The Relationship Between Polymorphisms on Chromosome 9p21 and Age of Onset of Coronary Heart Disease in Black and White Women

Theresa M. Beckie, Maureen W. Groër, and Jason W. Beckstead

Aim: Genome-wide association studies have identified variants on chromosome 9p21 that are associated with coronary heart disease (CHD). The relationship between these variants and the age of onset of CHD is less clear. The aim of this study was to examine the allelic frequencies and haplotype structure of eight single-nucleotide polymorphisms (SNPs) on chromosome 9p21 in ethnically diverse women. We also explored the relationship between 9p21 SNPs and the age of CHD onset. **Results:** There was considerable interethnic allelic and haplotype diversity across the 9p21 locus with only two SNPs (rs10757274 and rs4977574) in perfect linkage disequilibrium in both races, and only a small proportion of the haplotypes shared between the racial groups. With the exception of rs1333040, whites with at least one copy of the 9p21 SNP risk alleles were found to have CHD from 1.45 (rs10116277) to 4.77 (rs2383206) years earlier than those with the wild-type alleles. Blacks carrying at least one copy of the risk allele (92%) for rs1333040 had a CHD age of onset that was 6.5 years earlier than those with the wild-type alleles. **Conclusions:** Different variants on chromosome 9p21 may influence CHD age of onset in whites and blacks.

Introduction

CORONARY HEART DISEASE (CHD) is a polygenic disease resulting from complex interactions between multiple genes and environmental exposures (Arnett *et al.*, 2007; Roberts *et al.*, 2010). A considerable number of individuals develop CHD at a young age with risk factors that are predominantly genetic (Roberts, 2008; Kullo and Cooper, 2010). A history of early CHD in a first-degree relative confers double the risk of CHD with relative risks ranging from 1.3 to 11.3 (Kullo and Ding, 2007). Despite substantial heritability (Roberts *et al.*, 2010), the genetic foundation of CHD has been poorly characterized in women and ethnic minorities (Humphries *et al.*, 2010). Genome-wide association studies have identified common variants located in a 58 kilobase region on chromosome 9p21 that are associated with CHD (Helgadottir *et al.*, 2007; McPherson *et al.*, 2007; Samani *et al.*, 2007). These studies were largely conducted in populations of European ancestry (Rotimi and Jorde, 2010). A meta-analysis of studies of Northern Europeans provided compelling evidence linking chromosome 9p21 single-nucleotide polymorphisms (SNPs) and CHD (Schunkert *et al.*, 2008). These associations have been robustly replicated 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), South Asian (Saleheen *et al.*, 2010), and European (Karvanen *et al.*, 2009) populations. The few studies comprising blacks have found 9p21 variants to be less prevalent than in whites (McPherson *et al.*, 2007; Assimes *et al.*, 2008).

Comparisons across studies of the association between the 9p21 risk locus and early-onset CHD are challenging and results are mixed. Studies differ in the selection of 9p21 SNPs and the definitions of early-onset disease but uniformly include few women or non-white populations. Chromosome 9p21 SNPs were correlated with early onset CHD in several white (Helgadottir *et al.*, 2007; Abdullah *et al.*, 2008; Meng *et al.*, 2008; Ellis *et al.*, 2010) and Chinese (Chen *et al.*, 2009) populations but not in others (Broadbent *et al.*, 2008; Dehghan *et al.*, 2008). Approximately 25% of whites carry two copies of the 9p21 risk alleles with an estimated risk of early onset CHD that is twice that among individuals without these alleles (McPherson, 2010). Whites with early onset CHD who were homozygous for a 9p21 SNP risk allele (rs1333049-C) had a twofold increased risk for developing 3-vessel CHD compared to those with the nonrisk genotype (Dandona *et al.*, 2010). The Global Registry of Acute Coronary Events (GRACE) Genetics study, however, found no association between rs1333049 and three-vessel disease in whites with a mean age of 65 years (Buysschaert *et al.*, 2010). Others reported stronger correlations between 9p21 SNPs and the

broader phenotype of CHD than with myocardial infarction (MI) (Samani *et al.*, 2007; Broadbent *et al.*, 2008; Horne *et al.*, 2008).

Examining additional SNPs on chromosome 9p21 may refine the identification of the risk haplotype by determining which SNPs are most robust, and how ethnicity modulates the influence of these SNPs on CHD (Schunkert *et al.*, 2008). Given the conflicting finds regarding the relationship between 9p21 variants and early-onset CHD and the limited inclusion of ethnic minorities, additional replication studies are required before generalizations can be made across multiple populations. The purpose of this pilot study was to examine the allelic frequencies and haplotype structure of genetic variants on chromosome 9p21 in a cohort of black and white women with CHD. We also sought to explore the relationship between these genetic variants and the age of onset of CHD.

