Genetic Predictors of Coronary Heart Disease Risk Factors in Premenopausal African-American Women

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Purpose: This study examined polymorphisms at the loci HLA-*DQB1,* HLA-*DRB1, TNFa* microsatellite, and *D6S89,* all which lie within or telomeric to the major histocompatibility complex (MHC) and the apolipoprotein E (*APOE*) and *THO-1* loci in premenopausal African-American women (AAW) for their association with known coronary heart disease (CHD) risk factors. The sample, drawn from community and military sources, included premenopausal AAW ($\bar{x}$ age=34.18) who were at low risk (n=117) and high risk (n=173) for CHD.

Methods: In this case- (high risk) control (low risk) study, venous blood was used for DNA extraction. Polymorphisms were assessed by using a variety of standard polymerase chain reaction (PCR) methods. Allelic controls were used in all reactions, and two individuals sized and concurred on allele assignment in each analysis. Vertical auto profile (VAPI), glucose challenge tests, measurement of insulin levels, blood pressure, body mass index (BMI), and waist-hip ratio (WHR) assessments were conducted by using standardized procedures. Pearson's correlation coefficients and assessment of allele distributions via relative frequency and frequency variance were conducted in relation to military status, risk group, and risk factors by using exact *P* values and likelihood ratio chi-squared (lrchi^2) statistic. The significance level was set at .05.

Results: Of the 237 women (low and high risk), 116 of the women in the sample were in Stage I obesity or heavier. Of 237 women (low and high risk), 85 (36%) of the women in this sample were insulin resistant. The frequency of *D6S89* allele 185, *D6S89* allele 191, *TNFa* allele 97, and *TNFa* allele 103 alleles were higher in the high-risk than the low-risk group; and the *D6S89* 195 allele was higher in the low-risk group. Elevated systolic blood pressure (SBP) was associated with HLA-*DRB1**09 and *TNFa* 117 alleles. *APOE*4, TNFa* 109, and *DRB1**107 alleles were associated with increased relative risk for elevated total cholesterol to high-density lipoprotein (HDL) ratios. *APOE*4* and *D6S89* 193 alleles were associated with an elevated risk for low-density lipoprotein (LDL) or LDL sub-fraction levels. *APOE*2* was associated with a lower relative risk for total cholesterol to HDL ratios. Metabolic syndrome was identified in 26.6% of the sample and was associated with the presence of *DRB1**09, *DRB1**12, and *DRB1**15 alleles. Lp(a) levels were positively associated with risks for HDL, HDL2, HDL3, LDL, and total cholesterol. Lp(a) was negatively associated with risks for very low-density lipoprotein (VLDL), triglyceride, fasting blood sugar (FBS), insulin resistance, SBP, weight, and WHR.

Conclusion: The association of *APOE,* DRB1, D6589 and *TNFa* alleles with risk of CHD suggest that these are candidate genes or linked to genes for CHD in this cohort of AAW. Our data supported elevated plasma Lp(a) as a potential risk factor in AAW; however, its role is still unclear. The premenopausal AAW in this sample had a higher than expected rate of metabolic syndrome, which was associated with *DRB1* alleles. (*Ethn Dis.*2005;15:221–232)

Key Words: African-American Women, Coronary Heart Disease Risk Factors, Genotypes, Metabolic Syndrome, Phenotypes, Premenopausal

Introduction

Each year, 39% of all deaths in women are from coronary heart disease (CHD).[1] One of every five women in the United States has some form of cardiovascular disease (CVD).[2] In 2002, approximately 500,037 women died from CVD.[1–2] In 2001, the mortality for CHD in females was 236,468, representing 49.1% of all deaths for this disease.[2] Although CHD and related circulatory conditions continue to be the leading causes of deaths in the United States regardless of race,[1] limited research is conducted on women, regardless of age, who are at risk for this condition. Among women, premenopausal African-American women (AAW) have a death rate for CHD almost four times higher than their White counterparts. The death rate from CHD for AAW between the ages of 18–44 is 65.3 per 100,000 population, as compared to 18.10 per 100,000 population for White females in the same age range.[1] African-American women (AAW) between 18–45 years of age have been virtually neglected as a population at risk for CHD. Even with the comparatively high rate of CHD in premenopausal AAW, biologic risk factors for CHD (eg, family history of CHD, polymorphisms within or flanking candidate genes, differences in lipoprotein profiles, and areas of fat deposition) in this population have not been carefully examined and validated.

Background and Significance

The study of risk factors for CHD in premenopausal AAW is of significant interest because the mortality rates from CHD are significantly higher in AAW than in their White counterparts. This disparity has been clearly documented in the literature since the early 1940s.[3–5]

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Hypertension, hyperinsulinemia, and hypercholesterolemia are risk factors for CHD regardless of gender or race but tend to be manifested earlier among AAW. In fact, 40% of African Americans (AA) have documented hypertension by age 40; another 45% have documented diabetes (type 1 or type 2) by age 40, and still another 40% have had some evidence of hypercholesterolemia[2,8] since their early twenties. Although attempts have been made to reduce the prevalence of CHD risk factors in AAW, CHD remains the leading cause of death among this population.[2,6–8]

Until recently, CHD was viewed as a disease that predominately affected men. This false assumption has potentially contributed to the lack of adequate diagnosis and treatment and exacerbated problems associated with CHD in women regardless of race.[8] In addition to genetic risk factors, other known high risk factors for CHD among pre-menopausal AAW include: 1) smoking; 2) hypertension; 3) high density lipoprotein (HDL)<35 mg/dL; total cholesterol (TC)>240 mg/dL, low density lipoprotein (LDL)>130 mg/dL; 4) diabetes mellitus or potential to develop diabetes (gestational diabetes); 5) obesity; and 6) sedentary lifestyle.[9–10] Reducing the prevalence of risk factors associated with the development of CHD in AAW should be of paramount concern, since risk reduction may contribute to diminished quality of life and increased costs associated with the condition.

