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APOE polymorphism and angiographic coronary artery disease severity in the Women's Ischemia Syndrome Evaluation (WISE) study

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Abstract

Genetic variation in the apolipoprotein E (APOE) gene is a significant determinant of variation in plasma cholesterol levels and it also affects the risk of coronary artery disease (CAD). We examined the association of the APOE polymorphism with CAD severity in women from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. Quantitative coronary angiography was used to classify subjects as having normal/minimal CAD ($< 20\%$ stenosis), mild CAD (20–49% stenosis) and significant CAD ($\geq 50\%$ stenosis). The women with $\geq 50\%$ stenosis were further stratified according to the number of vessel disease they have (one, two, or three). In white subjects, the frequency of *APOE*4* carriers (3/4 and 4/4 genotypes) was significantly higher in the combined mild/significant CAD group ($\geq 20\%$ stenosis) compared with the normal/minimal CAD group ($< 20\%$ stenosis) (31.3 vs. 19.2%; $P = 0.025$) with an adjusted OR of 2.40 (95% CI: 1.47–3.93; $P = 0.0005$). Furthermore, the *APOE*4* allele was found to be significantly associated with the increased vessel disease number ($\chi^2 = 8.04$; $P = 0.0046$). This association of the *APOE*4* allele with CAD severity was present only in women with family history of CAD. APOE polymorphism also showed significant associations with increasing plasma total cholesterol ($P = 0.01$) and low-density lipoprotein (LDL)-cholesterol ($P < 0.001$) in whites. These data support the hypothesis that the *APOE*4* allele is an independent risk factor not only for the presence of CAD and hyperlipidemia, but also for the angiographic severity of CAD in white women with a family history of disease.

Keywords: Apolipoproteins; Coronary disease; Risk factors

1. Introduction

Coronary artery disease (CAD), a multifactorial disease, is the leading cause of death of women in industrialized countries. Because CAD results from the interaction of genes and environmental factors, one approach to understand the etiology of CAD is to study candidate genes, which are involved in lipoprotein metabolism and in the CAD pathogenesis. Apolipoprotein E (apoE, protein; APOE, gene) is one of the most studied candidate genes.

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ApoE, synthesized in the liver, is a constituent of chylomicron and its remnant, very low-density lipoprotein (VLDL) and a subclass of high-density lipoprotein (HDL) [1]. In lipoprotein metabolism, it serves as a receptor-binding ligand to mediate the binding and uptake of apoE-containing lipoproteins by both the low-density lipoprotein (LDL) receptor and LDL receptor-related protein [2,3], and thus it plays a prominent role in the transportation and redistribution in both the influx and efflux of cholesterol in the body [4]. Human ApoE exhibits a genetic polymorphism with the existence of three common alleles, *APOE*2*, *APOE*3*, and *APOE*4*. The basis for this heterogeneity arises as a result of cysteine–arginine interchange at residues 112 and 158. *APOE*2* has cysteines at both 112 and 158 residues, while *APOE*4* has arginines instead. *APOE*3* has cysteine at 112 and arginine at 158 [4] and is the most common isoform observed in all populations. This APOE polymorphism explains 4–15% of the variation in serum LDL-cholesterol level [5]. The *APOE*4* allele is thought to be associated with higher lipoprotein metabolism [6], increased plasma total and LDL-cholesterol levels, decreased apoE plasma levels, and consequently greater risk for CAD [7–9]. However, the *APOE*2* allele has defective receptor binding ability, is associated with reduced plasma total and LDL-cholesterol levels and higher apoE plasma levels, and is considered to be protective against the risk of CAD. The *APOE*4* and *APOE*2* alleles are also associated with an increased and decreased risk, respectively, of Alzheimer's disease [10]. Most of the published APOE–CAD association studies have utilized clinically assessed case-control cohorts with limited or no data on the relationship between the APOE polymorphism and angiographic stenosis severity as well as the number of affected vessels. In the present study we have examined the association between the APOE polymorphism and the severity of angiographic CAD in the Women's Ischemia Syndrome Evaluation (WISE) cohort.