Materials and Methods

Participants

Data for this study are from a subsample of women who completed a larger secondary prevention clinical trial. The university institutional review board approved the study and all participants provided written informed consent. Details of recruitment to and the methodological design of the parent trial are described elsewhere (Beckie, 2006; Beckie *et al.*, 2009). The study included women over the age of 21 years, with the diagnosis of an MI, unstable angina, or coronary revascularization within 3 months from the index event.

Clinical characteristics

Traditional risk factors for CHD, anthropomorphic measures, and medication use, were collected at baseline by a trained research assistant. Body mass index was calculated as weight (kg)/height (m²). Twelve-hour fasting lipid and glucose measurements were obtained utilizing the Cholestech LDX system (Hayward, CA). The presence of CHD was determined by coronary angiography and confirmation by the participant's cardiologist. Age of onset was defined as the first documented diagnosis of CHD.

Chromosome 9p21 genotype determination

Blood was overlaid on Histopaque (Sigma) and centrifuged at 1200 rpm for 25 min at room temperature with the brake off. The peripheral blood mononuclear cell (PBMC) layer was washed twice in RPMI-1640 with 10% fetal calf serum with 50 ug/ml gentamicin. Cell pellets were resuspended in freezing media with added 10% DMSO and transferred to liquid nitrogen. After quick thawing, PBMCs were centrifuged for 30 min at 4 degrees. The QIAamp DNA Micro Kits (Qiagen, Inc., Valencia, CA) were used to extract 50 µL of genomic DNA from each cell pellet. Samples were diluted to 20 ng of DNA/µL. Predesigned TaqMan[®] SNP genotyping assays (Applied Biosystems, Foster City, CA) were used to genotype eight SNPs spanning the 58 kb region of 9p21. The polymerase chain reactions were performed in a total volume of 25 µL containing 2.5 µL DNA, 12.5 µL TaqMan Genotyping Master Mix (Applied Biosystems), 1.25 µL of TaqMan assay, and 8.75 µL water. Reactions were performed in 96 well plates in triplicate using a Bio-Rad CFX quantitative polymerase chain reaction thermocycler. DNA was amplified under the

following conditions—Step 1: 50°C for 2 min; Step 2: 95°C for 10 min; Step 3: 40 cycles of 92°C for 15 s followed by 60°C for 1 min. Genotypes were generated by automatic calling using the CFX96 version 1.5 software and manually reviewed.

Haplotype construction

Pearson's chi-square (χ^2) test with 1 degree of freedom was used to assess whether the SNPs were in Hardy-Weinberg equilibrium among blacks and whites separately. Genotyping data were analyzed with PHASE version 2.1.1 (www.stat.washington.edu/stephens/software.html) that uses Bayesian statistics to infer phase at linked loci from the population genotype data, and reconstructs haplotypes with sample frequency estimates (Stephens *et al.*, 2001; Stephens and Donnelly, 2003). Haploview version 4.2 (www.broadinstitute.org/haploview/) was used to assess pair-wise linkage disequilibrium (LD) between the SNPs. Haplotype blocks were graphically identified from the LD intensity expressed as Lewontin's D' statistic (Lewontin, 1964) and r^2 (Devlin and Risch, 1995; Barrett *et al.*, 2005). The solid spine of LD method was used to search for strong LD from the first SNP to the last along the legs of the triangle in the LD plot using $D' > 0.5$ to define the LD blocks. Haploview's Tagger strategy was used to select tagging SNPs that best captured all alleles that were correlated at an $r^2 \geq 0.8$.

Statistical analysis

The PASW[®] Statistics 18 for Windows (SPSS, Inc., Chicago, IL) system was used for statistical analyses. Descriptive statistics, including means, standard deviations, and percentages, were used to describe the study variables. Pearson's χ^2 analysis was used to compare the distributions of allele frequencies by ethnicity. Analysis of variance was used to compare the mean age of CHD onset according to the presence versus absence of one or more copies of the risk alleles for each SNP. With a sample size of 146 women and $\alpha = 0.05$, the possibility of type II statistical error was apparent. Therefore, presentation 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

Participant characteristics

A total of 146 women with complete baseline genotypic and clinical data were included in this study. With a mean age of 63 ± 11 , 84% of women were white and 16% were black. The majority had undergone either percutaneous coronary interventions (30%) or coronary artery bypass grafting (27%) before enrolling in the parent study, and 31% were found to have an MI (Table 1). Most women were taking β blockers (82%), statins (88%), and aspirin (86%) with more blacks than whites on insulin therapy.