The Role of Genetics in CHD

Family history is a major risk factor for CHD, which could be due to a combination of inheritance of deleterious mutations, common environment, and lifestyle.[11–13] While a number of polymorphisms have been implicated in the etiology of CHD, it is the particular constellation of genes that an individual inherits that in part determines risk for CHD. Lipids, lipoproteins, glucose, and blood pressure levels have been the primary physiologic factors mediating the pathogenesis of CHD.[13] Human and animals studies, especially those of large epidemiologic designs, support each of these.

Apolipoprotein E

APOE is a member of the apolipoprotein gene family that codes for a polymorphic protein involved in the catabolism of chylomicron remnants.[14] Various *APOE* alleles, (E2, E3, and E4), are related to the development of combined hyperlipidemia and familial combined hyperlipidemia, total cholesterol and LDL cholesterol, and insulin levels and are associated with CHD, stroke, and type 1 and type 2 diabetes.[14–17] Critical gaps still exist in knowledge about the relationship of *APOE* to CHD in the African-American population.

Major Histocompatibility Complex

Within the major histocompatibility complex (MHC) lie several highly polymorphic loci which code for molecules involved in antigen presentation and inflammation.[18] Mounting evidence reveals that immune mechanisms play a role in CHD.[18–23] Several polymorphisms within the MHC on Ch6p have been implicated in this process. Several researchers have reported an association of atherosclerosis risk factors with various human leucocyte antigens (HLA).[18–31] Human leucocyte antigen (HLA) has also been reported to be associated with CHD[18–19] and hypertension.[19–31]

Tumor Necrosis Factor α Microsatellite

The tumor necrosis factor α *(TNFα)* gene lies within the MHC region 250 kb centromeric to HLA-B, codes for a cytokine important in inflammatory immune reactions, and has been implicated in a number of diseases.[32–34] Abnormal expression of TNFα appears to play a role in obesity-related insulin resistance.[33] The production of TNFα is related to polymorphisms within the promoter region of the gene, various *TNF* microsatellite polymorphisms within and flanking the *TNFα* locus and HLA alleles.[35–39] The *TNFa* microsatellite locus is located upstream of the *TNFB* locus, and various alleles have been associated with high and low production of TNFα.[38–39]A number of studies have observed associations between various *TNFα* and *TNF* microsatellite polymorphisms and risk factors for or susceptibility to CHD.[38–41]

Endothelin-1

Endothelin-1 (EDN-1) is a powerful vasoconstrictor secreted by endothelial cells.[40] Endothelin-1 (EDN-1) has been implicated either directly or indirectly in the etiology of hypertension, atherosclerosis, diabetes, CHD and heart failure.[41–46] The gene encoding the EDN-1 peptide is located 34–36 cM distal to the HLA region at 6p24-p23.[44–45] Endothelin-1 (EDN-1) is linked to the *D6S89* locus, which is a convenient highly polymorphic dinucleotide repeat marker for assessing this locus.[46–48]

HUMTHO-1

Hyperinsulinemia is associated with CHD, hypertension, dyslipidemia, and type 2 diabetes.[49] Flanking the 5′ end of the insulin gene located in the region, is a variable number of tandem repeat (VNTR) locus.[49–53] At this highly polymorphic locus are three discrete size classes of alleles, which in turn code for a transcription factor that in part governs the amount of insulin one is capable of producing.[51–56] The shortest, or class 1, allele is associated with type 1 diabetes mellitus.[57–58] A short tandem repeat (STR) locus, *HUMTHO1,* lies in the same region, and has been observed to be strongly associated with the class 1 5′ insulin VNTR.[57]

Genetics, Mortality, and CHD

Because mortality from CHD for African-American (AA) and White males is nearly equal, some researchers postulate that a possible protective

mechanism for AA males exists which does not exist in AAW.[58] This protective mechanism may be a result of genetically elevated high-density lipoprotein cholesterol (HDL-C) concentrations commonly found in AA men.[58–60] Genetically elevated HDL-C levels may protect AA males from CHD; however, this same protective effect may be lost in AAW. While the true cause of this protective loss among AAW, regardless of age, remains largely unknown, it may be primarily due to obesity, smoking, and sedentary behavior.[58, 60] A number of candidate genes have been identified that appear to influence these and other physiologic factors. Coronary heart disease is a multifactorial disease, where lifestyle factors interact with genetic predispositions. Thus, if one could identify individuals at genetic risk, early intervention could be implemented to reduce morbidity and mortality. However, little information exists about the relationship of these genes to CHD in AAs.

Because almost all diseases have a genetic link, we must understand the relationship of a particular gene or a constellation of genes to the development of disease. To develop an understanding of this relationship, critical gaps in the literature are addressed in this study, which details methods to further specify genetic risks and the association with known CHD risk factors.

