (Beckie, 2006; Beckie, Beckstead, & Groer, 2010; Beckie, Beckstead, Schocken, Evans, & Fletcher, 2010; Beckie et al., 2009). The Institutional Review Board of the university approved the study, and all participants provided written informed consent. The study included women over the age of 21 years with the diagnosis of a myocardial infarction (MI), unstable angina, or coronary revascularization who were within 3 months of the index event and had not yet begun a cardiac rehabilitation program.

Measures

Baseline clinical and physiological characteristics—Physiological assessment included CHD risk factors, anthropomorphic measures, medication use, and fasting lipids and glucose tests. We calculated body mass index as weight in kg/(height in m)². We obtained 12-hour fasting lipid and glucose measurements utilizing the Cholestech LDX system (Hayward, CA). Components of metabolic dysregulation were those specified in the revised National Cholesterol Education Program Adult Treatment Panel III criteria (Grundy et al., 2006).

Biomarkers—We centrifuged blood samples, obtained in heparinized tubes after a 12-hour fast, at 3,800 rpm for 25 minutes at 0°C. We then stored serum samples in 1.5-ml microcentrifuge tubes and immediately placed them at –80°C until analysis. We used the Luminox 200 IS system and LINCOplex Bio-assay kits (Millipore Corporation, St. Charles, MO) to analyze high-sensitivity CRP (hsCRP), IL-6, TNF- α , and ICAM-1 according to manufacturers' protocols. We ran samples in duplicate and analyzed concentrations using a 5-parameter logistic curve-fitting method. We set measures of cytokines that were below the minimum detectable concentration (<3.2 pg/ml) equal to half the detection level (Helsel, 2005). We measured serum hsCRP using a LINCOplex Human Cardiovascular Disease Panel 2 with an assay sensitivity of 6 minDC (pg/mL) and TNF- α and IL-6 in serum samples using the Human Cytokine LINCOplex two-plex kit. Sensitivities for TNF- α and IL-6 were 0.22 and 0.79 pg/mL, respectively. We used the Human Sepsis/Apoptosis LINCOplex kit to measure ICAM-1, at a minimum detectable concentration of 30.0 pg/mL. For all assays, we used manufacturer-provided quality controls for each plate. The intra-assay coefficients of variation (CV) for hsCRP, TNF- α , IL-6, and ICAM-1 were 8.0%, 8.0%, 13.6%, and 5%, respectively. The interassay CV for hsCRP, TNF- α , IL-6, and ICAM-1 were 17.5%, 10.9%, 12.7%, and 8.7%, respectively.

Genotype Determination

We overlaid blood samples on a Histopaque density gradient and centrifuged them at 1,200 rpm for 25 minutes at room temperature with the brake off. We washed the resultant

peripheral blood mononuclear cells (PBMCs) twice in RPMI-1640 with 10% fetal calf serum with 50 ug/ml gentamicin. We then slowly resuspended the cell pellets in a freezing solution of 10% DMSO in RPMI-1640 with 10% fetal calf serum and gentamicin. We placed the cryovials in propanol tanks at -80°C and transferred them within 24 hour to liquid nitrogen. For DNA extraction, we thawed the PBMCs, transferred them to microcentrifuge tubes, and centrifuged them for 30 minutes at 4°C to create a cell pellet. We used the QIAamp DNA Micro Kit (Qiagen Inc., Valencia, CA) to extract 50 uL of genomic DNA from each sample and measured DNA concentrations using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Nanodrop Technologies, Wilmington, DE). We diluted samples with RNase-free water to provide a concentration of 20 ng of DNA/uL and used predesigned TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) to genotype eight SNPs spanning the 58-kb region of 9p21 (rs10116277, rs1333040, rs4977574, rs10757274, rs2383206, rs2383207, rs10757278, and rs1333049), as reported in the literature (Broadbent et al., 2008). We amplified genomic DNA using PCR technology, performing the PCR reactions in a total volume of 25 uL containing 2.5 uL DNA, 12.5 uL TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA), 1.25 uL of TaqMan assay, and 8.75 uL water. We performed reactions in 96-well plates in triplicate using a Bio-Rad CFX96 real-time quantitative PCR thermocycler. PCR involves incubating the samples at three temperatures corresponding to the three steps in a cycle of amplification–denaturation, annealing, and extension. We amplified DNA under the following conditions: Step 1— 50°C for 2 minutes; Step 2— 95°C for 10 minutes; Step 3—40 cycles of 92°C for 15 s followed by 60° for 1 minutes. We generated genotypes by automatic calling using the CFX96 version 1.5 software. We also reviewed all genotypes manually and subsequently downloaded data to an excel spreadsheet for analysis.

Haplotype Construction

We employed Pearson's chi-square (χ^2) test with 1 degree of freedom to assess whether SNPs were in Hardy-Weinberg Equilibrium (HWE) among Blacks and Whites separately. We analyzed genotyping data with PHASE version 2.1.1 (<http://www.stat.washington.edu/stephens/software.html>), which uses Bayesian statistics to infer phase at linked loci from population genotype data and reconstructs haplotypes with sample frequency estimates (Stephens & Donnelly, 2003; Stephens, Smith, & Donnelly, 2001), and assessed pairwise linkage disequilibrium (LD) between the SNPs using Haploview version 4.2 (<http://www.broadinstitute.org/haploview>). We examined haplotypes separately for Blacks and Whites, given allelic frequency variability between populations (Silander et al., 2009). We identified haplotype blocks graphically from the LD intensity expressed as Lewontin's D' statistic (Lewontin, 1964) and r^2 (Barrett, Fry, Maller, & Daly, 2005; Devlin & Risch, 1995). Complete LD ($D' = 1$) occurs when the allelic association is as strong as possible, given the allele frequencies at the two sites (Carlson et al., 2004). When SNP minor allele frequencies (MAF) are identical, genotypes are perfectly correlated ($r^2 = 1$). We used the solid spine of LD method to search for a spine of strong LD from the first SNP to the last along the legs of the triangle in the LD plot. We used $D' > .5$ as the criterion the LD blocks. We used Haploview's Tagger strategy to select tagging SNPs that would best capture all alleles that were correlated at an $r^2 \geq .8$.

Statistical Analysis

We used PASW® Statistics 18 for Windows (SPSS Inc, Chicago, IL) for statistical analysis, calculating means, standard deviations, and percentages to describe the study variables and Pearson's χ^2 to compare the distributions of allele frequencies by race. We logarithmically transformed the inflammatory biomarker and triglyceride levels for analyses and then back-transformed them to their natural units for presentation purposes. We compared mean levels of inflammatory markers according to the presence versus absence of at least one copy of the risk allele (dominant genetic model) for each SNP within each race separately using analysis of variance (ANOVA). With a sample size of 91 and $\alpha = .05$, we considered the possibility of type II statistical error in our study. Moreover, controlling for multiple test comparisons between SNP genotypes and the inflammatory biomarkers rendered the reliance on statistical test probability values problematic. Therefore, our discussion of the results focus on the size of the effects observed indexed as η^2 (η^2). According to Cohen's convention, η^2 of .01, .06, and .14 represent small, medium, and large effects, respectively (Cohen, 1988).