The participants had numerous traditional risk factors, including hypertension (79%), dyslipidemia (84%), overweight (60%), and physical activity (87%) (Table 2). A greater proportion of whites, compared to blacks, had a family history of CHD, whereas more blacks were diabetic and had a history of previous stroke. Table 3 provides the baseline physiological characteristics of blacks and whites. While blacks

TABLE 1. BASELINE DIAGNOSTIC CATEGORY AND MEDICATIONS

Variable, n (%)	White, n = 122	Black, n = 24	χ^2	p
Diagnostic events/procedures at index hospitalization				
Percutaneous coronary intervention (PCI)	36 (29)	8 (33)	4.549 ^a	0.203
Coronary artery bypass graft surgery (CABG)	34 (28)	6 (25)		
Myocardial infarction → PCI	24 (20)	4 (17)		
Myocardial infarction → CABG	5 (4)	3 (12)		
Stable angina	15 (12)	1 (4)		
Myocardial infarction	8 (7)	1 (4)		
Medications				
β blockers	100 (82)	19 (79)	0.104	0.469
Diuretics	37 (30)	12 (50)	3.481	0.054
Angiotensin converting enzyme inhibitors	36 (29)	11 (46)	2.449	0.094
Lipid lowering Agents	109 (89)	19 (79)	1.922	0.148
Clopidogrel	78 (64)	17 (71)	0.420	0.345
Aspirin	107 (88)	19 (79)	1.237	0.209
Insulin	8 (7)	12 (50)	32.015	0.001
Oral hypoglycemic agents	34 (28)	6 (25)	0.083	0.495
Angiotensin receptor blocker	32 (26)	7 (29)	0.088	0.471
Nonsteroidal anti-inflammatory agents	30 (25)	7 (29)	0.222	0.405
Hormone replacement therapy	10 (8)	1 (4)	0.468	0.431

Data presented as number (percentage); chi-square (χ^2) test used to analyze discrete variables.

^adegrees of freedom = 5; for all other χ^2 tests, degrees of freedom = 1.

demonstrated higher fasting glucose and greater waist circumference, whites had higher triglyceride concentrations.

compared to 35.2% of whites; there was little interethnic genotypic variation for rs1333040.

Allelic and genotypic characteristics

The SNPs did not deviate from the Hardy-Weinberg equilibrium for whites or blacks (all $p > 0.2$). Table 4 presents the interethnic genotype frequencies of the participants and for relevant International HapMap Project populations (<http://hapmap.ncbi.nlm.gov>). Compared to whites, a larger proportion of blacks carried the risk genotype of two SNPs (rs10116277 and rs2383207). Few blacks, however, compared to whites, had the risk genotype for four of the eight SNPs (rs10757274, rs4977574, rs10757278, and rs1333049). Only 12.5% of blacks had the risk genotype (GG) for rs2383206

Linkage disequilibrium and haplotypes

The estimates of the haplotype frequencies of the 9p21 SNPs determine by PHASE are displayed in Table 5. The two most common and mutually exclusive haplotypes for whites were rs10116277-T, rs1333040-T, rs10757274-G, rs4977574-G, rs2383206-G, rs2383207-G, rs10757278-G, and rs1333049-C (46.9%) and G-C-A-A-A-A-A-G (30.5%), with the first haplotype representing all SNP risk alleles and the second reflecting the complementary wild-type alleles. Haplotype frequency estimates for blacks were much more diverse. The two most frequent haplotypes were rs10116277-T,

TABLE 2. BASELINE RISK FACTORS AND COMORBIDITIES

Risk factors, n (%)	Total, n = 146	White, n = 122	Black, n = 24	p
Physical inactivity	127 (87)	106 (87)	21 (87)	0.619
Overweight	88 (60)	71 (58)	17 (71)	0.357
Dyslipidemia	122 (84)	104 (85)	18 (75)	0.172
Familial coronary heart disease	65 (44)	58 (47)	7 (29)	0.075
Pack years smoked, mean (SD)	14 (21)	15 (22)	11 (15)	0.373
Lives with a smoker	32 (22)	29 (24)	3 (12)	0.172
Hypertension (or on antihypertensives)	115 (79)	94 (77)	21 (87)	0.194
Diabetes	54 (37)	39 (32)	15 (62)	0.005
Comorbidities				
Previous myocardial infarction	22 (15)	17 (14)	5 (21)	0.279
Previous coronary artery bypass graft surgery	10 (7)	10 (8)	0 (0)	0.156
Previous percutaneous coronary intervention	16 (11)	13 (11)	3 (12)	0.511
Previous stroke	8 (5)	3 (2)	5 (21)	0.003
Peripheral vascular disease	12 (8)	10 (8)	2 (8)	0.621
Degenerative arthritis	86 (59)	68 (56)	18 (75)	0.061

Continuous data are presented as means ± standard deviation; categorical data are presented as number and percentage. Chi-square (χ^2) test and t test used to analyze discrete and continuous variables, respectively.