2. Methods

2.1. Study population

The present study was carried out on 692 women [575 white, 117 black, mean age: 57.3 ± 11.6 (S.D.) years] participating in the WISE study. Samples were obtained with informed consent and the study was approved by the Institutional Review Board. WISE is a National, Heart, Lung and Blood Institute (NHLBI) sponsored cross-sectional population study involving four academic medical centers from three geographic locations (University of Alabama at Birmingham, Allegheny University of the Health Sciences at Pittsburgh, University of Florida at Gainesville and University of Pittsburgh at Pittsburgh). The WISE study is designed to address issues related to ischemic heart disease recognition and diagnosis in women. The complete study design and methodology of the WISE study has been described elsewhere [11]. In brief, the WISE participants are women 18 years and older (mean age: 57.3 years) who have undergone a clinically indicated coronary angiogram for chest pain symptoms or suspected myocardial ischemia. Major exclusion criteria were: pregnancy, cardiomyopathy, contraindications to provocative diagnostic testing, New York Heart Association class IV congestive heart test failure, recent myocardial infarction, significant valvular or congenital heart disease, language disability on questionnaire testing, or recent coronary angioplasty or coronary bypass surgery. Quantitative coronary angiography was performed using the WISE study protocol and the data were analyzed at the Angiography Core Laboratory at Brown University by investigators blinded to subject identifiers. Eligible subjects are categorized into following groups based on angiographic stenosis: normal/minimal disease ($< 20\%$ stenosis); mild disease (20–49% stenosis); and significant disease ($\geq 50\%$ stenosis) in any one major epicardial coronary artery. Patients with $\geq 50\%$ stenosis were further classified as having one, two, or three-vessel disease according to the number of vessel disease they have. Since the tests of stenosis and number of vessel disease were performed independently at two different times, the total number of women in the one, two, or three vessel disease groups did not exactly match the total number in the $\geq 50\%$ stenosis group. Lipid samples were analyzed at the WISE lipid core laboratory at the Cedars Sinai Medical Center, which is enrolled in the Centers for Disease Control and Prevention lipid standardization program [12]. Fasting total plasma cholesterol, triglyceride, and HDL-cholesterol were determined by enzymatic assays as previously published [13]. LDL-cholesterol was calculated using the Friedewald equation [14].

2.2. APOE polymorphism detection

Genomic DNA was isolated by using the Puregene™ System DNA purification kit (GENTRA). A 224 bp fragment, including the two ApoE polymorphic sites (codon 112 and 158) was amplified by PCR as described by Kamboh et al. [15].

2.3. Statistical analysis

Differences in mean values of age, BMI and lipid profile between white and black subgroups of WISE subjects were compared using a two-tailed t-test. Differences in proportions of discrete variables between these two ethnic groups were tested using a χ^2-test. APOE allele frequency was calculated by allele count-

ing. Hardy–Weinberg equilibrium was tested by a χ^2 goodness-of-fit test and comparisons of genotype distribution between groups were made by χ^2 contingency test. All lipid variables were transformed to ensure a more normal distribution prior to the analysis. Specifically, total- and LDL-cholesterol levels were square root transformed, and triglycerides and HDL-cholesterol levels were transformed by natural logarithms. All analyses were performed separately for white and black women.

χ^2-tests were applied to test significant linear trend in the association of APOE polymorphism with CAD severity [16]. Specifically, CAD severity levels assigned for stenosis groups are 1, 2, 5, and for number of vessel disease groups are 1, 2, 3, respectively, in the trend test. We tested for a significant trend in disease severity using a χ^2-test [16]. Then multiple logistic regressions were used to determine the odds ratios (ORs) and 95% confident intervals (CI) by adjusting for significant CAD risk factors, including: age, BMI, smoking history, alcohol use, family history of CAD, history of hypertension, history of diabetes, menopausal status, and plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Because the *APOE*2* and *APOE*4* alleles are associated with opposite plasma cholesterol levels and CAD risk, in the association study we excluded the 2/4 genotype (a total of 15 white and four black women) and comparisons were made between the E2 (2/2 and 2/3 genotypes), E3 (3/3 genotype) and E4 (3/4 and 4/4 genotypes) groups. We also tested whether there were any genotype by environment interaction effects of APOE genotypes on CAD severity using multiple logistic regression analyses. For example, we tested whether the association between APOE genotype and CAD severity was similar between smokers and non-smokers. In these analyses, we incorporated all covariates, and then tested each genotype × covariate interaction term separately.

Significant covariates for each dependent lipid variable were identified using the stepwise linear regression analyses with an overall 10% level of significance. After identification of significant covariates, we tested differences in lipid levels among APOE genotypes using ANOVA.