Results

Clinical Characteristics

We included a total of 91 women with complete baseline genotypic, inflammatory biomarker, and clinical data in this study. The mean age of the sample was 61.2 ± 10 years. Most were White (82%), married (51.6%), and retired (49.5%); Black women comprised 18% ($n = 16$) of the sample. The majority of women had undergone percutaneous coronary interventions (30%) or coronary artery bypass grafting (30%) just prior to enrolling in the parent study, and 11% had stable angina. Nearly 30% ($n = 27$) had been diagnosed with an MI during their index hospitalization. Most women had been prescribed statins (92%), aspirin (88%), and β -blockers (78%). About 26% of the women were taking nonsteroidal anti-inflammatory drugs (NSAIDs), and 6.6% were on estrogen replacement (see Table 1). Compared to Whites, a higher percentage of Blacks were taking insulin. There were no interethnic differences in the consumption of statins, aspirin, or NSAIDs.

Blacks and Whites were similarly sedentary, overweight, dyslipidemic, and hypertensive (see Table 2). Almost half of the participants reported a family history of CHD and nearly 18% had experienced a previous MI. Though the racial groups were not different on most traditional risk factors, more Blacks had a history of previous stroke. Table 3 summarizes the baseline lipid profiles, components of metabolic dysregulation, and inflammatory biomarkers that reveal few interethnic differences. White women demonstrated higher triglyceride and IL-6 concentrations than Black women. Secondary analysis of the use of prescribed medications known to influence the inflammatory response revealed that self-reported daily use of aspirin was associated with a reduced level of ICAM-1 (200.7 pg/mL vs. 289.7 pg/mL; $\eta^2 = .053$), while the reported daily use of statins did not affect the level of inflammatory biomarkers. Women reporting any use of nonspecific NSAIDs somewhat surprisingly demonstrated elevated hsCRP levels (10.2 mg/L vs. 5.3 mg/L; $\eta^2 = .084$).

Allelic and Genotypic Characteristics

The SNPs did not deviate from the HWE for either race ($p > .05$, details not shown). Table 4 presents the genotype frequencies for Black and White participants as well as for relevant International HapMap Project populations (available at <http://hapmap.ncbi.nlm.nih.gov/>). Compared to White women, few Blacks in our study were homozygous for the risk alleles of four of the eight SNPs (rs10757274-G, rs4977574-G, rs10757278-G, and rs1333049-C). However, compared to Whites, a larger proportion of Blacks carried two copies of the risk alleles of two SNPs (rs10116277-T and rs2383207-G). There was a trend for fewer Blacks to carry two copies of the risk allele for rs2383206 compared to White women. However, there was less interethnic variation in the genotypic distribution of rs1333040.

LD and Haplotypes

The SNPs examined span 44,106 base pairs of DNA at the 9p21 locus. Haplotype determination using PHASE 2.1.1 provided estimates of the sample haplotype frequencies, which we display in Table 5. The two most common haplotypes for White women were rs10116277-T, rs1333040-T, rs10757274-G, rs4977574-G, rs2383206-G, rs2383207-G, rs10757278-G, and rs1333049-C (51.1%) and G-C-A-A-A-A-A-G (26.3%), with the first haplotype representing all SNP risk alleles for CHD and the second reflecting the wild type alleles for each SNP. Haplotype frequency estimates for Black women were much more diverse. The two most frequent haplotypes for Black women were rs10116277-T, rs1333040-T, rs10757274-A, rs4977574-A, rs2383206-A, rs2383207-G, rs10757278-A, and rs1333049-G (40.6%), and T-T-A-A-G-G-A-G (10.9%). Notably, the first haplotype includes risk alleles for only three SNPs (rs10116277, rs1333040, and rs2383207), while the second haplotype includes risk alleles for four SNPs (rs10116277, rs1333040, rs2383206, and rs2383207). Only 5.8% of Blacks carried risk alleles for seven of eight SNPs and 6.3% carried risk alleles for five of eight SNPs.

We conducted an extended haplotype analysis using Haploview to provide further insights into the relationship between SNP LD patterns according to ethnicity. Figure 1A and B shows the position of the SNPs on chromosome 9p and the pairwise LD plot (scaled as D') for the White and Black women, respectively. Figure 1A illustrates the high pairwise LD of the eight SNPs in the haplotype block for Whites ($D' = 0.757 - 1.0$, $r^2 = 0.392 - 0.973$). Figure 1B shows that these same SNPs have dramatically lower pairwise LD in Blacks ($D' = 0.24 - 1.0$, $r^2 = 0.0 - 1.0$). The single haplotype block assigned to the SNPs of Whites shows two SNPs (rs10757274 and rs4977574) in perfect LD ($D' = 1$, $r^2 = 1$). The lowest LD was for SNPs rs1333040 and rs 2383207 ($D' = 0.694$, $r^2 = 0.428$). Four tagging SNPs that captured all eight SNPs for Whites included rs4977574, rs10757274, rs10757278, and rs1333049, with a mean maximum $r^2 = 0.937$. Haploview revealed two haplotype blocks for Blacks. The first block included rs10116277 and rs1333040 ($D' = 0.594$, $r^2 = 0.111$), and the second block comprised the six remaining SNPs. Two SNP pairs had the lowest LD (rs10116277 and rs2383206, $D' = 0$, $r^2 = 0$; rs2383206 and rs2383207, $D' = 0$, $r^2 = 0$). Tagging SNPs for Blacks included rs4977574 and rs10757274 ($D' = 1.0$, $r^2 = 1.0$).

Relationship Between Genotype and Inflammatory Markers

Because two SNPs were in perfect LD for both races (rs10757274 and rs4977574), we arbitrarily excluded rs4977574 from further analyses. The low frequency of various SNP genotypes in Blacks prompted the collapse of genotypes into carriers of the risk alleles and the wild type alleles for subsequent analyses. Table 6 provides the inflammatory biomarker data for Whites and Blacks with at least one copy of the SNP risk alleles compared to those with the wild type alleles (dominant genetic model). There was no evidence of an association between hsCRP and 9p21 SNPs in Whites. Blacks with the risk alleles for rs10757274 and rs10757278 (in high LD; $D' = 1$, $r^2 = 0.59$) demonstrated lower hsCRP levels (3.3 and 3.4 mg/L, respectively) compared to those with the wild type genotype (7.3 and 8.3 mg/L, respectively; $\eta^2 = .092$ and $.102$, respectively).

Across all SNPs among Whites, those with at least one copy of the risk alleles showed higher IL-6 levels (24.0–27.6 pg/mL) than did those with the wild type alleles (5.0–11.8 pg/mL). However, these differences must be interpreted relative to the standard deviations shown in parentheses in Table 6. The measure of effect size (η^2) puts these differences on a common metric. In Whites across all SNPs, the average η^2 was $.022$, suggesting that these mean differences in IL-6 levels are small. In Blacks, the η^2 for mean differences in IL-6 levels across all SNPs were much more varied, with some even counterintuitive. The sizes of the effects of two SNPs (rs2383206 and rs10757278) on IL-6 were much larger in Blacks ($\eta^2 = .158$ and $.112$, respectively) than in Whites ($\eta^2 = .032$ and $.026$, respectively). Similar to Whites, Blacks carrying the risk alleles for these SNPs demonstrated higher mean IL-6 levels (4.6 and 5.3 pg/mL, respectively) than did those carrying the wild type (1.6 and 3.1 pg/mL, respectively). Notably, few Blacks carried two copies of the risk alleles for these SNPs, and their IL-6 levels were lower than Whites.