OTHER ASSOCIATED RISK FACTORS FOR CHD

Plasma Values of Lp(a) as a Risk Factor for CHD

The Lp(a) molecule has a high degree of homology with plasminogen, by competing with plasminogen for receptors, thrombolysis may be inhibited and thrombosis promoted.[61–63] Lp(a) concentrations appear to be an independent risk factor for CHD, which is related to the particular Lp(a) isoform possessed by an individual.[61–63] Strong evidence indicates that Lp(a) levels are genetically determined.[61,63] Several polymorphisms flanking or within the gene coding for Lp(a) have been associated with Lp(a) levels and atherosclerosis in several racial and ethnic groups. Lp(a) levels vary among races, although the role of Lp(a) levels as an independent risk factor for CHD in AA remains quite controversial.[61–71]

Metabolic Syndrome

Metabolic syndrome (syndrome X) is a clustering of abnormalities characterized by the primary defects of compensatory insulin resistance, glucose intolerance, dyslipidemia, and centrally distributed obesity.[72–74] The executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in adults (Adult Treatment Panel III) established guidelines for diagnosis of metabolic syndrome.[73,75] These guidelines suggest that at least three of the following components must be present for a diagnosis of metabolic syndrome[73]:

- Waist circumference>102 cm in men and >88 cm in women;
- Serum triglyceride level >150 mg/dL (1.69 mmol/L);
- High density lipoprotein <40 mg/dL (1.04 mmol/L) in men and <50 mg/dL (1.29 mmol/L) in women;
- Blood pressure ≥130/85 mm Hg;
- Serum glucose level ≥110 mg/dL (6.1 mmol/L).[73]

Regardless of age, four factors contribute to the development of CHD, namely hyperinsulinemia, hypercholesterolemia/dyslipidemia, hypertension, and obesity.

Purpose of Study

The specific aim of this study was to determine if polymorphisms at the loci HLA-*DQB1,* HLA-*DRB1, TNFa,* and *D6S89,* all which lie within or telomeric to the MHC, as well as *APOE* and *THO-1,* are associated with known CHD risk factors, for example, hypertension and overt diabetes, in premenopausal AAW.

METHODS

Research Design

This was a multi-site study using a case-control design. Cases, composed of premenopausal AAW at risk for CHD (N=173) were compared with premenopausal AAW controls who were at low risk for CHD (N=117) to determine if candidate gene polymorphisms were associated with known CHD risk. This study was approved by the human subjects institutional review board (IRB) at the university and the institutional review committee (IRC) of the southeast catchments for the United States Army.

Sample and Setting

The sample consisted of 290 premenopausal AAW between 18 and 45 years of age who resided in either Jefferson County, Alabama, or Augusta, Georgia. The sample was a mixture of civilian (N=240) and active-duty Army military AAW (N=50). Premenopausal AA women were eligible to participate in the study if they met the following criteria: 1) 18 to 45 years of age; 2) had regular menstrual periods; 3) were not pregnant; 4) were at least 12 months postpartum at first screening contact if they had delivered a child; and 5) provided written informed consent. Women were excluded who were characterized by any of the following: 1) current pregnancy or plans to become pregnant over the course of participation in the study; 2) presence of a severe debilitating condition, such as cancer, AIDS, psychiatric disorder, or substance abuse, which might possibly limit full participation in the study; 3) the discontinuance of monthly menstrual cycles; or 4) unable to complete study forms and questionnaires.

A subject was considered high-risk if at least one of the following was present:

1) diagnosed with hypertension; 2) hypercholesterolemia; 3) diabetes; 4) family history of heart disease; or 5) current cigarette smoking (with or without birth control pill use). In addition, subjects who had at least two of the following were considered at risk for CHD: 1) overweight; 2) a positive glucose challenge test; 3) history of gestational diabetes; and 4) no regular physical activity. Similarly, subjects who were low-risk did not have a history of any of the following: 1) diagnosis of CHD; 2) hospitalization for an acute myocardial infarction; 3) history of percutaneous transluminal coronary angioplasty (PTCA) or angioplasty; 4) diagnosis of hypertension; 5) hypercholesterolemia; 6) diabetes; or 7) current cigarette smoking. In addition, subjects could not have two or more of the following: 1) overweight; 2) positive glucose challenge test; 3) history of gestational diabetes; and 4) no regular physical activity.

MEASURES AND INSTRUMENTS

As a quality measure, the persons performing the genetic and metabolic assays, assigning alleles or laboratory values, and entering the data into the computer database were blinded to the identity or risk status of the subjects being tested.

1. Genetic Typing
   a. DNA Extraction and Storage: Venous blood was drawn into EDTA-containing Vacutainers. An accession number was assigned to each sample and the information logged into the laboratory database. Genomic DNA was extracted from buffy coats by an inorganic extraction method[76–77] using Qiagen kits (Quiagen, Inc, Valencia, Calif). Purified DNA was aliquoted into cryotubes and stored at −70° C. until used. Polymerase Chain Reaction (PCR) Primers: All PCR primers were synthesized in house on a Beckman Oligo 1000 DNA Synthesizer.
   b. Molecular HLA Typing: Molecular HLA typing was conducted in a two-stage process. Low-resolution *DRB1* and high-resolution *DQB1* typing was conducted by PCR with sequence specific primers (SSP typing) specific for the *DRB1* and *DQB1* polymorphic exons.[76–77] The PCR-generated SSP products were subjected to electrophoresis on agarose gel, and the alleles were visualized by staining with ethidium bromide. Two readers independently assigned the HLA phenotypes. If there was discordance between the two readers, the assay was repeated.
   c. TNFa Microsatellite Genotyping: The *TNFa* microsatellite locus alleles were assessed by PCR analysis using end-labeled oligonucleotide primers modified under conditions previously described.[78–82] Unified conditions, given in several references for various dinucleotide repeats, were optimized to the equipment and reagents. Amplified products were electrophoresed on an 8% acrylamide/bis, 5.6 mol/L urea, 32% formamide 0.4mm sequencing gel maintained at a constant temperature of 47°C. Resultant gels were subjected to autoradiography. The resulting bands were read against a sequencing ladder run at flanking positions to the samples. Each run included a sample of DNA from an individual of known genotype. At least two individuals sized the alleles. If at least two persons did not agree on a size, the sample in dispute was analyzed again.
   d. D6S89 Typing: *D6S89* typing was conducted using the PCR primers previously described[47–48] and the electrophoretic conditions and autoradiography as described for *TNFa* microsatellite genotyping.
   e. THO-1 Typing: *THO-1* typing was performed using the PCR primers and conditions previously described.[70] The resulting bands were read against a sequencing ladder run at flanking positions to the samples. Each run included a sample of DNA from an individual of known genotype. At least two individuals sized the alleles. If at least two persons did not agree on a size, the sample in dispute was analyzed again.
   f. APOE Typing: *APOE* was assessed using the PCR primers and conditions previously described.[46] Allelic controls were included in all reactions. At least two individuals sized the alleles. If at least two persons did not agree on a size, the sample in dispute was analyzed again.
2. Metabolic Assays
   a. Glucose Challenge Test. Changes in glucose control served as a primary indicator of metabolic improvement rather than change in diabetic status. Not all subjects were diagnosed with diabetes, but may have had factors such as food intake, stress, hormonal fluctuations, and a variety of other environmental factors that may have impacted glycemic control and diabetic status. To standardize the protocol, blood-D glucose concentrations were determined, which is used universally as the "gold standard" for diagnosis of type 2 diabetes mellitus. Plasma glucose was measured after 10 hours of fasting and 2 hours after a 75-g glucose load.
   b. Blood Pressure (BP). Three