All descriptive statistical analyses were performed using SPSS version 10.0 for Windows™. Multiple logistic regressions were done in R software version 1.2.1 (Ihada, 1996).

3. Results

3.1. Characteristics of the WISE population

The mean levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides together with mean age, BMI, smoking history, alcohol use, history of CAD, history of hypertension and diabetes in white women and black women are given in Table 1. Within the WISE cohort, the black subjects were significantly younger, heavier, and had lower triglycerides levels, but

Table 1
Distribution of the characteristics in whites and blacks

	Whites ($n = 575$)	Blacks ($n = 117$)	P-value
Age (year)	57.92 ± 11.61	54.26 ± 11.42	0.0015
BMI (kg/m^2)	29.12 ± 6.48	31.82 ± 6.52	0.00004
Total-cholesterol (mg/dl)	196.74 ± 44.65	188.08 ± 44.52	ns
LDL-cholesterol (mg/dl)	112.71 ± 39.52	107.71 ± 38.04	ns
HDL-cholesterol (mg/dl)	53.46 ± 12.67	55.48 ± 13.72	ns
Triglyceride (mg/dl)	160.69 ± 120.22	124.40 ± 95.40	0.002
Smoking history (yes, %)	53.3%	57.4%	ns
Alcohol use (yes, %)	13.9%	12.3%	ns
Family history of CAD (yes, %)	67.6%	62.6%	ns
History of diabetes (yes, %)	20.5%	40.8%	< 0.001
History of hypertension (yes, %)	53.1%	83.6%	< 0.001
Menopause (yes, %)	82.7%	76.2%	ns
Number of disease vessels (%)			ns
None	66.5%	64.9%	
One	14.7%	18.9%	
Two	10.0%	9.0%	
Three	8.9%	7.2%	
Stenosis (%)			ns
< 20%	38.9%	40.0%	
20–49%	25.7%	22.5%	
≥ 50%	35.3%	37.5%	

ns, non-significant.

Table 2
Distribution of the APOE polymorphism in whites and blacks

Genotype	Whites		Blacks		
	n	%	*n*	%	
22	3	0.51%	2	1.68%	
23	69	11.79%	19	15.97%	
24	17	2.91%	2	1.68%	
33	343	58.63%	63	52.94%	
34	138	23.59%	29	24.37%	
44	15	2.56%	4	3.36%	
Total	585		119		
$\chi^2 = 4.64$; $P = 0.46$					
Allele frequency					*P*-value
*APOE*2*	0.0786		0.1050		0.215
*APOE*3*	0.7632		0.7311		0.308
*APOE*4*	0.1581		0.1639		0.826

had higher prevalence of diabetes and hypertension compared with the white subjects.

3.2. APOE polymorphism and CAD risk

Before pooling the data from three geographic locations, we performed homogeneity tests to determine if the APOE genotype distribution was comparable across the three locations within white and black groups, and observed no significant location effect within whites ($P = 0.18$) or blacks ($P = 0.10$). There were no significant differences in the distribution of APOE genotype frequencies between white and black women ($\chi^2_{5df} = 4.64$, $P = 0.46$), and the distributions of APOE genotypes were in Hardy–Weinberg equilibrium in both white and black groups (Table 2). Tables 3 and 4 show the distribution of APOE genotype and allele frequencies among different angiographic categories of stenosis ($< 20\%$, 20–49%, $\geq 50\%$) and among the number of significant vessel disease (one, two, or three) groups, respectively. In white women, the distributions of APOE genotypes were significantly different among stenosis (6×3 table, χ^2-test, $P = 0.047$) and number of vessel disease (6×3 table, χ^2-test, $P = 0.023$) groups. Next, the distribution of the E2 (2/2 and 2/3), E4 (3/4 and 4/4) and E3 (3/3) genotypes were compared among the stenosis and number of vessel disease groups. There was no significant difference in cardiovascular risk for E2 group compared with E3 group. However, compared with the E3 group, the *APOE*4* carriers (44+34) were significantly associated with the increasing stenosis risk ($\chi^2_T = 8.55$, $P = 0.0035$) as well as with the increasing risk of having multi vessel disease ($\chi^2_T = 8.04$, $P = 0.0046$). The adjusted OR for the E4 group compared with the E3

