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Atherosclerosis: National Heart, Lung, and Blood Institute Family Heart Study**

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Influence of Apolipoprotein E, Smoking, and Alcohol Intake on Carotid Atherosclerosis

National Heart, Lung, and Blood Institute Family Heart Study

Luc Djoussé, MD; Richard H. Myers, PhD; Michael A. Province, PhD; Steven C. Hunt, PhD; John H. Eckfeldt, MD; Gregory Evans, MA; James M. Peacock, MPH; R. Curtis Ellison, MD

Background—Apolipoprotein E (apoE) isoforms and lifestyle factors play an important role in the development of coronary heart disease. The association of apoE and carotid atherosclerosis remains controversial.

Methods—We investigated the relation of apoE, cigarette smoking, alcohol drinking, and their interaction with carotid atherosclerosis on 544 individuals free of coronary heart disease in the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study. Atherosclerotic lesions of the carotid arteries were detected through high-resolution ultrasound.

Results—Subjects in the apoE4 group had lower blood pressure, lower high-density lipoprotein cholesterol, and higher low-density lipoprotein cholesterol. In a multivariate logistic regression model, apoE isoforms and alcohol consumption were not significantly associated with the prevalence odds of carotid atherosclerosis ($P=0.94$ and 0.98 , respectively, for trend). In contrast, compared with those who never smoked, the prevalence odds ratios for carotid atherosclerosis were 1.7 [95% confidence interval (CI), 1.1 to 2.7], 2.8 (95% CI, 1.2 to 6.2), and 1.9 (95% CI, 0.7 to 5.5) for former smokers, current smokers of 1 to 20 cigarettes per day, and current smokers of >20 cigarettes day, respectively ($P=0.0018$ for trend). We did not find evidence of an interaction between apoE and alcohol consumption. Our data suggested a synergistic effect between the apoE allele ϵ_4 and smoking on carotid atherosclerosis: odds ratios were 1.7 (95% CI, 0.8 to 3.6) for smoking alone, 1.0 (95% CI, 0.6 to 1.8) for ϵ_4 alone, and 3.7 (95% CI, 1.1 to 3.6) for the joint presence of the apoE allele ϵ_4 and smoking.

Conclusions—Smoking but not alcohol consumption or ApoE is associated with an increased odds of carotid atherosclerosis. Our data suggest a synergistic effect between the apoE allele ϵ_4 and smoking on carotid atherosclerosis. (*Stroke*. 2002;33:1357-1361.)

Key Words: apolipoproteins ■ atherosclerosis ■ carotid arteries ■ cigarette smoking

The genetic polymorphism of apolipoprotein E (apoE), a protein found in low-density lipoprotein (LDL) and high-density lipoprotein (HDL), is common. The major isoforms E2, E3, and E4 are coded by the alleles ϵ_2 , ϵ_3 , and ϵ_4 , respectively.¹ The allele ϵ_4 has been associated with increased risk of coronary heart disease (CHD),²⁻⁶ raised LDL,⁷⁻¹⁰ higher triglycerides,⁷ and lower HDL.⁷ However, the relation between apoE isoforms and carotid atherosclerosis remains controversial. Although some studies have suggested an association between apoE isoforms and carotid artery intima-media thickness (IMT),¹¹⁻¹⁵ a measure of carotid atherosclerosis, others have failed to show an association.¹⁶⁻¹⁸

Cigarette smoking is a traditional risk factor for atherosclerosis and has been associated with an unfavorable lipid profile.¹⁰ Previous studies have demonstrated a positive

association between smoking and carotid IMT.¹⁹⁻²⁴ Furthermore, a strong interaction has been demonstrated between smoking and the apoE allele ϵ_4 on plasma LDL levels.¹⁰ However, it is not known whether smoking interacts with the apoE allele ϵ_4 to influence carotid atherosclerosis.

Light to moderate alcohol consumption is associated with a lower risk of mortality, coronary artery disease, and stroke.²⁵⁻³⁶ Little is known about alcohol consumption and carotid atherosclerosis. Furthermore, it is unclear whether the relation between apoE and carotid atherosclerosis is modified by alcohol consumption. Unlike apoE, both smoking and alcohol consumption are modifiable risk factors of cardiovascular disease and offer potential for primary and secondary prevention of CHD.