TABLE 3. BASELINE PHYSIOLOGICAL CHARACTERISTICS

Variable	Total sample, n = 146	White, n = 122	Black, n = 24	p	η^2
Age	62.7 ± 11	63.3 ± 11	60.0 ± 10	0.18	0.012
Total cholesterol, mg/dL	164.0 ± 40	164.3 ± 41	164.1 ± 33	0.98	0.000
LDL-cholesterol, mg/dL	90 ± 34	89 ± 35	97 ± 26	0.33	0.007
Total/HDL cholesterol ratio	3.9 ± 1.5	4.0 ± 1.5	3.8 ± 1.2	0.61	0.002
Body mass index, kg/m ²	31.4 ± 7	31.1 ± 7	32.8 ± 7	0.27	0.008
Body weight, pounds	181.5 ± 42	180.1 ± 43	189.1 ± 39	0.34	0.006
Percent body fat	37.8 ± 4	37.7 ± 4.6	38.8 ± 3.8	0.26	0.009
Resting heart rate, bpm	71 ± 10	70 ± 10	74 ± 12	0.09	0.020
HDL-cholesterol, mg/dL	44.9 ± 14	44.7 ± 14	46 ± 12	0.68	0.001
Triglycerides, mg/dL	145 ± 73	151 ± 76	116 ± 45	0.03	0.032
Fasting glucose, mg/dL	105 ± 30	102 ± 23	120 ± 50	0.006	0.051
Waist circumference, cm	100 ± 15	100 ± 16	102 ± 13	0.03	0.004
Systolic BP, mmHg	127 ± 17	126 ± 16	132 ± 18	0.11	0.018
Diastolic BP, mmHg	73 ± 11	72 ± 11	76 ± 11	0.09	0.020

Results are presented as mean ± SD; differences between whites and blacks calculated using *t*-test.

BP, blood pressure; bpm, beats per minute; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

TABLE 4. GENOTYPE OF EIGHT SINGLE-NUCLEOTIDE POLYMORPHISMS AT THE CHROMOSOME 9P21 LOCUS STRATIFIED BY RACE

Genotype	HapMap Populations ^b		White, n = 122	Black, n = 24	χ^2 (df = 2)	p
	CEU (%)	YRI (%)				
rs10116277						
GG	30.1	0.0	22 (18.0)	1 (4.2)		
GT	48.7	3.4	65 (53.3)	3 (12.5)		
TT ^a	21.2	96.6	35 (28.7)	20 (83.3)	25.505	0.001
rs1333040						
CC	19.5	7.5	15 (12.3)	2 (8.3)		
CT	49.5	51.7	60 (49.2)	11 (45.8)		
TT ^a	31.0	40.8	47 (38.5)	11 (45.8)	0.586	0.746
rs10757274						
AA	23.6	71.7	25 (20.5)	16 (66.7)		
AG	52.8	28.3	61 (50.0)	7 (29.2)		
GG ^a	23.6	0.0	36 (29.5)	1 (4.2)	22.177	0.001
rs4977574						
AA	29.2	81.6	25 (20.5)	16 (66.7)		
AG	49.6	17.7	60 (49.2)	7 (29.2)		
GG ^a	21.2	0.7	37 (30.3)	1 (4.2)	22.250	0.001
rs2383206						
AA	18.3	31.7	20 (16.4)	5 (20.8)		
AG	58.4	48.3	59 (48.4)	16 (66.7)		
GG ^a	23.3	20.0	43 (35.2)	3 (12.5)	17.374	0.089
rs2383207						
AA	25.7	0.0	21 (17.2)	0 (0.0)		
AG	52.2	3.4	55 (45.1)	4 (14.7)		
GG ^a	22.1	96.6	46 (37.7)	20 (83.3)	17.374	0.001
rs10757278						
AA	23.6	89.8	29 (23.8)	14 (58.3)		
AG	52.8	10.2	57 (46.7)	8 (33.3)		
GG ^a	23.6	0.0	36 (29.5)	2 (8.3)	12.397	0.002
rs1333049						
CC ^a	20.4	2.7	36 (29.5)	1 (4.2)		
CG	50.4	28.6	58 (47.5)	10 (41.7)		
GG	29.2	68.7	28 (23.0)	13 (54.2)	12.189	0.002

^aRisk genotype.

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

CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; YRI, Yoruba in Ibadan, Nigeria. Allelic frequencies can be calculated as the proportion of homozygotes plus one half of heterozygotes.