The associations between the SNPs and TNF- α concentrations were negligible in Whites despite a consistent trend for those with the risk alleles to demonstrate higher TNF- α levels (11.1–11.9 pg/mL) than those without (7.4–8.9 pg/mL). However, Blacks with at least one copy of the risk alleles of rs2383206 and rs10757274 revealed much larger effects on TNF- α levels ($\eta^2 = .156$ and $.234$, respectively) than those seen in Whites ($\eta^2 = .008$ and $.002$, respectively). Although few Blacks had the GG risk genotypes for these SNPs, we found inverse patterns of association between these two SNPs and TNF- α levels. Compared to Blacks with the wild type alleles, Blacks with risk alleles for rs2383206 demonstrated higher TNF- α levels (1.6 vs. 6.2 pg/mL), while those with risk alleles for rs10757274 had lower levels (2.8 pg/mL) than those with the wild type alleles (7.2 pg/mL).

Finally, for all SNPs, ICAM-1 levels were higher in Whites with at least one copy of the risk alleles (216.9–222.7 pg/mL) compared to those carrying the wild type alleles (145.6–189.9 pg/mL). This finding was particularly evident for rs1333040 (222.7 pg/mL vs. 145.6 pg/mL; $\eta^2 = .061$). The effects of the SNPs on ICAM-1 in Blacks were small (average $\eta^2 = .025$), with the exception of rs10757278 ($\eta^2 = .171$). Blacks with the wild type genotype (AA) for rs10757278 demonstrated higher ICAM-1 levels (248.3 pg/mL) compared to those with at least one copy of the risk allele (165.5 pg/mL).

Discussion

As an inflammatory metabolic disease, CHD develops through a complex interaction between multiple environmental, behavioral, and genetic factors contributing to the development of atherosclerotic plaques that lead to phenotypic presentations ranging from stable angina to MI (Roy, Bhardwaj, & Yla-Herttuala, 2009). Investigators have identified various common genetic variants associated with processes including lipid metabolism, oxidative stress, inflammation, and cell proliferation and apoptosis as risk factors for CHD (Yamada, Ichihara, & Nishida, 2008). Despite the consistently replicated association linking chromosome 9p21 variants and CHD, the biological mechanism of this association remains elusive (Palomaki, Melillo, & Bradley, 2010). About 50% of Whites carry at least one copy of the 9p21 risk alleles with an estimated 29% increased risk of CHD compared to noncarriers (Newton-Cheh et al., 2009). About 25% of Whites carry two copies of the risk alleles and have a twofold increased risk of early-onset CHD compared to those without these alleles (McPherson, 2010). The phenotypic heterogeneity of CHD presents a challenge for determining whether chromosome 9p21 variants are associated with the development of atherosclerosis or plaque rupture and/or thrombosis. Recent data suggest that 9p21 gene dose is associated with severity of coronary vessel involvement but not with MI in Whites (Dandona et al., 2010), lending support for the hypothesis that 9p21 is proatherogenic. Because of the polygenic and multifactorial nature of CHD, determining the unique contribution and biological validity of genetic variants, inflammation, and traditional risk factors for atherosclerosis and altered arterial biology continues to challenge biobehavioral researchers.

Researchers have published few replication studies of the 9p21 locus in non-European populations for CHD-related phenotypes to date. To address this gap in the literature, we sought to examine the allelic frequencies and haplotype structure of polymorphisms on chromosome 9p21 in Black and White women with CHD. Given that both the biological relationship between 9p21 and CHD and the direct causal link between inflammatory biomarkers and CHD are tentative, particularly for African descendants, we explored the potential relationship between inflammatory and genetic markers of CHD in a sample of ethnically diverse women.

The risk factor profiles, clinical characteristics, and comorbidities of Black and White women were similar in our study. Moreover, the majority of women in both racial categories consumed a variety of anti-inflammatory medications including aspirin and lipid-lowering therapy. Although statin therapy had no effect on biomarker levels, aspirin use was associated with lower ICAM-1 levels; the 24 women reporting any NSAID use, inexplicably, had higher hsCRP concentrations. However, because these women were plausibly consuming NSAIDs on a temporary or intermittent schedule, it is unclear whether any exposure had occurred on the days preceding the blood draw or whether, in fact, the women were using NSAIDs for pain relief due to a chronic or acute inflammatory condition. In our sample of women with CHD, Whites had higher baseline triglyceride, CRP and IL-6 levels compared to Blacks (see Table 3). Carroll and colleagues (2009) examined the ethnic differences in CRP and IL-6 levels of primary-care practice patients older than 45 years and without a current or previous history of CHD. In comparison to our findings, Carroll and

colleagues found that Black women ($n = 68$) had higher CRP and IL-6 levels than did White women ($n = 47$) after controlling for visceral fat and age. IL-6 is a recognized prognostic risk factor for future cardiovascular events in patients with CHD (Koenig & Khuseyinova, 2007). Investigators of the Women's Health Initiative study (89% White) found that of 18 biomarkers assessed, only increased IL-6 was independently associated with CHD (Kim et al., 2010). IL-6 promotes endothelial dysfunction and smooth muscle proliferation and perpetuates vascular inflammation through recruitment and activation of inflammatory cells (Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). The few studies that have examined interethnic differences in inflammation reveal inconsistent findings. Although investigators have reported that apparently healthy White women have higher ICAM-1 levels (Albert et al., 2009) and higher TNF- α levels than Black women (Hyatt et al., 2009), others found that apparently healthy Blacks had higher inflammatory biomarker concentrations than Whites (Albert, 2007; Carroll et al., 2009; Ford, Giles, Mokdad, & Myers, 2004; Walston et al., 2007). Some studies investigating the association between cytokine polymorphisms and race suggest the potential for systemic inflammatory upregulation in Blacks (Ness, Haggerty, Harger, & Ferrell, 2004), while others do not (Van Dyke, Cote, Wenzlaff, Land, & Schwartz, 2009). Thus, despite reported cytokine allelic, genotypic, and haplotype frequency differences by race, a pro-inflammatory genetic profile remains ambiguous.

We found considerable interethnic allelic and haplotype diversity across the 9p21 locus with only two SNPs in perfect LD in both races (rs10757274 and rs4977574) and only a small proportion of the haplotypes shared between the racial groups. We found significant LD among the SNPs in White, but not in Black women. Our data corroborate previous findings of extensive LD in variants on chromosome 9p21 in Northern and Western European (CEU) populations that is diminished in populations of African ancestry (YRI) for both pairwise LD and the size of the haplotype blocks (International HapMap Consortium, 2005; International HapMap Project, 2003). We found that White women displayed two common haplotypes, with one representing risk alleles for all SNPs examined and the other representing wild type alleles for all SNPs. Investigators have previously described these risk and protective haplotypes as a perfect complementary, or yin-yang, pattern (Broadbent et al., 2008; Zhang, Rowe, Clark, & Buetow, 2003).

Despite the exponential advances in understanding the genetic basis of CHD, progress in uncovering the interethnic variations have been slower, and validation studies are few. In this pilot study, we more completely characterized the 9p21 genetic locus by examining all eight SNPs most frequently identified in the literature and by including an ethnic group other than White. The interethnic variations in risk alleles and the LD structure of the eight 9p21 SNPs reported herein contribute to the limited body of knowledge regarding the contributions of race to the genetic determinants of CHD. A more extensive understanding of the interethnic differences in genotype frequencies may inform how the combination of SNPs and distinct haplotypes affect the susceptibility to CHD. Replication studies with diverse populations are needed to clarify the biological link of the 9p21 locus to CHD.