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In this study, we used data collected on 544 individuals (from 346 families who were randomly selected) in the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study who were free of CHD and diabetes mellitus to evaluate (1) whether apoE isoforms, smoking, and current alcohol intake are associated with carotid atherosclerosis and (2) whether the apoE allele ϵ_4 interacts with smoking or alcohol intake to influence carotid atherosclerosis.

Materials and Methods

Study Population

The NHLBI Family Heart Study is a multicenter, population-based study designed to identify and evaluate genetic and nongenetic determinants of CHD, preclinical atherosclerosis, and cardiovascular risk factors. A detailed description of the methods and design has been given elsewhere.³⁷ Data were collected on 592 families who were randomly selected and 661 families who were chosen because of higher-than-expected CHD rates among family members. Each subject gave informed consent, and the study protocol was reviewed and approved by the institutional review boards of each participating institution. Of 681 individuals with information on both apoE genotype and carotid ultrasound, 137 were excluded from the present analyses for the following reasons: (1) E2/E4 genotype resulting from a classification problem ($n=14$), (2) prevalent CHD ($n=62$), (3) prevalence of diabetes mellitus ($n=42$), (4) cholesterol-lowering medication ($n=7$), or (5) missing covariates ($n=12$).

Assessment of Carotid Artery Lesions

Using high-resolution B-mode ultrasound, trained technicians measured carotid IMT according to the Atherosclerosis Risk in Communities (ARIC) protocol.³⁸ Measurements of IMT were derived in the far wall of 3 segments of the right and left extracranial carotid arteries: the common carotid artery (1 cm proximal to the dilatation of the carotid bulb), the bifurcation (the 1-cm segment proximal to the flow divider), and the internal carotid artery (the 1-cm segment in the internal branch distal to the flow divider). B-mode images were recorded on high-resolution, 3/4-in, high-end cassettes. At the central reading station, trained readers measured IMT at various points in each of the 3 carotid artery segments according to a standardized protocol. Estimates of the site-specific reliability coefficients were 0.77, 0.73, and 0.70 for mean carotid far-wall IMT at the carotid bifurcation, internal artery, and common carotid artery, respectively. Based on the ultrasound images, it was determined whether an atherosclerotic lesion was present in the segment visualized. For each subject, the total number of lesions was recorded. For these analyses, carotid atherosclerosis was present if at least 1 lesion was detected.

Blood Collection and Assays

Blood samples were collected from fasting subjects and spun at 3000g for 10 minutes at 4°C. Sera were stored at -70°C until shipped periodically to a central laboratory at the Fairview-University Medical Center in Minneapolis, Minn, for processing. LDL was estimated by the method of Friedewald et al³⁹ except in subjects with triglycerides >400 mg/dL, whose LDL was measured by ultracentrifugation. Total cholesterol and triglyceride concentrations were measured with a Roche COBAS FARA high-speed centrifugal analyzer (Roche Diagnostic Systems).⁴⁰ HDL cholesterol was measured after precipitation of the other lipoprotein fractions by dextran sulfate.⁴¹

ApoE genotyping was performed with polymerase chain reaction to amplify a 267-base pair fragment from exon 4 of the apoE gene.⁴² The polymerase chain reaction product was digested with the *HhaI* restriction endonuclease (an isoschizomer of *CfoI*), which results in a specific banding pattern for the 3 isoforms of the apoE protein when separated by polyacrylamide gel electrophoresis and silver stained. ApoE categories were created as followed: E2 (E2/E2 or E2/E3), E3 (E3/E3), and E4 (E4/E4 or E3/E4).

Smoking and Alcohol Data

Information on cigarette smoking was obtained by questionnaire. Participants were asked if they had ever smoked cigarettes and, if so, whether they were current smokers. The number of cigarettes smoked per day was obtained for current smokers. This information was used to classify each participant as never smoked, former smoker, or current smoker of 1 to 20 or >20 cigarettes per day. Alcohol information was self-reported. The average number of drinks (beer, spirits, and wine) consumed per week on average over the past 12 months was recorded. This information was used to compute the total ethanol content. Each subject was classified as current nondrinker or current drinker of 1 to 12, 12.1 to 24, or >24 g/d ethanol.