Table 3
Distribution of the APOE polymorphism by stenosis

APOE genotype[a]		Stenosis					
		$< 20\%$		20–49%		$\geq 50\%$	
		n	%	*n*	%	*n*	%
White	E2	24	11.1%	22	15.4%	25	12.4%
	E3	149	69.0%	76	53.1%	112	55.7%
	E4	43	19.9%	45	31.5%	64	31.8%
	Total	216		143		201	
Trend test (E2 vs. E3)	$\chi^2_T = 0.50$; $P = 0.48$						
Trend test (E4 vs. E3)	$\chi^2_T = 8.55$; $P = 0.00345$						
Black	E2	9	19.6%	5	20.8%	7	15.6%
	E3	26	56.5%	12	50.0%	23	51.1%
	E4	11	23.9%	7	29.2%	15	33.3%
	Total	46		24		45	
Trend test (E2 vs. E3)	$\chi^2_T = 0.08$; $P = 0.77$						
Trend test (E4 vs. E3)	$\chi^2_T = 0.67$; $P = 0.41$						

[a] The adjusted OR for the E4 group compared with the E3 group to develop 20% stenosis was 2.40 (95% CI: 147, 3.93; $P = 0.0005$) and 1.81 (95% CI: 1.14, 2.88; $P = 0.012$) to develop 50% stenosis in white women.

Table 4
Distribution of the APOE polymorphism by number of vessel disease

APOE genotype[a]		Number of vessel disease					
		One		Two		Three	
		n	%	*n*	%	*n*	%
White	E2	13	16.9%	7	13.2%	2	4.7%
	E3	46	59.7%	28	52.8%	18	41.9%
	E4	18	23.4%	18	34.0%	23	53.5%
	Total	77		53		43	
Trend test (E2 vs. E3)	$\chi^2_1 = 1.17$; $P = 0.28$						
Trend test (E4 vs. E3)	$\chi^2_1 = 8.04$; $P = 0.0046$						
Black	E2	2	9.5%	2	20.0%	1	12.5%
	E3	14	66.7%	2	20.0%	4	50.0%
	E4	5	23.8%	6	60.0%	3	37.5%
	Total	21		10		8	
Trend test (E2 vs. E3)	$\chi^2_1 = 0.53$; $P = 0.46$						
Trend test (E4 vs. E3)	$\chi^2_1 = 1.59$; $P = 0.21$						

[a] The adjusted OR for the E4 group compared with E3 group to develop multiple vessel disease was 3.91 (95% CI: 1.81, 8.46; $P = 0.00054$) in white women.

group to develop 20% stenosis was 2.40 (95% CI: 147, 3.93; $P = 0.0005$) and 1.81 (95% CI: 1.14, 2.88; $P = 0.012$) to develop 50% stenosis. The adjusted OR for the E4 group compared with E3 group to develop multiple vessel disease was 3.91 (95% CI: 1.81, 8.46; $P = 0.00054$). Though similar trends were observed in blacks, no statistically significant differences were noted, probably due to the small sample size.

3.3. APOE polymorphism and quantitative lipid levels

Fig. 1 presents the mean values ±S.D. of lipid levels among APOE genotypes (E2, E3, and E4 groups) in whites and blacks. As expected, the total cholesterol ($P = 0.007$) and LDL-cholesterol ($P < 0.001$) levels were significantly different among the E2, E3 and E4 groups in whites. Specifically, the *APOE*2* carriers were