Cognizant of the consequences of multiple statistical tests being conducted with a small sample size, in our analyses of the relationship between the 9p21 variants and inflammatory

biomarkers we concentrated on the size of the associations (η^2) rather than on tests of significance. The homogeneity among the 9p21 SNPs observed in Whites (Figure 1A) but not in Blacks (Figure 1B) parallels the associations observed between the SNP genotypes and the inflammatory markers. For all SNPs examined in White women, those with at least one copy of the risk allele had higher biomarker levels than those with the wild type allele. This pattern was not consistent across SNP genotypes for Black women. We observed interethnic differences in the relationships between all biomarkers and genotypes of both rs10757274 and rs2383206. Few Blacks carried two copies of the risk alleles of these SNPs. However, Blacks with at least one risk allele compared to those with the wild type generally had lower biomarker levels. Conversely, Whites with at least one copy of the risk alleles, compared to those with wild type genotypes, demonstrated higher biomarker concentrations. Similarly, compared to the wild type alleles, the risk alleles of rs10116277, rs1333040, and rs1333049 were generally associated with lower biomarker levels in Blacks but higher levels in Whites. While intriguing, these results require replication. Also warranted is further investigation of the interethnic discrepancies in the strength and direction of the associations between rs10757278 and both IL-6 and ICAM-1. The influence of both prescribed and over-the-counter medications on inflammatory biomarkers also requires closer consideration. Current evidence suggests that environmental factors contribute to inflammatory biomarker levels to a greater degree than genetic influences (Schnabel et al., 2009), but this remains to be replicated in ethnically diverse populations.

Caution is warranted when generalizing these results for a number of reasons—first, because participants were from a single institution in the southeastern United States. Second, while all women had established CHD, phenotypic heterogeneity of this small convenience sample may present a limitation. Third, the effect sizes of the variants were small, plausibly related to phenotypic differences, but this makes them no less interesting. The effect sizes (η^2) for Blacks reported in Table 6 ranged from .001 to .234 with a median value of .039. Using the median effect size (an optimistic assumption), an α .05, and power of .80, a sample size of 200 Blacks with CHD would be sufficient for examining the association between the 9p21 risk alleles and the inflammatory biomarkers. The most conservative estimate of sample size, using the smallest η^2 of .001, indicates that approximately 7,800 Blacks would be required. Fourth, although we identified several interesting interethnic patterns between 9p21 genotypes and inflammatory biomarkers, the available sample, with few Blacks, precluded examination of plausible clinical predictor variables of these biomarkers in multivariate models. Finally, because CHD is a complex polygenic condition, multiple unmeasured polymorphisms may interact to influence inflammatory levels. This pilot study served to refine the biobehavioral methodological protocol, obtain estimates of effect sizes, and generate additional hypotheses for a future longitudinal case-control study that examines gene-gene and gene-environment interactions with a larger sample of women.

Implications and Future Prospects

The aspirations for personalized healthcare, the development of evidence-based treatment strategies, and improved CHD risk prediction are gaining traction as interdisciplinary researchers strive to characterize the genomic, environmental, and behavioral influences underlying the mechanisms of CHD. Although evidence suggests that genetic susceptibility

at the 9p21 locus to CHD is not mediated by known pathogenic mechanisms such as altered lipid metabolism (Broadbent et al., 2008; Schunkert et al., 2008), inflammation (Paynter et al., 2009), or traditional risk factors (Assimes et al., 2008; Brautbar et al., 2009), these possibilities cannot be ruled out, given the limited investigations to date. The lifetime accumulation of traditional risk factors in older age groups may overshadow the genetic effects on CHD (Walston et al., 2007). All women in the current study had CHD, yet only 51% of Whites and 7% of Blacks carried the risk alleles for all 9p21 variants. Additional work is required to examine the common SNPs associated with inflammatory biomarkers and their role in CHD in diverse ethnic and age groups.

The exponential growth of genomic discoveries confers fundamental implications for nursing science and practice. Nurse scientists are playing an increasingly integral role in contributing to the understanding of the biological mechanisms of complex diseases. In this era of genomic science, nurses have a crucial role to play in translating genetics and genomics research through customizing therapies designed to improve clinical outcomes and in counseling individuals and families with identified genetic risks for disease. Through the conduct of translational research, nurse scientists are fostering the integration of genomic findings into evidence-based and culturally sensitive clinical health care. Clinical translation of 9p21 SNP genotypes may prove useful for risk stratification of patients with established CHD (Dandona et al., 2010). Early genetic CHD risk prediction could potentially motivate early and aggressive lifestyle modification before the disease manifests (Johansen, Lanktree, & Hegele, 2010). Nursing research has a long and accomplished history of examining the psychosocial, environmental, cultural, and behavioral influences on health and disease. As integral members of interdisciplinary research teams, nurse scientists are delineating the extent to which genes and health behaviors such as smoking, diet, exercise, and stress interact to influence health and illness. Genetic risks, such as the 9p21 variants, plausibly require specific environmental contexts or stressors in order to manifest phenotypically. Greater characterization of ethnically specific gene–environment interactions is likely to improve our understanding of how changing environmental exposures modify the effects of genetic risk factors. Interdisciplinary research involving the genetic triggers of CHD in consort with the allostatic load model (the cumulative physiological dysregulation in response to environmental stressors; McEwen, 1998a, 1998b) is a promising approach for developing evidence-based interventions to lessen the burden of CHD.

In summary, we found interethnic differences in the haplotype structure of chromosome 9p21 polymorphisms in women with CHD. The ethnically diverse pattern of relationships between 9p21 SNPs and inflammatory biomarkers identified warrants further investigation. Although currently, CHD risk is most accurately assessed using conventional risk factors including knowledge of family history and lifestyle behaviors, the concept of personalized medicine where health care is tailored to environmental and biological conditions is an important future goal (Ferro et al., 2010).

Acknowledgments

TMB carried out the biomarker and genetic analyses. JWB and MWG participated in the data analyses and the manuscript preparation. This work was supported with resources and the use of facilities in the Biobehavioral Laboratory of the College of Nursing, University of South Florida.

Funding: The author(s) disclosed receipt of the following financial support for the research and/or authorship of this article: This study was funded by the National Institute of Nursing Research (R01-NR007678; TMB), the University of South Florida (USF) Established Researcher Grant (TMB), The USF Signature Interdisciplinary Research Program in Cardiovascular Diseases Grant (TMB), and the USF Nursing Faculty Pilot Research Grant (TMB).