Other Variables

Anthropometric data were collected with subjects wearing scrub suits. A balance scale was used to measure body weight, and height was measured with a wall-mounted vertical ruler. Body mass index was computed as weight (kilograms) divided by the height (meters) squared. Hypertension was defined as systolic blood pressure of at least 140 mm Hg, diastolic blood pressure of at least 90 mm Hg, or use of medication for hypertension. Information on physical activity (minutes per day of leisure activity) was obtained by interview.

Statistical Analysis

Because the apoE allele ϵ_4 has been associated with a higher risk of CHD,²⁻⁶ we used the presence or absence of allele ϵ_4 (yes/no) and dichotomized current smoking (yes/no) and current drinking (yes/no) to evaluate interaction. For the apoE and smoking interaction, we created the following mutually exclusive categories: (1) absence of ϵ_4 allele and no smoking, (2) absence of ϵ_4 allele and smoking, (3) presence of ϵ_4 allele and no smoking, and (4) presence of ϵ_4 allele and smoking. Similar categories were generated for allele ϵ_4 and alcohol intake.

We used analysis of variance to compare characteristics across apoE isoforms for continuous variables and χ^2 analysis for categorical variables. We fitted a logistic regression model to assess the relation of different exposure variables and the presence of carotid atherosclerosis. Because of the familial clustering among individuals in this study, we used generalized estimating equations to account for familial clustering. The simple model adjusted for age and sex. The multivariate model controlled for age, sex, body mass index, physical activity, LDL, HDL, smoking, systolic blood pressure, and history of hypertension. Further adjustment for education, field center, and exercise did not alter the results.

Results

Of the 544 participants analyzed, 299 (55%) were women and 245 (45%) were men. The mean \pm SD age was 56.4 \pm 10.1 years (range, 26.4 to 78.0 years). As expected, E3/E3 was the most prevalent genotype ($n=337$), followed by E3/E4 ($n=130$), E2/E3 ($n=65$), E4/E4 ($n=10$), and E2/E2 ($n=2$). The allele frequency distributions of ϵ_2 , ϵ_3 , and ϵ_4 were 6%, 80%, and 14%, respectively, and the observed genotype distributions of E3/E3 (61.9%), E3/E4 (23.9%), E2/E3 (11.9%), E2/E2 (0.4%), and E4/E4 (1.8%) were in Hardy-Weinberg equilibrium ($P=0.61$). Table 1 gives characteristics of the study participants. Compared with other isoforms, subjects with allele ϵ_4 had lower blood pressure, lower HDL cholesterol, higher LDL cholesterol, and lower prevalence of hypertension.

In a logistic regression model adjusted for age and sex, apoE isoforms were not associated with a significantly increased odds of carotid atherosclerosis ($P=0.85$, Table 2). Additional adjustment for other covariates did not alter the results (Table 2). There was no significant association between alcohol consumption and prevalence odds of carotid atherosclerosis ($P=0.98$ for trend; Table 2). Smoking was related to the prevalence odds of carotid atherosclerosis. Compared with those who never smoked, mul-

TABLE 1. Characteristics of the Study Population

	E2 (E2/E2 or E2/E3; n=67)	E3 (E3/E3; n=337)	E4 (E4/E4 or E3/E4; n=140)	P*
Age, y	55.7±11.1	57.2±9.8	55.0±10.1	0.08
Body mass index, kg/m ²	27.5±4.9	27.9±5.8	28.0±5.0	0.78
Systolic BP, mm Hg	117.7±13.6	118.5±17.6	114.1±15.3	0.03
Diastolic BP, mm Hg	70.9±9.1	68.8±9.3	67.7±9.6	0.07
HDL cholesterol, mmol/L	1.3±0.4	1.4±0.4	1.2±0.3	0.0007
LDL cholesterol, mmol/L	2.9±0.8	3.3±0.8	3.4±0.9	0.0001
Alcohol intake, g/d	4.6±10.2	4.7±9.9	3.9±9.7	0.72
Exercise, min/d	25.9±33.9	30.4±34.3	25.5±35.2	0.30
Male sex, %	41.8	44.2	48.6	0.58
Hypertension, %	19.4	30.6	17.1	0.004

BP indicates blood pressure.