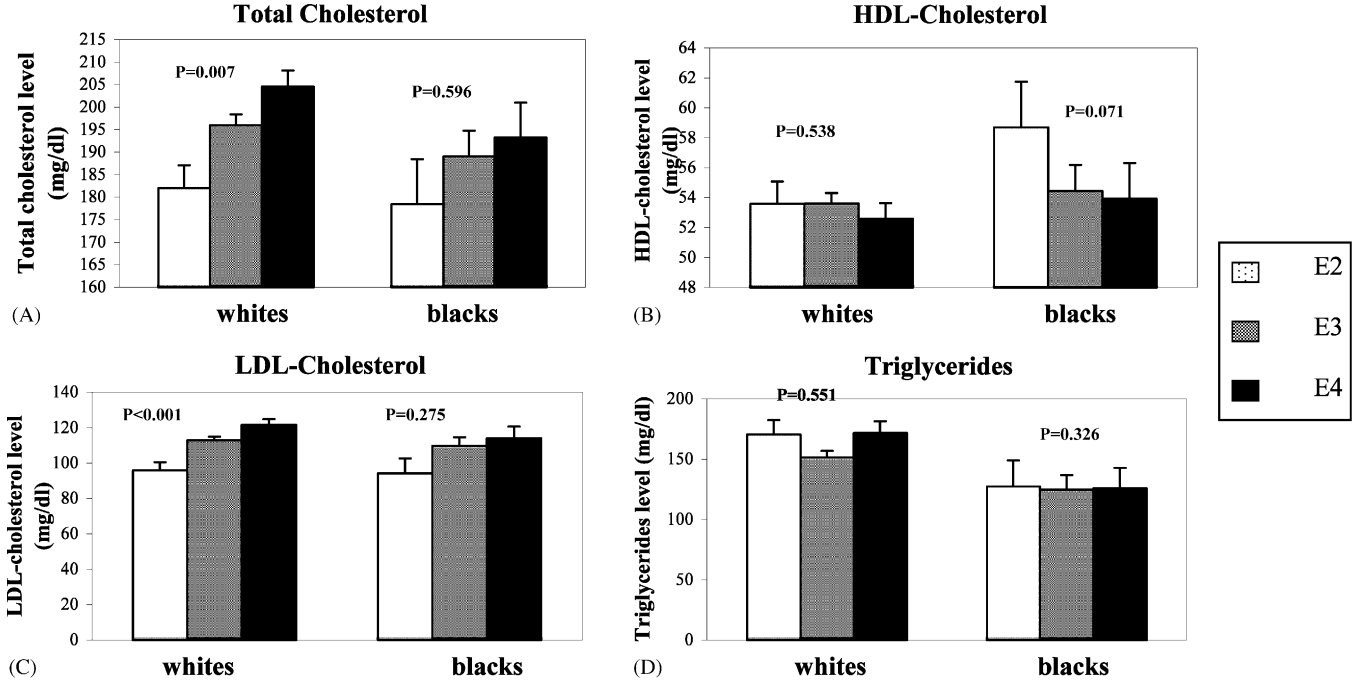

Fig. 1. Mean levels of four lipid traits among APOE genotype groups: E2 (22, 23 genotypes), E3 (33 geneotype) and E4 (34, 44 genotypes) in whites and blacks. (A) Total cholesterol; (B) HDL-cholesterol; (C) LDL-cholesterol; (D) Triglycerides. Comparisons were made between the E2, E3 and E4 groups for each lipid trait, and *P*-values were estimated by general linear regression analysis adjusted by significant covariates for each lipid level. The total number was 570 for whites and 116 for blacks.

associated with significantly lower total (182.13 ± 5.03 mg/dl) and LDL-cholesterol (95.90 ± 4.64 mg/dl), while the *APOE*4* carriers were associated with significantly higher total (204.56 ± 3.48 mg/dl) and LDL-cholesterol (121.42 ± 3.09 mg/dl) than the E3/3 genotype (total cholesterol: 196.06 ± 2.32 mg/dl; LDL-cholesterol: 112.92 ± 1.93 mg/dl). The impact of the APOE polymorphism on total and LDL-cholesterol was not significant in blacks; however, they showed similar trend as observed in whites. No significant association was found between the APOE polymorphism and HDL-cholesterol and triglycerides levels in either whites or blacks.

3.4. APOE genotype by covariate effect on stenosis

The potential effect of interactions of the APOE genotypes by covariates on stenosis severity was analyzed among the white women. We performed 12 interaction analyses between the APOE polymorphism and each covariate, including: age, BMI, smoking history, alcohol using, family history of CAD, history of diabetes, history of hypertension, menopausal status, and plasma lipid profiles (triglycerides, total-, LDL-, and HDL-cholesterol). Significant interaction was observed between the APOE polymorphism and CAD family history ($P = 0.025$). Based on this observation, we stratified the white women with and without CAD family history to further examine the relationship with APOE genotype (Table 5). As can be seen, no significant association was observed between the APOE polymorphism and CAD severity among individuals without CAD family history (stenosis groups: $P = 0.43$; number of vessel disease groups: $P = 0.11$). However, among women with CAD family history, there was a significant relationship between the APOE genotype and stenosis (3×3 contingency $\chi^2 = 29.97$, $P < 0.001$), as well as number of vessel disease (3×3 contingency $\chi^2 = 11.19$, $P = 0.025$). In particular, the E4 allele is significantly associated with increased risk of CAD stenosis ($\chi^2_T = 14.65$, $P = 0.0001$), and is also associated with the increasing number of vessel disease among the significant stenosis patients ($\chi^2_T = 6.37$, $P = 0.01$). Therefore, in white women with positive family history of CAD, the adjusted OR for *APOE*4* carriers compared with the APOE*33 individuals for the development of CAD is 6.91 (95% CI: 2.44, 19.60; $P = 0.0003$).