TABLE 5. RELATIVE FREQUENCIES OF ESTIMATED EIGHT 9p21 SINGLE-NUCLEOTIDE POLYMORPHISM HAPLOTYPES IN WHITE AND BLACK WOMEN

rs10116277	rs1333040	rs10757274	rs4977574	rs2383206	rs2383207	rs10757278	rs1333049	White, n = 122	Black, n = 24
T	T	G	G	G	G	G	C	0.469	0.146
G	C	A	A	A	A	A	G	0.305	0.000
G	T	A	A	A	A	A	G	0.067	0.000
G	C	G	G	G	G	G	C	0.035	0.000
T	T	A	A	G	G	A	G	0.026	0.102
T	T	G	G	G	G	A	G	0.017	0.000
T	T	G	G	G	G	G	G	0.016	0.000
T	T	A	A	A	G	A	G	0.000	0.387
T	T	A	A	G	G	G	G	0.000	0.021
T	C	A	A	A	G	A	G	0.000	0.134
T	C	A	A	G	G	A	G	0.000	0.044
T	C	G	G	G	G	G	C	0.000	0.058
T	C	A	A	G	G	A	C	0.000	0.042
G	C	G	G	G	A	G	C	0.000	0.010
G	T	G	G	G	A	G	C	0.000	0.032
T	C	A	A	G	G	G	G	0.000	0.021
Cumulative relative frequency =								0.935	0.997

The first row represents the risk haplotype (TTGGGGGC) and is in boldface; the second row represents the protective haplotype (GCAAAAAG). Haplotypes with >5% frequency are shaded.

rs1333040-T, rs10757274-A, rs4977574-A, rs2383206-A, rs2383207-G, rs10757278-A, and rs1333049-G (38.7%) and T-T-G-G-G-G-A-C (14.6%). Notably, the first haplotype includes risk alleles for only three SNPs (rs10116277, rs1333040, and rs2383207), whereas the second haplotype includes risk alleles for all eight SNPs.

The location of the 9p21 SNPs and the pair-wise LD plot (scaled as D') for whites and blacks are shown in Figure 1A and B, respectively. The high pair-wise LD of the SNPs in the haplotype block for whites is illustrated in Figure 1A. Figure 1B shows that these same SNPs for blacks have dramatically lower pair-wise LD. A single haplotype block was assigned to the SNPs of whites with rs10757274 and rs4977574 in perfect LD ($D' = 1$, $r^2 = 1$). The SNPs rs1333040 and rs2383207 had the lowest LD ($D' = 0.715$, $r^2 = 0.453$). Four tagging SNPs captured all eight SNPs for whites (rs4977574, rs10757274, rs10757278, and rs1333049). Haploview revealed two haplotype blocks for blacks. The first block included rs10116277 and rs1333040 ($D' = 0.651$, $r^2 = 0.108$), and the second comprised the six remaining SNPs. Three SNP pairs had the lowest LD (rs1333040 and rs2383206, $D' = 0.037$, $r^2 = 0.001$; and rs10116277 with both rs4977574 and rs10757274, $D' = 0.048$, $r^2 = 0.001$). Tagging SNPs for blacks included rs4977574 and rs10757274 ($D' = 1.0$, $r^2 = 1.0$).

The relationship between genotype and age of CHD onset

Because two SNPs were perfect surrogates for each other in both races (rs10757274 and rs4977574), rs4977574 was arbitrarily excluded from further analyses. The dominant genetic model, in which the presence of any at-risk allele is compared to the wild-type allele, was used for subsequent analyses due to infrequency of various genotypes in blacks. Table 6 presents the mean age of onset of CHD for risk versus nonrisk genotypes for seven 9p21 SNPs stratified by ethnicity. On the one hand, the 92% of blacks carrying at least one copy of the rs1333040 risk allele were found to have CHD 6.5 years earlier

than those with the wild-type alleles ($\eta^2 = 0.031$); this was not the case for whites ($\eta^2 = 0.002$). On the other hand, whites with at least one copy of the rs2383206 risk alleles were found to have CHD nearly 5 years earlier compared to those with the nonrisk genotype ($\eta^2 = 0.025$); this was not the case for blacks ($\eta^2 = 0.008$). The 42% of blacks with heterozygous or homozygous risk genotypes for rs10757278 were found to have CHD about 3 years earlier compared to blacks with the wild-type (AA) genotype ($\eta^2 = 0.021$); the effect of this SNP in whites was similar in magnitude and direction ($\eta^2 = 0.013$).

Discussion

The polygenic and multifactorial nature of CHD presents a challenge in determining the unique contribution and biological validity of genetic variants and traditional risk factors. Despite the consistently replicated association between chromosome 9p21 variants and CHD, the biological mechanisms by which 9p21 variants influence CHD have yet to be elucidated (Palomaki *et al.*, 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); there have been few replication studies in blacks with CHD phenotypes. Thus, we examined the haplotype structure of chromosome 9p21 SNPs in black and white women with CHD. Given that the link between 9p21 and early-onset disease are conflicting, we explored the potential relationship between 9p21 SNPs and age of CHD onset in ethnically diverse women.