*Probability values were obtained by use of ANOVA for continuous variables and χ^2 for categorical variables.

tivariate adjusted prevalence odds ratios (ORs) were 1.7 [95% confidence interval (CI), 1.1 to 2.7], 2.8 (95% CI, 1.2 to 6.2), and 1.9 (95% CI, 0.7 to 5.5) for former smokers and current smokers of 1 to 20 and >20 cigarettes per day, respectively ($P=0.0018$ for trend; Table 2).

Compared with subjects without the ϵ_4 allele who were non-smokers, smoking alone was associated with 1.7-times-greater odds of carotid atherosclerosis, and the apoE allele ϵ_4 alone was not related to carotid atherosclerosis. The joint presence of allele ϵ_4 and current smoking was associated with 3.7-times-greater odds of carotid atherosclerosis (OR, 3.7; 95% CI, 1.1 to 3.6; Table 3),

TABLE 2. Prevalence ORs and 95% CIs of Carotid Artery Lesion According to ApoE Isoforms, Smoking, and Alcohol Consumption

	Subjects/ Total, n	OR (95% CI)	
		Adjusted for Age and Sex	Multivariate Model
ApoE isoform*			
E3	90/337	1.0	1.0
E2	18/67	1.0 (0.6–2.1)	1.2 (0.6–2.2)
E4	37/140	1.2 (0.7–1.9)	1.1 (0.7–1.9)
P for trend		0.85	0.94
Cigarette smoking†			
Never	69/330	1.0	1.0
Former	56/160	1.7 (1.1–2.6)	1.7 (1.1–2.7)
Current, 1–20 cigarettes/d	15/39	2.8 (1.3–5.9)	2.8 (1.2–6.2)
Current, >20 cigarettes/d	5/15	1.8 (0.6–5.6)	1.9 (0.7–5.5)
P for trend		0.0023	0.0018
Alcohol intake*			
Current nondrinker	96/369	1.0	1.0
Current drinker, 1–12 g/d	26/105	1.0 (0.6–1.7)	0.8 (0.5–1.5)
Current drinkers, 12.1–24 g/d	12/39	1.4 (0.6–3.1)	1.0 (0.4–2.4)
Current drinker, >24 g/d	11/31	1.3 (0.6–3.2)	1.1 (0.4–2.8)
P for trend		0.43	0.98

*Multivariate model adjusted for age, sex, body mass index, exercise, smoking, LDL and HDL-cholesterol, systolic blood pressure, and hypertension.

†Multivariate model adjusted for age, sex, body mass index, exercise, LDL and HDL cholesterol, systolic blood pressure, and hypertension.

suggesting a synergistic effect between smoking and the ϵ_4 allele. There was no significant evidence for an interaction between alcohol consumption and the ϵ_4 allele (Table 3).

Discussion

Although the genetic polymorphism of apoE is an important factor for cardiovascular risk and the development of CHD, data are mixed on whether apoE is associated with carotid artery atherosclerosis. The most important effect of apoE seems to be on the level of LDL cholesterol, in which a strong interaction between apoE and smoking has been demonstrated.¹⁰ Although epidemiological studies have shown that cigarette smoking is associated with increased risk of carotid atherosclerosis and that moderate alcohol intake may lower the risk of cardiovascular disease, it is not known whether the apoE allele ϵ_4 interacts with smoking and/or alcohol to influence the risk of carotid atherosclerosis. In this study, we examined participants free of CHD and diabetes mellitus and found evidence that smoking but not alcohol consumption or apoE isoforms was significantly related to the prevalence odds of carotid atherosclerosis. Although there was no interaction between alcohol and the apoE allele ϵ_4 , our findings suggested that smoking and the ϵ_4 allele may have synergistic effects on the prevalence odds of carotid atherosclerosis. These effects of smoking alone and its interaction with the apoE allele ϵ_4 were independent of LDL cholesterol.