References

- Albert MA. Inflammatory biomarkers, race/ethnicity and cardiovascular disease. *Nutrition Reviews*. 2007; 65:S234–238. [PubMed: 18240555]
- Albert MA, Pare G, Morris A, Rose L, Buring J, Ridker PM, Zee RY. Candidate genetic variants in the fibrinogen, methylenetetrahydrofolate reductase, and intercellular adhesion molecule-1 genes and plasma levels of fibrinogen, homocysteine, and intercellular adhesion molecule-1 among various race/ethnic groups: Data from the Women's Genome Health Study. *American Heart Journal*. 2009; 157:777–783. [PubMed: 19332210]
- Arnett DK, Baird AE, Barkley RA, Basson CT, Boerwinkle E, Ganesh SK, O'Donnell CJ. Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: A scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group. *Circulation*. 2007; 115:2878–2901. [PubMed: 17515457]
- Assimes T, Knowles J, Basu A, Iribarren C, Southwick A, Tang H, Quertermous T. Susceptibility locus for clinical and subclinical coronary artery disease at chromosome 9p21 in the multi-ethnic ADVANCE study. *Human Molecular Genetics*. 2008; 17:2320–2328. [PubMed: 18443000]
- Barrett J, Fry B, Maller J, Daly M. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265. [PubMed: 15297300]
- Beckie TM. A behavior change intervention for women in cardiac rehabilitation. *Journal of Cardiovascular Nursing*. 2006; 21:146–153. [PubMed: 16601534]
- Beckie TM, Beckstead JW, Groer MW. The influence of cardiac rehabilitation on inflammation and metabolic syndrome in women with coronary heart disease. *Journal of Cardiovascular Nursing*. 2010; 25:52–60. [PubMed: 19935427]
- Beckie TM, Beckstead JW, Schocken DD, Evans ME, Fletcher GF. The effects of a tailored cardiac rehabilitation program on depressive symptoms in women: A randomized clinical trial. *International Journal of Nursing Studies*. 2011; 48:3–12. [PubMed: 20615504]
- Beckie TM, Mendonca MA, Fletcher GF, Schocken DD, Evans ME, Banks SM. Examining the challenges of recruiting women into a cardiac rehabilitation clinical trial. *Journal of Cardiopulmonary Rehabilitation and Prevention*. 2009; 29:13–21. [PubMed: 19158582]
- Brautbar A, Ballantyne CM, Lawson K, Nambi V, Chambless L, Folsom AR, Boerwinkle E. Impact of adding a single allele in the 9p21 locus to traditional risk factors on reclassification of coronary heart disease risk and implications for lipid-modifying therapy in the Atherosclerosis Risk in Communities study. *Circulation Cardiovascular Genetics*. 2009; 2:279–285. [PubMed: 20031596]
- Broadbent H, Peden J, Lorkowski S, Goel A, Ongen H, Green F, Watkins H. Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. *Human Molecular Genetics*. 2008; 17:806–814. [PubMed: 18048406]
- Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *American Journal of Human Genetics*. 2004; 74:106–120. [PubMed: 14681826]
- Carroll JF, Fulda KG, Chiapa AL, Rodriguez M, Phelps DR, Cardarelli KM, Cardarelli R. Impact of race/ethnicity on the relationship between visceral fat and inflammatory biomarkers. *Obesity*. 2009; 17:1420–1427. [PubMed: 19197255]
- Chen SN, Ballantyne CM, Gotto AM Jr, Marian AJ. The 9p21 susceptibility locus for coronary artery disease and the severity of coronary atherosclerosis. *BMC Cardiovascular Disorders*. 2009; 9:3. [PubMed: 19173706]
- Chen Z, Qian Q, Ma G, Wang J, Zhang X, Feng Y, Yao Y. A common variant on chromosome 9p21 affects the risk of early-onset coronary artery disease. *Molecular Biology Reports*. 2009; 36:889–893. [PubMed: 18459066]

- Cohen, J. Statistical power analysis for the behavioral sciences. 3rd. New York, NY: Academic Press; 1988.
- Cook NR, Buring JE, Ridker PM. The effect of including C-reactive protein in cardiovascular risk prediction models for women. *Annals of Internal Medicine*. 2006; 145:21–29. [PubMed: 16818925]
- Dandona S, Stewart AF, Chen L, Williams K, So D, O'Brien E, Roberts R. Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. *Journal of the American College of Cardiology*. 2010; 56:479–486. [PubMed: 20670758]
- Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature*. 2006; 444:881–887. [PubMed: 17167477]
- Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics*. 1995; 29:311–322. [PubMed: 8666377]
- Elliott P, Chambers JC, Zhang W, Clarke R, Hopewell JC, Peden JF, Kooner JS. Genetic loci associated with C-reactive protein levels and risk of coronary heart disease. *Journal of the American Medical Association*. 2009; 302:37–48. [PubMed: 19567438]
- Ellis KL, Pilbrow AP, Frampton CM, Doughty RN, Whalley GA, Ellis CJ, Cameron VA. A common variant at chromosome 9P21.3 is associated with age of onset of coronary disease but not subsequent mortality. *Circulation Cardiovascular Genetics*. 2010; 3:286–293. [PubMed: 20400779]
- Espinola-Klein C, Rupprecht HJ, Bickel C, Post F, Genth-Zotz S, Lackner K, Blankenberg S. Impact of metabolic syndrome on atherosclerotic burden and cardiovascular prognosis. *American Journal of Cardiology*. 2007; 99:1623–1628. [PubMed: 17560864]
- Feinberg MS, Schwartz R, Behar S. Impact of metabolic syndrome in patients with acute coronary syndrome. *Advances in Cardiology*. 2008; 45:114–126. [PubMed: 18230959]
- Ferro W, Geada D, Guevara Y, Padilla S, Conde A, Alvarez T, Valdes R. Prolonged stability of the plantibody HB-01 directed against the hepatitis B surface antigen in cryo-preserved tobacco leaves. *Biologicals*. 2010; 38:415–419. [PubMed: 20303783]
- Ford ES, Giles WH, Mokdad AH, Myers GL. Distribution and correlates of C-reactive protein concentrations among adult US women. *Clinical Chemistry*. 2004; 50:574–581. [PubMed: 14709450]
- Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM. Metabolic syndrome and risk of incident cardiovascular events and death: A systematic review and meta-analysis of longitudinal studies. *Journal of the American College of Cardiology*. 2007; 49:403–414. [PubMed: 17258085]
- Grundey SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Costa F. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005; 112:2735–2752. [PubMed: 16157765]
- Grundey SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Costa F. Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Current Opinion in Cardiology*. 2006; 21:1–6. [PubMed: 16355022]
- Helgadóttir A, Thorleifsson G, Manolescu A, Gretarsdóttir S, Blondal T, Jonasdóttir A, Stefansson K. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science*. 2007; 316:1491–1493. [PubMed: 17478679]
- Helsel DR. More than obvious: Better methods for interpreting nondetect data. *Environmental Science and Technology*. 2005; 39:419A–423A.
- Hinohara K, Nakajima T, Takahashi M, Hohda S, Sasaoka T, Nakahara K, Kimura A. Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. *Journal of Human Genetics*. 2008; 53:357–359. [PubMed: 18264662]
- Horne BD, Carlquist JF, Muhlestein JB, Bair TL, Anderson JL. Association of variation in the chromosome 9p21 locus with myocardial infarction versus chronic coronary artery disease. *Circulation Cardiovascular Genetics*. 2008; 1:85–92. [PubMed: 19956784]