There was considerable interethnic allelic and haplotype diversity across the 9p21 locus with only two SNPs in perfect LD in both races, and only a small proportion of the haplotypes shared between the racial groups. These findings substantiate the extensive LD for both pair-wise LD and the size of the haplotype blocks found in this region on chromosome 9p21 in the Northern and Western European (CEU) HapMap populations that is reduced in populations of African ancestry

FIG. 1. Linkage disequilibrium (LD) and haplotype blocks on chromosome 9p21 in (A) white ($n = 122$) and (B) black ($n = 24$) women. LD patterns between eight single-nucleotide polymorphisms (SNPs), rs10116277, rs1333040, rs10757274, rs4977574, rs2383206, rs2383207, rs10757278, rs1333049, were derived from genotyping data from women with coronary heart disease. The pair-wise correlation between SNPs was measured as D' and are 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, $r^2 = 1$.

(YRI) (Gibbs *et al.*, 2003; The International HapMap Consortium, 2005; Frazer *et al.*, 2007). Whites in the current study displayed two common haplotypes with one representing risk alleles and the other representing wild-type alleles for all SNPs. These risk and protective haplotypes have previously been described as a perfect complementary or yin-yang pattern (Zhang *et al.*, 2003; Shen *et al.*, 2008). Although all study

participants had CHD, less than half of whites and 15% of blacks carried the risk alleles for all 9p21 variants.

Despite little interethnic genotypic variation for rs1333040, the impact of ethnicity on age of CHD onset was more dramatic. The largest difference in the age of CHD onset was among blacks with at least one copy of the risk alleles for rs1333040 who were diagnosed 6.5 years earlier compared to those with the wild-type alleles; in whites this effect was essentially absent. The second largest interethnic difference in the age of CHD onset was in whites with risk alleles for rs2383206 who were diagnosed nearly 5 years earlier than those with wild-type alleles; for blacks this effect was near zero. Disparate interethnic findings were also evident for rs10116277 and rs1333049 risk alleles, both of which were associated with an earlier age of CHD diagnosis in whites but not in blacks. In both ethnic groups, rs10757278 risk genotypes were associated with a CHD diagnosis that was 3 years younger than the nonrisk genotypes. Assimes *et al.* (2008) examined three SNPs common to our study (rs10757274, rs2383206, and rs10757278). Whereas they found these SNPs unrelated to CHD in blacks or age of onset in women regardless of race, we found that the effect of rs2383206 on age of CHD onset was strongest in whites.

Previous research with whites, homozygous for the risk allele for rs1333049 compared to those with wild-type alleles, demonstrated a 2–5 years earlier onset of CHD (Ellis *et al.*, 2010) and a strong association with 3-vessel disease in those with early onset CHD (Dondona *et al.*, 2010). This supported previous findings that other SNPs in the same haplotype block (rs10757274, rs2383206, rs2383207, and rs10757278) were associated with premature CHD in whites (Helgadóttir *et al.*, 2007; Abdullah *et al.*, 2008). Dehghan *et al.* (2008), however, found no relationship between rs10757274 and rs10757278 and CHD in whites with a mean age of onset of 68.6 ± 7.4 . Finding no age effect, they speculated that 9p21 variants invoked only early onset CHD with the effects too small to detect in their older cohort.

Reduction and treatment of traditional risk factors remains the cornerstone of secondary prevention for reducing the burden of CHD. Although women in our study demonstrated multiple behavioral and environmental risk factors for CHD, we found few interethnic differences in their physiological characteristics. The associations between 9p21 SNPs and CHD appear to be largely independent of those mediated through traditional risk factors (Assimes *et al.*, 2008; Brautbar *et al.*, 2009; Karvanen *et al.*, 2009). However, genetic risk variants for CHD manifestation may require the presence of specific combinations of traditional risk factors. By clarifying the gene–environment interactions, we may better understand how modifying environmental exposures modified the effects of genetic risk factors. Future studies may refine the risk assessment of CHD by screening for the 9p21 variants in ethnically diverse young and older patients. This may resolve the question of whether 9p21 variants are involved in the initiation of atherosclerosis, the formation of atheroma, or plaque rupture (Broadbent *et al.*, 2008; Horne *et al.*, 2008; Dondona *et al.*, 2010).

Caution is warranted when generalizing these results because participants were from a single institution in the southeastern United States. Although all women had established CHD, phenotypic heterogeneity and the limited sample size with few blacks may have contributed to the small effect