Hixson⁴³ found that apoE was associated with atherosclerosis of the aorta in young men. In another study of nondiabetic subjects free of cardiovascular disease, apoE4 was associated with increased thickness of the carotid artery wall.⁴⁴ In both studies, the observed effects were independent of serum cholesterol. Other investigators have reported a positive association between apoE and carotid IMT.^{11–15} In contrast, Guz et al¹⁸ found no association between the apoE polymorphism and carotid IMT in 269 hemodialysis patients. In a study of young adults (n=150) free of cardiovascular disease, Sass et al¹⁷ found no association between the apoE allele ϵ_4 and carotid IMT. Furthermore, Kogawa and colleagues¹⁶ found no evidence of an association between the apoE allele ϵ_4 and carotid IMT. The heterogeneity of these results merits comment. It is possible that chance may have played a role in some of the positive studies. In addition, other determinants of atherosclerosis such as smok-

TABLE 3. Prevalence ORs and 95% CIs of Carotid Artery Lesion According to the ApoE Allele ϵ_4 , Current Smoking, and Current Drinking

ϵ_4 Allele		Subjects/ total, n	OR (95% CI)	
			Adjusted for Age and Sex	Multivariate Model
Current smoking*				
Absent	No	94/362	1.0	1.0
Absent	Yes	14/42	1.7 (0.8–3.4)	1.7 (0.8–3.6)
Present	No	31/128	1.1 (0.6–1.8)	1.0 (0.6–1.8)
Present	Yes	6/12	3.9 (1.1–3.6)	3.7 (1.1–3.6)
Current drinking†				
Absent	No	57/200	1.0	1.0
Absent	Yes	51/204	0.8 (0.5–1.4)	0.8 (0.5–1.4)
Present	No	13/75	0.6 (0.3–1.3)	0.6 (0.3–1.3)
Present	Yes	24/65	1.5 (0.7–2.9)	1.5 (0.7–2.9)

*Adjusted for age, sex, body mass index, exercise, LDL and HDL cholesterol, systolic blood pressure, and hypertension.

†Adjusted for age, sex, body mass index, exercise, smoking, LDL and HDL cholesterol, systolic blood pressure, and hypertension.

ing, LDL cholesterol, blood pressure, and other lifestyle factors might have been unbalanced between apoE isoforms and that inadequate control of such risk factors partially explained the findings. Finally, nondifferential measurement error of the IMT may have biased the results toward the null.

Our results for smoking and carotid atherosclerosis are consistent with published data and indicate a detrimental effect of smoking on carotid atherosclerosis^{19–24}. Limited data exist on the relation of alcohol intake and carotid atherosclerosis. Although Kiechl et al⁴⁵ reported a U-shaped association between alcohol intake and carotid atherosclerosis, Demirovic et al⁴⁶ reported no association between alcohol intake and carotid atherosclerosis. Data from the Kuopio Ischemic Heart Disease Risk Factor Study⁴⁷ found that binge drinking was associated with a progression in carotid artery wall thickness. To the best of our knowledge, no previous study has evaluated the interaction between apoE and smoking or apoE and alcohol intake on carotid atherosclerosis.

The fact that we found an association between smoking and carotid atherosclerosis after adjustment for LDL and HDL cholesterol suggests that mechanisms other than lipids may play a role in the atherosclerosis process. The suggested interaction between smoking and the apoE allele ϵ_4 indicates that subjects with allele ϵ_4 may be more susceptible to the detrimental effects of cigarette smoking on carotid atherosclerosis. In addition, smoking has been shown to impair endothelial function.^{48,49} Because of a lack of data on endothelial function, we were unable to test whether subjects with the apoE allele ϵ_4 showed greater impairment of the endothelial function than those without the allele. The clinical implication of our findings is that subjects with the apoE allele ϵ_4 who smoke may be at greater risk of carotid atherosclerosis and thus higher risk of stroke. If confirmed by future studies, this particular subgroup may benefit from preventive measures.

In conclusion, our findings indicated that smoking but not alcohol consumption or apoE isoforms was associated with a higher prevalence odds of carotid atherosclerosis. The effects of

smoking were independent of LDL cholesterol and were greater in the presence of the apoE allele ϵ_4 .

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