Table 5
ApoE genotype distributions by family history of CAD in whites

ApoE genotype	Stenosis					
	< 20%		20–49%		≥ 50%	
	n	%	*n*	%	*n*	%
Family history negative						
E2	9	12.33%	3	6.98%	11	17.19%
E3	42	57.53%	29	67.44%	40	62.50%
E4	22	30.14%	11	25.58%	13	20.31%
Total	73		43		64	
Family history positive						
E2	15	10.71%	19	19.39%	13	9.77%
E3	105	75.00%	46	46.94%	69	51.88%
E4	20	14.29%	33	33.67%	51	38.35%
Total	140		98		133	
ApoE genotype	Number of vessel disease					
	One		Two		Three	
	n	%	*n*	%	*n*	%
Family history negative						
E2	5	18.52%	2	16.67%	2	12.50%
E3	16	59.26%	10	83.33%	7	43.75%
E4	6	22.22%	0	0.00%	7	43.75%
Total	27		12		16	
Family history positive						
E2	7	14.58%	5	12.50%	0	0.00%
E3	29	60.42%	17	42.50%	11	40.74%
E4	12	25.00%	18	45.00%	16	59.26%
Total	48		40		27	

4. Discussion

Nearly 250 000 women die of ischemic heart disease each year in the US, making it the number one cause of death in women. Compared with men, heart disease in women is still identified less often and at a more advanced stage [17]. The purpose of the WISE study is to develop a more accurate algorithm for the risk stratification of women for CAD, including an evaluation of the role of genes as predictors of the presence of CAD in women. In an effort to delineate the genetic basis of CAD in women, we evaluated the role of the APOE polymorphism on the risk of angiographically documented CAD in the WISE cohort.

To our knowledge, this is the first study, which has examined the association between the APOE polymorphism and CAD severity in terms of both angiographically detected stenosis and number of significantly diseased vessels in a large number of women. Our study comprised 575 white and 117 black women. The frequency of the *APOE*4* allele was similar in black (16.4%) and white (15.8%) women. Our *APOE*4* allele frequency in whites is similar to those reported previously [18,19]. Although US blacks have been reported to have significantly higher frequency of the *APOE*4* allele than US whites in some studies [20,21], others have found comparable frequencies between the two groups [22,23]. The variation in APOE allele frequencies in US black populations could be due to variable non-African admixture. No significant association was observed between APOE polymorphism and the CAD severity in black women, most likely due to the relatively small

sample sizes in each of the stenosis and diseased vessel subgroups.

Among white women in the WISE population, the *APOE*4* allele exhibited significant associations with both the severity of stenosis as well as the number of diseased vessels, as measured by quantitative angiography. The risk for having a moderate to significant CAD ($>20\%$ stenosis) was 2.4-fold higher among *APOE*4* carriers than the other genotype carriers. Similarly, the risk for having a significant three vessel CAD was 3.9-fold higher among *APOE*4* carriers. Because we adjusted the OR by all the known risk factors including age, BMI, smoking, alcohol use, family history of CAD, history of hypertension, diabetes, and the lipid levels, our result suggests that the overall association between the *APOE*4* allele and CAD extent and severity is independent of known risk factors. Previously, Wang et al. [9] have reported the association of the *APOE*4* allele with the number of disease vessels in 424 white Australians. Similar to our findings, they found that the frequency of the *APOE*4* allele was significantly higher in the three vessel CAD group (24.7%) than the one and two vessel CAD groups (13.6–16.5%). However, unlike our data, Wang et al. [9] did not report the association of the APOE polymorphism among groups with varying severity of angiographic stenoses.