- Humphries SE, Drenos F, Ken-Dror G, Talmud PJ. Coronary heart disease risk prediction in the era of genome-wide association studies: Current status and what the future holds. *Circulation*. 2010; 121:2235–2248. [PubMed: 20497987]
- Hyatt TC, Phadke RP, Hunter GR, Bush NC, Munoz AJ, Gower BA. Insulin sensitivity in African-American and White women: Association with inflammation. *Obesity*. 2009; 17:276–282. [PubMed: 19039315]
- International HapMap Consortium. A haplotype map of the human genome. *Nature*. 2005; 437:1299–1320. [PubMed: 16255080]
- International HapMap Project. *Nature*. 2003; 426:789–796. [PubMed: 14685227]
- Johansen CT, Lanktree MB, Hegele RA. Translating genomic analyses into improved management of coronary artery disease. *Future Cardiology*. 2010; 6:507–521. [PubMed: 20608823]
- Karvanen J, Silander K, Kee F, Tiet L, Salomaa V, Kuulasmaa K, Evans A. The impact of newly identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. *Genetic Epidemiology*. 2008; 33:237–246. [PubMed: 18979498]
- Kim HC, Greenland P, Rossouw JE, Manson JE, Cochrane BB, Lasser NL, Robinson JG. Multimarker prediction of coronary heart disease risk: The Women's Health Initiative. *Journal of the American College of Cardiology*. 2010; 55:2080–2091. [PubMed: 20447530]
- Koenig W, Khuseynova N. Biomarkers of atherosclerotic plaque instability and rupture. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007; 27:15–26.
- Lewontin RC. The interaction of selection and linkage. I. General considerations; heterotic models. *Genetics*. 1964; 49:49–67. [PubMed: 17248194]
- Libby P. Inflammation in atherosclerosis. *Nature*. 2002; 420:868–874. [PubMed: 12490960]
- Libby P, Okamoto Y, Rocha VZ, Folco E. Inflammation in atherosclerosis: Transition from theory to practice. *Circulation Journal*. 2010; 74:213–220. [PubMed: 20065609]
- Maitra A, Dash D, John S, Sannappa PR, Das AP, Shanker J, Kakkar VV. A common variant in chromosome 9p21 associated with coronary artery disease in Asian Indians. *Journal of Genetics*. 2009; 88:113–118. [PubMed: 19417554]
- McEwen BS. Protective and damaging effects of stress mediators. *New England Journal of Medicine*. 1998a; 338:171–179. [PubMed: 9428819]
- McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of the New York Academy of Sciences*. 1998b; 840:33–44. [PubMed: 9629234]
- McPherson R. Chromosome 9p21 and coronary artery disease. *New England Journal of Medicine*. 2010; 362:1736–1737. [PubMed: 20445187]
- McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Cohen J. A common allele on chromosome 9 associated with coronary heart disease. *Science*. 2007; 316:1488–1491. [PubMed: 17478681]
- Meng W, Hughes AE, Patterson CC, Belton C, Kee F, McKeown PP. Chromosome 9p21.3 is associated with early-onset coronary heart disease in the Irish population. *Disease Markers*. 2008; 25:81–85. [PubMed: 18957718]
- Ness RB, Haggerty CL, Harger G, Ferrell R. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. *American Journal of Epidemiology*. 2004; 160:1033–1038. [PubMed: 15561982]
- Newton-Cheh C, Cook NR, VanDenburgh M, Rimm EB, Ridker PM, Albert CM. A common variant at 9p21 is associated with sudden and arrhythmic cardiac death. *Circulation*. 2009; 120:2062–2068. [PubMed: 19901189]
- Novack V, Cutlip DE, Jotkowitz A, Lieberman N, Porath A. Reduction in sex-based mortality difference with implementation of new cardiology guidelines. *American Journal of Medicine*. 2008; 121:597–603. [PubMed: 18538296]
- Palomaki GE, Melillo S, Bradley LA. Association between 9p21 genomic markers and heart disease: A meta-analysis. *Journal of the American Medical Association*. 2010; 303:648–656. [PubMed: 20159873]
- Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR, Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. *Annals of Internal Medicine*. 2009; 150:65–72. [PubMed: 19153409]

- Pischon T, Hu FB, Rexrode KM, Girman CJ, Manson JE, Rimm EB. Inflammation, the metabolic syndrome, and risk of coronary heart disease in women and men. *Atherosclerosis*. 2008; 197:392–399. [PubMed: 17681508]
- Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: An 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003; 107:391–397. [PubMed: 12551861]
- Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine*. 2000; 342:836–843. [PubMed: 10733371]
- Roberts R, Wells GA, Stewart AF, Dandona S, Chen L. The genome-wide association study – A new era for common polygenic disorders. *Journal of Cardiovascular Translational Research*. 2010; 3:173–182. [PubMed: 20560037]
- Roy H, Bhardwaj S, Yla-Herttuala S. Molecular genetics of atherosclerosis. *Human Genetics*. 2009; 125:467–491. [PubMed: 19301036]
- Saleheen D, Alexander M, Rasheed A, Wormser D, Soranzo N, Hammond N, Danesh J. Association of the 9p21.3 locus with risk of first-ever myocardial infarction in Pakistanis: Case-control study in South Asia and updated meta-analysis of Europeans. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2010; 30:1467–1473.
- Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, Schunkert H. Genome-wide association analysis of coronary artery disease. *New England Journal of Medicine*. 2007; 357:443–453. [PubMed: 17634449]
- Samani NJ, Raitakari OT, Sipila K, Tobin MD, Schunkert H, Juonala M, Lehtimäki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2008; 28:1679–1683.
- Schnabel RB, Lunetta KL, Larson MG, Dupuis J, Lipinska I, Rong J, Benjamin EJ. The relation of genetic and environmental factors to systemic inflammatory biomarker concentrations. *Circulation Cardiovascular Genetics*. 2009; 2:229–237. [PubMed: 20031590]
- Schütt H, Luchtfeld M, Grothausen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. *Thrombosis and Haemostasis*. 2009; 102:215–222. [PubMed: 19652871]
- Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, Samani NJ. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. *Circulation*. 2008; 117:1675–1684. [PubMed: 18362232]
- Silander K, Tang H, Myles S, Jakkula E, Timpson N, Cavalli-Sforza L, Peltonen L. Worldwide patterns of haplotype diversity at 9p21.3, a locus associated with type 2 diabetes and coronary heart disease. *Genome Medicine*. 2009; 1:51. [PubMed: 19463184]
- Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics*. 2003; 73:1162–1169. [PubMed: 14574645]
- Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*. 2001; 68:978–989. [PubMed: 11254454]
- Van Dyke AL, Cote ML, Wenzlaff AS, Land S, Schwartz AG. Cytokine SNPs: Comparison of allele frequencies by race and implications for future studies. *Cytokine*. 2009; 46:236–244. [PubMed: 19356949]
- Walston JD, Fallin MD, Cushman M, Lange L, Psaty B, Jenny N, Reiner A. IL-6 gene variation is associated with IL-6 and C-reactive protein levels but not cardiovascular outcomes in the Cardiovascular Health Study. *Human Genetics*. 2007; 122:485–494. [PubMed: 17851695]
- Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007; 447:661–678. [PubMed: 17554300]
- Yamada Y, Ichihara S, Nishida T. Molecular genetics of myocardial infarction. *Genomic Medicine*. 2008; 2:7–22. [PubMed: 18704761]
- Yan E, Chen S, Hong K, Kim WS, Bajpai A, Treyzon L, Heber D. Insulin, hs-CRP, leptin, and adiponectin. An analysis of their relationship to the metabolic syndrome in an obese population

with an elevated waist circumference. *Metabolic Syndrome and Related Disorders*. 2008; 6:64–73. [PubMed: 18370838]

Zhang J, Rowe WL, Clark AG, Buetow KH. Genomewide distribution of high-frequency, completely mismatching SNP haplotype pairs observed to be common across human populations. *American Journal of Human Genetics*. 2003; 73:1073–1081. [PubMed: 14560401]

Figure 1.

Analysis of linkage disequilibrium (LD) in A. White ($n = 75$) and B. Black ($n = 16$) women. LD patterns between eight single nucleotide polymorphisms (SNPs) rs10116277, rs1333040, rs10757274, rs4977574, rs2383206, rs2383207, rs10757278, rs1333049 were derived from genotyping data from a sample of women with established coronary heart disease. The pairwise correlation between SNPs was measured as D' and is shown ($\times 100$) in each diamond. Diamonds without numbers represent $D' = 1$. The color scheme is white, $r^2 = 0$; shades of gray, $0 < r^2 < 1$; black, $r^2 = 1$.