measurements were taken after a five-minute seated rest period, and the last two measurements were averaged. Pulse was recorded along with BP readings in the event they were required to help interpret BP results.

c. Vertical Auto Profile (VAPI) (all lipoprotein classes). This cholesterol panel measures total cholesterol, LDL, HDL and subfractions of HDL, VLDL, Lp(a) and other lipoproteins. The reliability range was 0.95–0.97 on all of the sub-fractions. The lowest observed reliability was in intermediate-density lipoprotein (IDL), which is considered by some scientists to not be a well-defined lipoprotein class and difficult to separate from other classes.[83] Given the stability over time, the VAP1 was a reliable indicator of lipoprotein classes and was used to measure blood plasma values for Lp(a).

d. Insulin Levels. Insulin levels were measured with standard radioimmunoassay procedures.[84–85] Plasma insulin levels were measured after 12 hours of fasting (FINS) and two hours after a 75-g glucose challenge.

3. Family History of Disease Questionnaire. This instrument ascertained whether an individual had any first- or second-degree relatives who had one or more of the following conditions: family history based on one or more first-degree blood relatives having one or more of the following: 1) diabetes (type 1 or type 2); 2) hypertension; 3) cardiovascular disease; 4) heart attack; 5) stroke; 6) high cholesterol; or 7) high triglycerides.

Analysis of Data

Means and standard deviations were calculated to describe the distributions of interval- and ratio-scale variables. Frequencies and percentages were calculated to describe distribution of nominal variables. Allele distributions (relative frequency and variance) were calculated by using the technique described by Weir.[86] Allele associations with: 1) military status; 2) risk group; and 3) overall and selected individual family history risk factor variables (coronary heart disease [CHD], diabetes [DIAB], high blood pressure [HBP], high cholesterol [HCHOL], heart attack [HRTATAK], insulin dependence [INSDP], and stroke), were tested by using exact *P* values from a likelihood ratio chi-squared (lrchi^2) statistic. In addition, exact 95% confidence intervals were estimated for odds of exhibiting a high risk phenotype, given presence of a specific allele. Those exact estimates were produced by using SAS version 8.2 (SAS, Inc., Cary, NC) and are based on the statistical theory of exact conditional inference for contingency tables, as reviewed by Agresti.[87] Pearson's product moment coefficient was used to measure relationships among Lp(a) and selected risk factors. A .05 type 1 error rate was used for all inferential tests.

Assessment of Caro and Matthews Insulin Resistance Associations

Associations between selected alleles, specifically, *TNFa* and *THO-1* variants, and the Caro[88] and Matthews[84] measures of insulin resistance were tested by using 1) two-by-two contingency tables incorporating an exact test for the lrchi^2 statistic for the dichotomized Caro measure; and 2) independent sample *t* tests for differences in means for the continuous Caro and Matthews measures. Both Caro and Matthews' formulas[84,88] for insulin resistance were used to validate and confirm the presence of insulin resistance.

RESULTS

Descriptive Data

The typical subject who participated in this study was 34 years old (Mean=34.18, SD=6.66), not married (70.8%), attended one year of college (41.4%), had a body mass index (BMI) of 30.4 (SD=7.9), engaged in moderate physical activity (70.3%), was normotensive (80.3%), and did not smoke (88.9%). A history of diabetes was present in 10.7% of the subjects, while 52.8% reported a history of gestational diabetes, and 34.2% were found to be insulin resistant. Seventy-nine out of 267 subjects (29.6%) had a family history of heart disease as defined by having one or more of the conditions found on a Family History of Disease Questionnaire. On average, the subjects had normal total cholesterol (mean=170.79 mg/dL, SD=34.57), HDL cholesterol (mean=43.64 mg/dL, SD=11.54), LDL cholesterol (mean=108.31 mg/dL, SD=29.92), and triglycerides (mean=90.97 mg/dL, SD=46.02).

The descriptive statistics for some key variables in our sample that have been identified as risk factors for CHD are found in Table 1. These data indicate that the average woman in the sample was in Stage I Obesity (NHLBI 1998), and insulin resistant based on Matthews'[84] formula (fasting insulin × fasting glucose / 22.5).