Previously, several studies have linked the presence of the *APOE*4* allele with a greater risk of CAD [8,24]. A meta-analysis based on 14 published reports (nine clinical CAD and five coronary angiography) estimated *APOE*4*-associated ORs to be 1.26 (95% CI: 1.13–1.41) for clinical CAD and 1.11 (95% CI: 0.9–1.4) for coronary angiography [25,26]. However, the meta-analysis did not find a protective effect associated with the *APOE*2* allele. Another study, based on autopsy material in the PDAY study, showed that the *APOE*4* allele was associated with atherosclerosis and it accounted for about 5.9% of the observed variation in atherosclerotic lesions in aorta and this association was independent of cholesterol levels [27].

The strong association of the *APOE*4* allele and increased CAD risk has been confirmed in this study. However, as CAD is due to complex interaction between multiple factors, the APOE polymorphism may not function alone to affect the disease. Thus, we investigated whether the APOE effect on CAD was modified by other CAD risk factors. Our results suggest that the positive family history of CAD in conjunction with the *APOE*4* allele are the most significant predictors of CAD (OR: 6.91; 95% CI: 2.44, 19.60; $P = 0.0003$). Since positive family history is a known risk factor for developing CAD due to shared genes and environmental factors, the interaction between the family history of CAD and APOE genotype may not be surprising. The family history of CAD in this study was defined as first-degree relative (mother, father or sibling) having CAD or sudden death before age 55/65 (male/female). The significant increased risk of CAD seen among *APOE*4* carriers with family history of CAD appears to be due to the interaction between the APOE polymorphism with other genetic variants present in these individuals. Recently, an interaction between the *APOE*4* allele and smoking has been reported to increase the risk of CAD among middle aged men [28]. However, we did not observe such an interaction in our women subjects.

The precise mechanism behind the association of the *APOE*4* allele with angiographic CAD is not certain. One of the mechanisms may involve the effect of APOE on plasma lipoprotein profile, as apoE is ligand for the LDL receptor and LRP1, by which it mediates the uptake of the apoE-containing lipoprotein particles. In the general population, the *APOE*4* allele is associated with elevated levels of total and LDL-cholesterol as compared with *APOE*3*, while the reverse is true for *APOE*2* [18,29]. It has been suggested that the presence of *APOE*4* allele may lead increased level of LDL-cholesterol because of the enhanced up-take of apoE4-containing chylomicron remnants and consequently down-regulation of the LDL receptor, while the *APOE*2* allele results in decreased level of LDL-cholesterol level because its lower receptor binding ability delays the removal of chylomicron remnants and upregulation of LDL receptor activity [26]. To evaluate the association of the APOE polymorphism with angiographic CAD presentation through its effect on plasma cholesterol levels, we also examined the impact of the APOE polymorphism on plasma lipid profile in the WISE cohort. Indeed, the *APOE*4* allele was associated with significantly elevated total cholesterol ($P = 0.043$) and LDL-cholesterol ($P = 0.027$), while the *APOE*2* allele was associated with low total cholesterol ($P = 0.01$) and LDL-cholesterol ($P < 0.001$). However, the contribution of the APOE allele-specific effect on risk of CAD via its effects on plasma cholesterol levels seems minimal, because the *APOE*4* allele was a significant independent risk factor for angiographic CAD even after inclusion of plasma lipid profile. APOE appears to be a multifunctional protein, as evidenced by the strong association of *APOE*4* allele with Alzheimer's disease [10]. Thus, the mechanism of the association of the *APOE*4* allele may involve additional aspects other than its established role in lipid metabolism. Several lines of evidence suggest that oxidation of LDL and its uptake by macrophages can lead to the formation of foam cells, which initiates the process of atherosclerosis [30,31]. Increased LDL and VLDL peroxidation has been observed in apoE-deficient mice [32] and antioxidant treatment has been shown to slow the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbits [33,34]. In this regard, APOE allele-specific antioxidant activity and allele-specific protection of cultured cells from

oxidative cell death have been demonstrated with *APOE*2 > APOE*3 > APOE*4* [35]. Thus, it appears that the association of the *APOE*4* allele with CAD and Alzheimer's disease could be due to its default action in protecting lipoproteins and neuronal cells from oxidative damage.

In summary, our data on a large number of WISE women confirm the hypothesis that genetic variation in the APOE gene is a significant determinant of plasma cholesterol levels, and CAD risk. Furthermore, we show that APOE is associated with increasing disease severity, and that positive family history enhances the association of the APOE polymorphism with CAD.

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