Table 1
Baseline Diagnostic Category and Medications (*n* [%])

Variable	White <i>n</i> = 75	Black <i>n</i> = 16	χ^2	<i>P</i>
Diagnostic events/procedures at index hospitalization				
Percutaneous coronary intervention (PCI)	22 (29)	5 (31)	5.064 ^a	.652
Coronary artery bypass graft surgery (CABG)	22 (29)	5 (31)		
Myocardial infarction → PCI	14 (19)	3 (19)		
Myocardial infarction → CABG	3 (4)	2 (13)		
Stable angina	10 (13)	0 (0)		
Myocardial infarction	4 (5)	1 (6)		
Medications				
β blockers	59 (79)	12 (75)	.103	.488
Diuretics	24 (32)	7 (44)	.811	.267
Angiotensin converting enzyme inhibitors	22 (29)	7 (44)	1.262	.202
Lipid lowering agents	70 (93)	13 (81)	2.401	.143
Clopidogrel	49 (65)	12 (75)	.558	.332
Aspirin	67 (89)	13 (81)	.811	.098
Insulin	6 (8)	6 (37)	10.024	.006
Oral hypoglycemic agents	24 (32)	3 (19)	1.109	.230
Angiotensin receptor blocker	22 (29)	4 (6)	.121	.494
Nonsteroidal anti-inflammatory agents	18 (24)	6 (38)	1.238	.209
Hormone replacement therapy	5 (7)	1 (6)	.004	.716

Note. Chi-square (χ^2) test used to analyze discrete variables.

^aDegrees of freedom (*df*) = 5; for all other χ^2 tests, *df* = 1.

Table 2
Baseline Risk Factors and Comorbidities

Risk Factors and Comorbidities	Total N = 91	White n = 75	Black n = 16	P
Risk factors				
Physical inactivity	80 (88)	66 (88)	14 (88)	.616
Overweight	63 (69)	53 (71)	10 (62)	.357
Dyslipidemia	75 (82)	63 (84)	12 (75)	.297
Familial coronary heart disease	43 (47)	38 (51)	5 (31)	.128
Pack years smoked, $m \pm SD$	14 $\pm$ 19	14 $\pm$ 19	13 $\pm$ 17	.761
Lives with a smoker	16 (18)	14 (19)	2 (13)	.431
Hypertension (or on antihypertensives)	75 (82)	61 (89)	14 (88)	.431
Diabetes	34 (37)	26 (35)	8 (50)	.192
Comorbidities				
Previous myocardial infarction	16 (18)	13 (17)	3 (19)	.569
Previous coronary artery bypass graft surgery	7 (8)	7 (9)	0 (0)	.245
Previous percutaneous coronary intervention	10 (11)	8 (11)	2 (13)	.558
Previous stroke	4 (4)	1 (1)	3 (19)	.016
Peripheral vascular disease	10 (11)	8 (11)	2 (13)	.558
Degenerative arthritis	53 (58)	42 (56)	11 (69)	.257

Note. Continuous data are presented as means $\pm$ standard deviation; categorical data are presented as number and percentage. Chi-square (χ^2) test and *t*-test were used to analyzed discrete and continuous variables, respectively.

Table 3

Baseline Physiological Characteristics

Variable	Total Sample N = 91	White n = 75	Black n = 16	P	η^2
Age	61.2 ± 10	62.3 ± 10	57.2 ± 9.6	.06	.040
Total cholesterol (mg/dL)	162 ± 38	163.5 ± 39	156 ± 34	.48	.006
LDL-cholesterol (mg/dL)	92 ± 34	93 ± 35	93 ± 26	.96	.000
Total/HDL cholesterol ratio	4 ± 2	4.1 ± 1.6	3.7 ± 1.1	.39	.008
Body mass index (kg/m ²)	32 ± 7	32 ± 7	32 ± 7	.79	.001
Body weight (lb)	183 ± 43	186 ± 44	184 ± 40	.89	.000
Percent body fat	38.9 ± 3	38.8 ± 3.4	39.4 ± 2.8	.51	.005
Resting heart rate (bpm)	72 ± 9	72 ± 9	75 ± 11	.13	.026
Peak exercise capacity (METs)	6 ± 2	6 ± 2	5 ± 2	.36	.010
Peak treadmill time (min)	8.7 ± 3	8.7 ± 3	7.9 ± 3	.36	.009
Metabolic syndrome components					
HDL-cholesterol (mg/dL)	43.4 ± 13	43.1 ± 12.4	44.7 ± 13	.66	.002
Triglycerides (mg/dL)	132 ± 65	137 ± 67	101 ± 36	.04	.049
Fasting glucose (mg/dL)	104 ± 31	102 ± 24	111 ± 53	.26	.014
Waist circumference (cm)	101 ± 15	101 ± 16	100 ± 13	.87	.000
Systolic BP (mm Hg)	126 ± 16	126 ± 15	128 ± 18	.67	.002
Diastolic BP (mm Hg)	73 ± 10	72 ± 10	77 ± 11	.08	.034
HsCRP (mg/L)	6.6 ± 10.5	6.7 ± 11	5.8 ± 6	.68	.002
Interleukin-6 (pg/mL)	19 ± 48	22 ± 53	4 ± 3	.04	.047
TNF-α (pg/mL)	10 ± 21	11 ± 23	6 ± 5	.16	.022
ICAM-1 (pg/mL)	211.5 ± 117	212 ± 122	207 ± 98	.98	.000

Note. BP = blood pressure; bpm = beats per minute; HDL = high-density lipoprotein; HsCRP = high-sensitivity C-reactive protein; ICAM-1 = intercellular adhesion molecule-1; LDL = low-density lipoprotein; METs = metabolic equivalent units; TNF-α = tumor necrosis factor-α. Results are presented as means ± SD; differences between Whites and Blacks calculated using *t*-test.

Table 4
Genotype Counts for Eight SNPs at the Chromosome 9p21 Locus, Stratified by Race (*n* [%]).

Genotype	HapMap Populations ^b			White <i>n</i> = 75	Black <i>n</i> = 16	χ^2 (<i>df</i> = 2)	<i>P</i>
	CEU (%)	YRI (%)					
rs10116277							
GG	30.1	0.0	13 (17.3)	1 (6.2)			
GT	48.7	3.4	36 (48.0)	2 (12.5)			
TT ^a	21.2	96.6	26 (34.7)	13 (81.3)	11.710	.003	
rs1333040							
CC	19.5	7.5	10 (13.0)	1 (6.2)			
CT	49.5	51.7	30 (40.0)	8 (50.0)			
TT ^a	31.0	40.8	35 (47.0)	7 (43.8)	.887	.642	
rs10757274							
AA	23.6	71.7	16 (21.3)	10 (62.5)			
AG	52.8	28.3	33 (44.0)	6 (37.5)			
GG ^a	23.6	0.0	26 (34.7)	0 (0.0)	13.498	.001	
rs4977574							
AA	29.2	81.6	16 (21.3)	10 (62.5)			
AG	49.6	17.7	32 (42.7)	6 (37.5)			
GG ^a	21.2	0.7	27 (36.0)	0 (0.0)	13.666	.001	
rs2383206							
AA	18.3	31.7	11 (14.7)	2 (12.5)			
AG	58.4	48.3	33 (44.0)	12 (75.0)			
GG ^a	23.3	20.0	31 (41.3)	2 (12.5)	5.629	.060	
rs2383207							
AA	25.7	0.0	12 (16.0)	0 (0.0)			
AG	52.2	3.4	30 (40.0)	3 (18.7)			
GG ^a	22.1	96.6	33 (44.0)	13 (81.3)	7.822	.020	
rs10757278							
AA	23.6	89.8	19 (25.3)	8 (50.0)			