Genetic Indicators

Of the 290 participants in the study, 265 had blood samples available for genetic typing. The military population was less likely to have the *TNFa 117* allele than the civilian population (lrchi^2=5.32; *df*=1; *N*=262; *P*=.033). With the large number of inferential tests performed, the investigators concluded that with only one statistically significant finding, no appreciable evidence showed that the military and civilian populations differed in terms of their genetic makeup at the loci assessed.

The frequencies for a number of polymorphisms were observed to be statistically different between the high-risk and low-risk groups: 1) *D6S89* 185 allele (OR=3.46; lrchi^2=8.59; *df*=1;

Table 1. CHD risk factors in African-American women

Variable	*N*	Mean	SD	Variable	*N*	Mean	SD
BMI kg/m²	237	30.43	7.86	Weight (lbs)	287	186.11	51.73
WHR (cm)	285	0.797	0.084	FBS (mg/dL)	282	114.32	63.32
FINS (uiU/mL)	280	21.13	29.16	HOMA (insulin resistance)	278	109.7	172.14
SBP (mm Hg)	289	119.00	17.17	DBP (mm Hg)	289	76.59	12.26
Total cholesterol (mg/dL)	280	170.79	34.57	HDL (mg/dL)	280	43.64	11.54
HDL2 (mg/dL)	279	12.16	7.19	HDL3 (mg/dL)	279	31.41	6.31
LDL (mg/dL)	280	108.31	29.92	Triglycerides (mg/dL)	279	90.97	46.02

Units of measurement are consistent for all Tables that follow.
CHD = coronary heart disease; SD = standard deviation; BMI = body mass index; WHR = waist-to-hip-ratio; FBS = fasting blood sugar; FINS = 12 hours of fasting; HOMA = homeostasis model assessment; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high density lipoprotein; LDL = low density lipoprotein.

N=231; *P*=.005); 2) *D6S89* 191 allele (OR not available due to zero cell; lrchi²=9.02; *df*=1; *N*=231; *P*=.012); 3) *TNFa* 97 allele (OR not available due to zero cell; lrchi²=7.15; *df*=1; *N*=262; *P*=.032); and 4) *TNFa* 103 allele (OR=2.62; lrchi²=6.68; *df*=1; *N*=262; *P*=.012), with the high risk group subjects being more likely to have the variant. On the other hand, the relative frequency of the *D6S89* 195 allele was higher in the low-risk group compared to the high-risk group (OR=0.270; lrchi²=13.30; *df*=1; *N*=231; *P*<.001). While not statistically significant, the low risk group was more likely to have the *DQB1**302 allele (OR=0.323; lrchi²=4.65; *df*=1; *N*=239; *P*=.057) than the high risk group.

Twelve subjects were homozygous and 91 were heterozygous for the *APOE**4 allele (allele proportion of 21.7%; allele frequency variance of 0.0003206). One subject was homozygous for *APOE**2, and 61 were heterozygous for *APOE**2 (allele proportion of 11.89%; allele frequency variance of 0.0001977).

Analyses for possible associations between the specific alleles assessed and known CHD risk factors were also computed. Table 2 illustrates significant relationships between those variables. If the OR is >1, subjects with that allele have a higher odds of exhibiting that particular risk factor than those without the allele. However, if the OR is <1, then subjects with this particular allele are less likely to exhibit that risk factor than subjects who do not have that allele.

As can be seen in Table 2, elevated systolic blood pressure (SBP) was positively associated with the alleles *DRB1**09 and *TNFa* 117. Subjects possessing the *DQB1**604 or *DRB1**08 alleles had a lower risk for being insulin resistant, by using Caro's formula. Several alleles were found to be associated with serum lipid CHD risk factors. While none of the alleles examined were associated solely with risk for elevated total cholesterol, *APOE**4, *TNF*a109, and *DRB1**07 alleles were associated with an increased risk for elevated total cholesterol to HDL ratios, while the *APOE*2* allele tended to be associated with a lower risk for elevated total cholesterol to HDL ratios. Elevated risk for high LDL or LDL subfraction levels was associated with the presence of *APOE*4*

Table 2. Significant associations between alleles and CHD risk factors

Allele	Risk Factor	Odds Ratio (95% CI)	Irchi²	*df*	*P* Value	Allele	Risk Factor	Odds Ratio (95% CI)	Irchi²	*df*	*P* Value
*APOE*2*	LDL	0.28 (0.09, 0.71)	9.65	1	.003	*APOE*2*	TC/HDL	0.26 (0.12, 0.52)	17.74	1	<.001
*APOE*2*	LDL/HDL	0.26 (0.12, 0.53)	17.16	1	<.001	*APOE*2*	LDL-R	0.24 (0.09, 0.58)	13.92	1	<.001
*APOE*2*	LDL-R/HDL	0.36 (0.19, 0.68)	11.42	1	<.001	*APOE*4*	LDL	2.19 (1.18, 4.06)	7.14	1	.011
*APOE*4*	TC/HDL	2.03 (1.18, 3.47)	7.56	1	.007	*APOE*4*	LDL/HDL	1.95 (1.14, 3.35)	6.76	1	.010
*APOE*4*	LDL-R	2.00 (1.12, 3.56)	6.38	1	.013	*APOE*4*	LDL-R/HDL	2.08 (1.20, 3.64)	7.74	1	.007
D6S89 193	LDL-R	2.06 (0.99, 4.22)	4.36	1	.047	D6S89 195	LDL	0.22 (0.04, 0.74)	8.59	1	.007
DQB1*501	HDL	0.41 (0.16, 0.96)	5.19	1	.034	DQB1*604	CARO	0.34 (0.12, 0.90)	5.79	1	.022
DQB1*604	HDL	2.67 (0.98, 7.04)	4.46	1	.038	DQB1*604	HDL2	2.99 (1.09, 7.92)	5.46	1	.033
DRB1*07	HDL	0.16 (0.03, 0.53)	13.71	1	>.001	DRB1*07	TC/HDL	2.55 (1.28, 5.18)	8.40	1	.006
DRB1*07	HDL2	0.33 (0.10, 0.91)	5.98	1	.022	DRB1*08	CARO	0.39 (0.15, 1.0)	4.67	1	.044
DRB1*08	VLDL	0.28 (0.07, 0.86)	6.61	1	.016	DRB1*09	SBP	4.52 (1.38, 13.51)	7.46	1	.006
DRB1*12	HDL	0.31 (0.06, 1.07)	4.52	1	.047	DRB1*13	HDL	2.18 (1.14, 4.11)	6.48	1	.012
DRB1*13	HDL2	2.66 (1.37, 5.12)	9.76	1	.002	DRB1*13	LDL-R/HDL	0.50 (0.28, 0.89)	6.46	1	.014
TNFa109	TC/HDL	1.79 (1.02, 3.15)	4.66	1	.043	TNFa105	HDL2	2.36 (1.11, 4.91)	5.74	1	.019
TNFa117	SBP	2.70 (1.04, 6.58)	4.97	1	.038						