TABLE 6. MEAN AGE OF CORONARY HEART DISEASE ONSET AS A FUNCTION OF CHROMOSOME 9p21 POLYMORPHISMS AND ETHNICITY

White, n = 122				Black, n = 24			
rs10116277							
GG	GT/TT ^a	Difference	η^2	GG	GT/TT ^a	Difference	η^2
62.50 (9.3)	61.05 (11.5)	−1.45	0.003	56.00 (−)	57.13 (10.7)	+1.13	0.002
rs1333040							
CC	CT/TT ^a			CC	CT/TT ^a		
59.93 (7.8)	61.50 (11.5)	+1.57	0.002	64.00 (11.3)	57.50 (10.5)	−6.5	0.031
rs10757274							
AA	AG/GG ^a			AA	AG/GG ^a		
64.08 (8.8)	60.59 (11.6)	−3.49	0.016	58.31 (11.1)	57.50 (9.7)	−0.81	0.001
rs2383206							
AA	AG/GG ^a			AA	AG/GG ^a		
65.3 (8.3)	60.53 (11.5)	−4.77	0.025	59.80 (11.7)	57.58 (10.4)	−2.22	0.008
rs2383207							
AA	AG/GG ^a			AA	AG/GG ^a		
63.48 (9.3)	60.86 (11.5)	−2.62	0.008	—	58.04 (10.5)	—	—
rs10757278							
AA	AG/GG ^a			AA	AG/GG ^a		
63.59 (9.7)	60.60 (11.5)	−2.99	0.013	59.28 (11.5)	56.30 (9.2)	−2.98	0.021
rs1333049							
GG	GC/CC ^a			GG	GC/CC ^a		
63.75 (9.8)	60.58 (11.4)	−3.17	0.014	57.08 (11.5)	59.18 (9.5)	+2.1	0.010

Data are presented as means (SD).

^aRisk genotype.

sizes of the variants. The sample size also precluded examination of plausible clinical predictor variables of early-onset CHD in multivariate models. This pilot study served to enhance the methodological protocol, obtain effect size estimates, and generate additional hypotheses for a larger, more ethnically diverse future study.

To conclude, this study found interethnic diversity in the risk alleles and the haplotype structure of chromosome 9p21 variants. The results suggest that different variants on chromosome 9p21 may influence CHD age of onset in whites and blacks. Had we examined each SNP in isolation, we would have reached three different conclusions. Greater understanding of the interethnic differences in genotypes may contribute to the limited knowledge of how combination of SNPs and distinct haplotypes affect the variability in susceptibility to CHD.

Acknowledgments

This study was funded by the National Institute of Nursing Research (NR007678), the University of South Florida (USF) Established Researcher Grant, The USF Signature Interdisciplinary Research Program in Cardiovascular Diseases Grant, and The USF Nursing Faculty in Pilot Research Grant. T.M.B. carried out the genetic analyses. J.W.B. and M.W.G. participated in the data analyses and article preparation. This work was supported with resources and the use of facilities in the Biobehavioral Laboratory of the College of Nursing, USF.

Disclosure Statement

No competing financial interests exist.

References

- Abdullah KG, Li L, Shen GQ, *et al.* (2008) Four SNPs on chromosome 9p21 confer risk to premature, familial CAD and MI in an American Caucasian population (GeneQuest). *Ann Hum Genet* 72:654–657.
- Arnett DK, Baird AE, Barkley RA, *et al.* (2007) 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* 115:2878–2901.
- Assimes TL, Knowles JW, Basu A, *et al.* (2008) Susceptibility locus for clinical and subclinical coronary artery disease at chromosome 9p21 in the multi-ethnic ADVANCE study. *Hum Mol Genet* 17:2320–2328.
- Barrett J, Fry B, Maller J, *et al.* (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263–265.
- Beckie TM (2006) A behavior change intervention for women in cardiac rehabilitation. *J Cardiovasc Nurs* 21:146–153.
- Beckie TM, Mendonca MA, Fletcher GF, *et al.* (2009) Examining the challenges of recruiting women into a cardiac rehabilitation clinical trial. *J Cardiopulm Rehabil Prev* 29:13–21.
- Brautbar A, Ballantyne CM, Lawson K, *et al.* (2009) 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. *Circ Cardiovasc Genet* 2:279–285.
- Broadbent HM, Peden JF, Lorkowski S, *et al.* (2008) Susceptibility to coronary artery disease and diabetes is encoded by distinct,