HapMap Populations ^b					
Genotype	CEU (%)	YRI (%)	White n = 75	Black n = 16	χ^2 (df = 2) P
AG	52.8	10.2	29 (38.7)	7 (43.8)	
GG ^a	23.6	0.0	27 (36.0)	1 (6.2)	6.583 .037
rs1333049					
CC ^a	20.4	2.7	27 (36.0)	0 (0.0)	
CG	50.4	28.6	30 (40.0)	9 (56.2)	
GG	29.2	68.7	18 (24.0)	7 (43.8)	8.445 .015

Note. CEU = Utah residents with Northern and Western European ancestry; SNP = single nucleotide polymorphism; YRI = Yoruba in Ibadan, Nigeria. Allelic frequencies can be calculated as the proportion of homozygotes plus one-half of heterozygotes.

^aRisk allele.

^bHapMap populations (<http://hapmap.ncbi.nlm.nih.gov/>).

Table 5
Estimated Relative Frequencies of Eight SNP Haplotypes for the 9p21 Locus in White and Black Women

rs10116277	rs1333040	rs10757274	rs4977574	rs2383206	rs2383207	rs10757278	rs1333049	White n = 75	Black n = 16
T	T	G	G	G	G	G	C	.511	.074
G	C	A	A	A	A	A	G	.263	.000
G	T	A	A	A	A	A	G	.070	.000
T	T	A	A	G	G	A	G	.035	.109
G	C	G	G	G	G	G	C	.029	.000
T	T	G	G	G	G	A	G	.020	.000
T	T	A	A	A	G	A	G	.000	.406
T	T	A	A	G	G	G	G	.000	.063
T	C	A	A	A	G	A	G	.000	.056
T	C	A	A	G	G	A	G	.000	.048
T	C	G	G	G	G	G	C	.000	.058
T	C	A	A	G	G	A	C	.000	.063
G	C	G	G	G	A	G	C	.000	.019
G	T	G	G	G	A	G	C	.000	.036
Cumulative relative frequency									.932

Note. The first row, represents the risk haplotype (TTGGGGGC). The second row represents the protective haplotype (GCACAAAAG).

Table 6
Relationship Between Inflammatory Biomarkers and Genotype of Polymorphisms on Chromosome 9p21, Stratified by Race

Phenotype	White <i>n</i> = 75		Black <i>n</i> = 16	
	rs10116277			
	GG	GT/TT ^a	GG	GT/TT ^a
HsCRP mg/L	4.4 (4.7)	7.2 (12.2)	3.7 (-)	6.0 (6.0)
		η^2		η^2
IL-6 pg/mL	9.7 (13.8)	25.0 (57.0)	7.0 (-)	4.0 (3.2)
		η^2		η^2
TNF- α pg/mL	8.3 (7.4)	11.3 (25.4)	7.8 (-)	5.4 (4.7)
		η^2		η^2
ICAM-1 pg/mL	175.4 (126.5)	220.2 (120.3)	244.3 (-)	204.4 (100.9)
		η^2		η^2
	rs1333040			
	CC	CT/TT ^a	CC	CT/TT ^a
HsCRP mg/L	5.4 (6.1)	6.9 (11.9)	3.7 (-)	5.9 (6.0)
		η^2		η^2
IL-6 pg/mL	11.8 (15.2)	24.0 (55.9)	7.0 (-)	4.0 (3.2)
		η^2		η^2
TNF- α pg/mL	8.9 (7.9)	11.1 (24.8)	7.8 (-)	5.4 (4.7)
		η^2		η^2
ICAM-1 pg/mL	145.6 (58.4)	222.7 (125.9)	244.3 (-)	204.4 (100.9)
		η^2		η^2
	rs10757274			
	AA	AG/GG ^a	AA	AG/GG ^a
HsCRP mg/L	6.1 (6.9)	6.9 (12.2)	7.3 (6.8)	3.3 (2.4)
		η^2		η^2
IL-6 pg/mL	6.3 (6.2)	26.7 (58.3)	4.4 (3.5)	3.8 (2.7)
		η^2		η^2
TNF- α pg/mL	8.5 (6.6)	11.4 (26.0)	7.2 (4.9)	2.8 (2.1)
		η^2		η^2
ICAM-1 pg/mL	183.3 (121.2)	220.3 (121.7)	228.2 (116.4)	171.3 (44.3)
		η^2		η^2
	rs2383206			
	AA	AG/GG ^a	AA	AG/GG ^a
HsCRP mg/L	4.7 (5.1)	7.1 (12.0)	11.1 (15.2)	5.1 (4.0)
		η^2		η^2
IL-6 pg/mL	5.0 (3.4)	25.3 (56.2)	1.6 (0)	4.6 (3.2)
		η^2		η^2
TNF- α pg/mL	7.4 (8.2)	11.4 (24.9)	1.6 (0)	6.1 (4.6)
		η^2		η^2
ICAM-1 pg/mL	186.2 (132.4)	216.9 (120.4)	271.8 (159.8)	197.6 (91.5)
		η^2		η^2
	rs2383207			
	AA	AG/GG ^a	AA	AG/GG ^a
HsCRP mg/L	3.4 (3.5)	7.4 (12.1)	-	5.8 (5.8)
		η^2		η^2
IL-6 pg/mL	6.2 (6.2)	25.4 (56.6)	-	4.2 (3.2)
		η^2		η^2

Phenotype	White <i>n</i> = 75		Black <i>n</i> = 16	
TNF- α pg/mL	7.7 (7.8)	11.4 (25.2)	.005	– 5.6 (4.6)
ICAM-1 pg/mL	179.9 (131.1)	218.6 (119.9)	.042	– 206.9 (98.0)
			rs10757278	
	AA	AG/GG ^a	η^2	AA AG/GG ^a η^2
HsCRP mg/L	6.1 (6.9)	7.0 (12.4)	.000	8.3 (7.4) 3.4 (2.1) .102
IL-6 pg/mL	7.1 (6.3)	27.6 (59.8)	.026	3.1 (1.7) 5.3 (4.0) .112
TNF- α pg/mL	7.6 (6.5)	11.9 (26.7)	.002	6.9 (5.5) 4.3 (3.3) .060
ICAM-1 pg/mL	189.9 (115.2)	220.1 (123.9)	.029	248.3 (111.7) 165.5 (64.6) .171
			rs1333049	
	GG	GC/CC ^a	η^2	GG GC/CC ^a η^2
HsCRP mg/L	5.6 (6.7)	7.1 (12.4)	.000	7.5 (8.0) 4.5 (3.2) .014
IL-6 pg/mL	7.1 (6.5)	27.2 (59.3)	.025	4.2 (4.0) 4.1 (2.6) .002
TNF- α pg/mL	7.6 (6.6)	11.8 (26.4)	.002	5.5 (3.9) 5.6 (5.3) .001
ICAM-1 pg/mL	189.3 (118.5)	219.7 (122.8)	.032	216.7 (138.2) 199.3 (59.3) .006

Note. HsCRP = high sensitivity C-reactive protein; ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; TNF- α = tumor necrosis factor alpha. Data are presented as means (SD).

^aRisk genotype.