CHD = coronary heart disease; HDL = high density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; TC = total cholesterol; Caro = Caro measure[88].

or *D6S89* 193 alleles. The presence of *APOE*2, DRB1**08, or *D6S89* 195 alleles was associated with a lower risk for high LDL or LDL subfraction levels. The risk for low HDL or HDL subfraction levels was higher in subjects having the *DQB1**604, *DRB1**13, or *TNFa* 105 alleles. Subjects having the *DQB1**501, *DRB1**07, or *DRB1**12 alleles had lower relative risks for low HDL or HDL subfraction levels than subjects not having one of those alleles. In terms of ratios between LDL or LDL subfraction and HDL, subjects with the *APOE*4* allele had higher risks for an elevated ratio, while subjects with the *APOE*2* or *DRB1**13 alleles exhibited lower risks (Table 2).

No statistically significant associations were found between the dichotomized Caro[80] measure and any of the *TNFa* or *THO1* allele variants. Although subjects with the *THO1* 108 allele were more likely to be insulin resistant this association was not statistical significance (chi^2=4.46, *df*=1, *N*=83, *P*=.061. Subjects with the *TNFa* 101 allele had higher mean Matthews values[76] (mean=290.27, SD=266, *t*=2.47, *df*=250, *P*=.014) than subjects not exhibiting *TNFa* 101 (mean=110.58, SD=174). This finding should be interpreted cautiously, however, as only six subjects out of 252 (2%) possessed the *TNFa* 101 allele. Subjects with *TNFa* 109, however, exhibited lower mean Matthews values (*t*=2.35, *df*=246, *P*=.020) than subjects without that allele.

Family History Association with Military Status

In terms of military status, military subjects had a lower likelihood of having a positive family history of CHD, diabetes, heart attack, high blood pressure, stroke, or high cholesterol (χ^2=8.09, *df*=1, *N*=267, *P*=.006). This finding was also observed for individual risk factors (CHD, DIAB, HBP, HCHOL, INSDP, and STROKE), though zero cells in those tables make interpretation problematic.

Family History Association with Selected Alleles

A review of selected alleles indicated that subjects with *DRB1**03 (χ^2=4.88, *df*=1, *N*=239, *P*=.036) and *DRB1**12 (χ^2=7.45, *df*=1, *N*=239, *P*=.010) were more likely to have a family history of heart disease, while subjects with *D6S89* 195 (χ^2=9.58, *df*=1, *N*=216, *P*=.003) and *DRB1**13 (χ^2=4.74, *df*=1, *N*=239, *P*=.037) were less likely to have a family history of heart disease (Table 3). Subjects with a CHD family history risk were more likely to have the *DQB1**201 allele (χ^2=5.29, *df*=1, *N*=222, *P*=.036), while subjects exhibiting the *APOE*4* allele were less likely to have a family history of CHD (χ^2=4.79, *df*=1, *N*=247, *P*=.035). Subjects with a family history of high blood pressure (HBP) tended to be less likely to exhibit the *DRB1**11 allele (χ^2=5.61, *df*=1, *N*=239, *P*=.021), and subjects with a family history of STROKE were less likely to have the *DRB1**13 allele (χ^2=4.75, *df*=1, *N*=239, *P*=.042) (Table 3).

Other Potential Risks and Family History

Other potential associations between selected individual risk factors and selected alleles were also identified, though low expected cell sizes in those tables dictate that they be considered tentative. Findings which fall into that category were as follows: subjects with the *D6S89* 209 allele were more likely to have a family risk for diabetes (DIAB) (χ^2=6.50, *df*=1, *N*=216, *P*=0.037) and hypercholesterolemia (HCHOL) (χ^2=6.52, *df*=1, *N*=216, *P*=.012) than subjects who did not possess that allele. Subjects exhibiting the *DQB1**302 allele were less likely to have a family history of DIAB (χ^2=9.46, *df*=1, *N*=222, *P*=.010), and subjects with the *D6S89* 199 allele were less likely to have a family history of HCHOL (χ^2=6.99, *df*=1, *N*=216, *P*=.035). Subjects with a family history of HRTATAK were more likely to exhibit the *TNFa* 105 (χ^2=5.90, *df*=1, *N*=244, *P*=.048), *DRB1**16 (χ^2=4.49, *df*=1, *N*=239, *P*=.035), and *D6S89* 207 (χ^2=9.37, *df*=1, *N*=216, *P*=.002) alleles, while they were less likely to exhibit *DRB1**11 (χ^2=4.82, *df*=1, *N*=239, *P*=.052). Subjects describing a family history of insulin dependence (INSDP, type 1) were more likely to have the *TNFa* 97 (χ^2=4.10, *df*=1, *N*=244, *P*=.044) allele. Subjects with a family history of stroke were more likely to have the *D6S89* 189 allele (χ^2=4.74, *df*=1, *N*=216, *P*=.045) (Table 3).