- tightly linked SNPs in the ANRIL locus on chromosome 9p. *Hum Mol Genet* 17:806–814.
- Buysschaert I, Carruthers KF, Dunbar DR, *et al.* (2010) A variant at chromosome 9p21 is associated with recurrent myocardial infarction and cardiac death after acute coronary syndrome: the GRACE Genetics Study. *Eur Heart J* 31:1132–1141.
- Chen Z, Qian Q, Ma G, *et al.* (2009) A common variant on chromosome 9p21 affects the risk of early-onset coronary artery disease. *Mol Biology Rep* 36:889–893.
- Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.
- Dandona S, Stewart AF, Chen L, *et al.* (2010) Gene dosage of the common variant 9p21 predicts severity of coronary artery disease. *J Am Coll Cardiol* 56:479–486.
- Dehghan A, van Hoek M, Sijbrands EJ, *et al.* (2008) Lack of association of two common polymorphisms on 9p21 with risk of coronary heart disease and myocardial infarction; results from a prospective cohort study. *BMC Med* 6:30.
- Devlin B, Risch N (1995) A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 29:311–322.
- Ellis KL, Pilbrow AP, Frampton CM, *et al.* (2010) A common variant at chromosome 9P21.3 is associated with age of onset of coronary disease but not subsequent mortality. *Circ Cardiovasc Genet* 3:286–293.
- Frazer KA, Ballinger DG, Cox DR, *et al.* (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449:851–861.
- Gibbs RA, Belmont JW, Hardenbol P, *et al.* (2003) The International HapMap Project. *Nature* 426:789–796.
- Helgadóttir A, Thorleifsson G, Manolescu A, *et al.* (2007) A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* 316:1491–1493.
- Hinohara K, Nakajima T, Takahashi M, *et al.* (2008) Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations. *J Hum Genet* 53:357–359.
- Horne BD, Carlquist JF, Muhlestein JB, *et al.* (2008) Association of variation in the chromosome 9p21 locus with myocardial infarction versus chronic coronary artery disease. *Circ Cardiovasc Genet* 1:85–92.
- Humphries SE, Drenos F, Ken-Dror G, *et al.* (2010) Coronary heart disease risk prediction in the era of genome-wide association studies: current status and what the future holds. *Circulation* 121:2235–2248.
- Karvanen J, Silander K, Kee F, *et al.* (2009) The impact of newly identified loci on coronary heart disease, stroke and total mortality in the MORGAM prospective cohorts. *Genet Epidemiol* 33:237–246.
- Kullo IJ, Cooper LT (2010) Early identification of cardiovascular risk using genomics and proteomics. *Nat Rev Cardiol* 7:309–317.
- Kullo IJ, Ding K (2007) Mechanisms of disease: the genetic basis of coronary heart disease. *Nat Clin Pract Cardiovasc Med* 4:558–569.
- Lewontin RC (1964) The Interaction of selection and linkage. I. General considerations; heterotic models. *Genetics* 49:49–67.
- Maitra A, Dash D, John S, *et al.* (2009) A common variant in chromosome 9p21 associated with coronary artery disease in Asian Indians. *J Genet* 88:113–118.
- McPherson R (2010) Chromosome 9p21 and coronary artery disease. *N Engl J Med* 362:1736–1737.
- McPherson R, Pertsemlidis A, Kavaslar N, *et al.* (2007) A common allele on chromosome 9 associated with coronary heart disease. *Science* 316:1488–1491.
- Meng W, Hughes AE, Patterson CC, *et al.* (2008) Chromosome 9p21.3 is associated with early-onset coronary heart disease in the Irish population. *Dis Markers* 25:81–85.
- Newton-Cheh C, Cook NR, VanDenburgh M, *et al.* (2009) A common variant at 9p21 is associated with sudden and arrhythmic cardiac death. *Circulation* 120:2062–2068.
- Palomaki GE, Melillo S, Bradley LA (2010) Association between 9p21 genomic markers and heart disease: a meta-analysis. *JAMA* 303:648–656.
- Roberts R (2008) Genetics of premature myocardial infarction. *Curr Atheroscler Rep* 10:186–193.
- Roberts R, Wells GA, Stewart AF, *et al.* (2010) The genome-wide association study—a new era for common polygenic disorders. *J Cardiovasc Transl Res* 3:173–182.
- Rotimi CN, Jorde LB (2010) Ancestry and disease in the age of genomic medicine. *N Engl J Med* 363:1551–1558.
- Saleheen D, Alexander M, Rasheed A, *et al.* (2010) 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. *Arterioscler Thromb Vasc Biol* 30:1467–1473.
- Samani NJ, Erdmann J, Hall AS, *et al.* (2007) Genomewide association analysis of coronary artery disease. *N Engl J Med* 357:443–453.
- Schunkert H, Gotz A, Braund P, *et al.* (2008) Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. *Circulation* 117:1675–1684.
- Shen G, Li L, Rao S, Abdullah K, *et al.* (2008) Four SNPs on chromosome 9p21 in a South Korean population implicate a genetic locus that confers high cross-race risk for development of coronary artery disease. *Arterioscler Thromb Vasc Biol* 28:360–365.
- Stephens M, Donnelly P (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 73:1162–1169.
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978–989.
- The International HapMap Consortium (2005) A haplotype map of the human genome. *Nature* 437:1299–1320.
- Zhang J, Rowe WL, Clark AG, *et al.* (2003) Genomewide distribution of high-frequency, completely mismatching SNP haplotype pairs observed to be common across human populations. *Am J Hum Genet* 73:1073–1081.

Address correspondence to:

Theresa M. Beckie, Ph.D.

College of Nursing

University of South Florida

12901 Bruce B. Downs Blvd., MDC22

Tampa, FL 33612

E-mail: tbeckie@health.usf.edu