Metabolic Syndrome Associations

Of the 267 subjects entered into the study, 71 (26.6%) were identified as having metabolic syndrome. Associations between metabolic syndrome and selected alleles were tested by using two-by-two contingency tables incorporating an exact test for the lrchi^2 statistic. Association with military status could not be interpreted because of the presence of zero frequencies in the table, as no military subject evidenced metabolic syndrome.

Subjects who had alleles *DRB1**09 (χ^2=6.01, *df*=1, *N*=239, *P*=.029), *DRB1**12 (χ^2=4.76, *df*=1, *N*=239, *P*=.043), and *DRB1**15 (χ^2=5.49, *df*=1, *N*=239, *P*=.022) were more likely to have metabolic syndrome than those subjects who did not have those alleles. Subjects having alleles *DQB1**604 (χ^2=7.95, *df*=1, *N*=222, *P*=.011), DRB1*11 (χ^2=5.19, *df*=1, *N*=239, *P*=.031), and *DRB1**13 (χ^2=7.39, *df*=1, *N*=239, *P*=.008) were less likely to exhibit metabolic syndrome than subjects who did not have those alleles.

Lp(a) and Risk Association

Correlations between selected known CHD risk factors and Lp(a) levels were also examined, since Lp(a) is considered by some researchers to be a valid indicator for CHD risk in AAs.[66]

Table 3. Significant allele relationships with selected family history heart disease risk factors

Risk Factor		Odds Ratio (Exact 95% CI)	χ^2	Exact *P*
Overall family history	Military	0.285 (0.084, 0.774)	8.09	.006
	DRB1*03	1.993 (1.032, 3.813)	4.88	.036
	DRB1*12	3.006 (1.264, 7.210)	7.45	.010
	DRB1*13	0.508 (0.254, 0.984)	4.74	.037
	D6S89 195	0.229 (0.057, 0.699)	9.58	.003
CHD family history	DQB1*201	2.605 (1.057, 6.696)	5.29	.036
	APOE4	0.396 (0.139, 0.999)	4.79	.035
HPN family history	DRB1*11	0.460 (0.218, 0.929)	5.61	.021
Stroke family history	DRB1*13	0.362 (0.105, 1.015)	4.75	.042
	D6S89 189	14.385 (0.709, 164.254)	4.74*	.045
Diabetes family history	D6S89 209	5.019 (1.208, 24.118)	6.50†	.037
	DQB1*302	N/A‡	9.46†	.010
Insulin dependent diabetes family history	TNFα97	4.619 (0.818, 25.634)	4.10†	.044
Hypercholesterolemia family history	D6S89 209	6.75 (1.288, 29.629)	6.52†	.012
	D6S89 199	N/A‡	6.99†	.035
Heart attack family history	TNFα105	3.889 (1.147, 12.481)	5.90†	.048
	DRB1*16	5.732 (0.872, 27.183)	4.49†	.035
	D6S89 207	7.052 (1.761, 26.668)	9.37†	.002
Metabolic syndrome	DRB1*09	3.333 (1.288, 8.626)	6.01	.029
	DRB1*11	0.438 (0.185, 0.959)	5.19	.031
	DRB1*12	2.469 (1.015, 5.870)	4.76	.043
	DRB1*13	0.399 (0.181, 0.830)	7.39	.008
	DRB1*15	2.094 (1.077, 4.020)	5.49	.022
	DQB1*604	0.118 (0.003, 0.773)	7.95	.011

* 50% of cells have expected counts <5; χ^2 may not be valid.
† 25% of cells have expected counts <5; χ^2 may not be valid.
‡ Zero frequency cell precludes odds ratio calculation.

Significant associations were observed for all of the VAPI lipoprotein variables, as well as for fasting blood sugar levels, systolic blood pressure, weight, waist/hip ratio (WHR), and insulin resistance based on Matthews' criterion. In Table 4 the statistically significant correlations between risk factors and plasma Lp(a) levels observed in this sample of premenopausal AAW are displayed.

DISCUSSION

The relationships between CHD risk and *APOE* alleles observed in this sample provides additional support that this gene is involved in the etiology of CHD. APOE is believed to participate in the receptor-mediated clearance of blood lipids such as cholesterol, which is considered a major risk factor for CHD.[66,88–90] In North America, the variability of the *APOE* gene is estimated to account for up to 6% of the variation in CHD.[91] Some but not all studies have observed that the frequencies of the *APOE* alleles *APOE*2, APOE*3, APOE*4* vary between Whites and Blacks in the United States. In most studies *APOE*3* is the most frequent allele (≈0.73) and *APOE*2* the lowest (≈0.10).[92] While no significant difference in distribution of *APOE*2* ($\chi^2=2.26$, $df=1$, exact $P=.172$), *APOE*3* ($\chi^2=0.03$, $df=1$, exact $P=1.00$), or *APOE*4* ($\chi^2=0.015$, $df=1$, exact $P=1.00$) between high- and low-risk groups was found in this study, *APOE*4* has been observed to be significantly associated with increased number of coronary artery vessels that had stenosis in White women with a family history of CAD.[92–93] An association between *APOE*4* and increased plasma levels of total cholesterol and low-density LDL cholesterol has been observed in White women. *APOE*4* was not associated with these variables in the Black women studied. The authors concluded that this finding may have been due to the small number of Black women included in the study. In our study, which investigated a larger cohort, *APOE*4* was significantly associated with TC/HDL, LDL, LDL-R, LDL/HDL AND LDL-R/HDL.

Plasma Lp(a) level is considered by some researchers to be a valid indicator for CHD risk in AAs,[93–94] although controversy exists.[93] Moliterno et al[91] found that elevated plasma concentrations of Lp(a) are more strongly associated with coronary atherosclerosis in Whites than AAs. African Americans (AAs) tend to have a plasma concentration of Lp(a) at least two times higher than Whites.[92–93] While Hayden[93] found a strong inverse relationship between Lp(a) and HDL and a positive direct relationship between Lp(a) and LDL and triglycerides, this present study is not totally consistent with Hayden's findings.[93]

The high prevalence of metabolic syndrome (26.6%) in this population

Table 4. Significant correlations of selected risk factors with Lp(a)

Variable	*N*	*r*	Value of *P*	Variable	*N*	*r*	Value of *P*
HDL	280	0.544	<.001	Total cholesterol	280	0.315	<.001
HDL2	280	0.523	<.001	FBS	275	−0.234	<.001
HDL3	280	0.396	<.001	Insulin resistance (HOMA)	271	−0.140	.021
LDL	280	0.196	.001	SBP	279	−0.123	.40
VLDL	280	−0.121	.043	Weight	277	−0.144	.16
Trigly.	279	−0.150	.012	WHR	275	−0.250	<.001

HDL = high density lipoprotein; LDL = low density lipoprotein; FBS = fasting blood sugar; HOMA = homeostasis model assessment; SBP = systolic blood pressure; VLDL = very low density lipoprotein; WHR = waist-to-hip-ratio.

with a mean age of 34.18 is of interest. However, this explanation might be plausible based on the fact that the cases in the study were selected for their high risk for cardiovascular disease. Nonetheless, the age-adjusted prevalence for metabolic syndrome among 8814 US adults in the National Health and Nutrition Survey suggest that among women of all races in the age range of 20 to 29, the prevalence is only about 6%. Similarly, findings from this study also suggest that for women of all races between 30 to 39 years of age, the prevalence is only 13%. Given the high prevalence among Black US adults, the prevalence of metabolic syndrome may be significantly higher in AAW than found in other US groups.[94]

A great deal of controversy still exists over an acceptable standard definition for metabolic syndrome. For example, Ninomiya et al[95] identified five conditions: hypertriglyceridemia, low HDL cholesterol, hypertension, abdominal obesity, and insulin resistance as major contributory factors and thereby providing plausible rationale for the grouping of these conditions as a syndrome. Using their definition for metabolic syndrome in a seminal study, Ninomiya and colleague[95] examined the association of nonfatal myocardial infarctions and stroke with metabolic syndrome and its accompanying conditions. Findings from this study suggest a strong, consistent relationship between metabolic syndrome and myocardial infarction and stroke. Gene associations with metabolic syndrome identified in this study also need to be examined using other definitions of metabolic syndrome to see if they still hold.

The present study identified a number of alleles at several loci associated with various risk factors for CHD in a group of AA women. Subjects with some of the alleles exhibited higher odds for presence of certain risk factors, while other alleles seemed to be protective, in that subjects having those alleles tended to have lower odds for certain CHD risk factors. With the large number of statistical tests done on these data, the overall type 1 error rate is invariably larger than the 5% used to evaluate individual tests. Ideally, Bonferroni adjustments would be employed to control the increased type 1 rate. However, applying such adjustment in this study would be impractical because of the detrimental effect on power. The alleles associated with risk for CHD in this study can be interpreted in two ways. The genes could be directly involved in the etiology of CHD. This interpretation is plausible for *APOE* alleles, considering the role of *APOE* in lipid metabolism[17] and for HLA-*DRB1,* HLA-*DQB1* and *TNFα,* which are involved in immune response and inflammation. Inflammation is considered to contribute to the development of CHD.[18–39] Moreover, TNFα has been observed associated with obesity, insulin resistance, glucose intolerance, and diabetes.[81,96–98] Inflammation has also been implicated in all of these conditions. Another explanation is that the alleles assessed are not directly involved in the etiology of CHD but are in linkage with alleles that are. The loci assessed within the MHC and the *D6S89* are all in linkage disequilibrium.[48] Thus the HLA-*DRB1,* HLA-*DQB1, TNFα* and *D6S89* alleles observed in the present study associated with risk factors for CHD could be linked to other genes within this chromosome region that are actual culprits. This link would also apply to the *THO1* locus which is in linkage with the insulin gene.[50–55]

SUMMARY

Premenopausal African-American women are at risk for coronary heart disease, myocardial infarction, and stroke, given the data available. While this study adds a genetic component to the literature, the identified relationships need to be validated in additional studies. In addition, the meaning of the genetic associations identified in this study for the pathophysiology of CHD, interactions with behavioral and environmental factors, and design of health promotion interventions needs to be investigated. Our study suggests that AAW have several genetic and lifestyle risk factors for CHD. The association of *APOE* and *TNFα* alleles with risk of CHD suggests that these are candidate genes or linked to genes for CHD in this cohort of AAW. Our data supported elevated plasma Lp(a) as a potential risk

factor in AAW; however, its role is still unclear. The premenopausal AAW in this sample had a higher than expected rate of metabolic syndrome, which was associated with *DRB1* alleles. The association of genes within the MHC adds further support for the role of inflammation in risk of CHD. Although further studies are needed on larger cohorts of AAW, selective preventative interventions to modify behavioral, metabolic, and inflammatory risks should be considered for those possessing the indicated risk factors for CHD.

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