Association of *APOE* genotype with carotid atherosclerosis in men and women: the Framingham Heart Study

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Abstract **The aim of this study was to determine the association between *APOE* genotype and carotid atherosclerosis, defined as intimal-medial thickness (IMT) and stenosis, and to assess if other cardiovascular risk factors modify this association. A total of 1,315 men and 1,408 women from the Framingham Offspring Study underwent carotid ultrasound during examination cycle 6 and had complete data on *APOE* genotype. Three *APOE* genotype groups were defined: *APOE2* (including *E2/E2*, *E3/E2* genotypes), *APOE3* (*E3/E3*), and *APOE4* (including *E4/E3*, *E4/E4* genotypes). Carotid IMT and the presence of carotid stenosis > 25% were determined by ultrasonography. In women, the *APOE2* group was associated with lower carotid IMT (0.67 vs. 0.73 mm) and lower prevalence of stenosis (odds ratio = 0.49; 95% confidence interval = 0.30–0.81) compared with the *APOE3* group. In men, *APOE* genotype was not associated with carotid IMT or stenosis in the whole group; however, diabetes modified the association between *APOE* genotype and carotid IMT (*P* for interaction = 0.044). Among men with diabetes, the *APOE4* group was associated with a higher internal carotid artery IMT (1.22 mm) than the *APOE3* group (0.90 mm) or the *APOE2* group (0.84 mm).** The *E2* allele was associated with lower carotid atherosclerosis in women, and the *E4* allele was associated with higher internal carotid IMT in diabetic men.**—Elosua, R., J. M. Ordovas, L. A. Cupples, C. S. Fox, J. F. Polak, P. A. Wolf, R. A. D'Agostino, Sr., and C. J. O'Donnell. **Association of *APOE* genotype with carotid atherosclerosis in men and women. The Framingham Heart Study. *J. Lipid Res.* 2004.** 45: **1868–1875.**

Supplementary key words genetics • atherosclerosis • carotid artery • lipoprotein • apolipoprotein E genotype

The *APOE* locus is one of the candidate gene regions proposed to play an important role in atherosclerosis. Apolipoprotein E (apoE) is a plasma protein that modulates the metabolism of lipoproteins. Genetic variation in the polymorphic *APOE* locus significantly affects plasma lipoprotein concentrations. Three common *APOE* alleles have been identified: *APOE*E2* (*E2*), *APOE*E3* (*E3*), and *APOE*E4* (*E4*). The presence of the *E4* allele is associated with increased LDL cholesterol, whereas the presence of the *E2* allele is associated with decreased LDL cholesterol (1). Both *E2* and *E4* are also associated with increases in plasma triglycerides (2) and increased cardiovascular disease (CVD) risk (1, 3), although the *E2* allele was associated with a lower CVD risk in women in the Framingham Study (1, 4).

Carotid intimal-medial thickness (IMT) and plaque measured by ultrasound are associated with prevalent CVD (5), incident myocardial infarction and stroke (6), premature parental coronary heart disease (7), and peripheral arterial disease (8). Therefore, carotid IMT is widely used as a surrogate measure of atherosclerosis burden. Data from the Framingham Heart Study (9) and other studies (10) demonstrate that there is a substantial heritable component to carotid atherosclerosis.

The association between carotid atherosclerosis and *APOE* genotype has been examined previously, with discordant results (11–25). The aim of the current analyses was to determine the association between *APOE* genotype and carotid atherosclerosis and to assess whether other

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CVD risk factors modify this association in the Framingham Heart Study.

METHODS

Study population

The Framingham original cohort began enrollment in 1948, with the recruitment of 5,209 men and women between the ages of 30 and 62 years. Subjects included in this analysis were participants in the offspring cohort of the Framingham Heart Study, which began in 1971 with the recruitment of 5,124 men and women who were offspring and spouses of offspring of the original cohort and ranged in age from 5 to 70 years. The study design of the Framingham original and offspring studies has been described in detail (26). There were 3,532 participants in offspring study examination cycle 6 (1995–1998). A total of 3,378 (96%) of these participants underwent B-mode carotid ultrasonography; *APOE* genotype data were available for 2,723 participants (81%). The research protocol was approved by the Institutional Review Board of Boston University. All participants provided informed consent.

Carotid ultrasonography

Ultrasound studies were acquired and images analyzed according to a standard protocol (27, 28). Imaging was conducted using a high-resolution 7.5 MHz transducer for the common artery and a 5.0 MHz transducer for the internal carotid artery (Toshiba Medical System). Two images were obtained at the distal common carotid artery (CCA) and two in the proximal 2 cm of the internal carotid artery (ICA). A single trained sonographer made all of the measurements and was overread by one of the investigators (J.F.P.).

For each site, the maximal IMT measurements in the near and far walls were averaged. CCA and ICA IMT were defined as the mean of the maximal IMT measurements for the right and left sides. Images were gated to diastole. Data for the mean of the mean IMT measurements for CCA and ICA IMT were also calculated (9). Based upon 25 readings by two separate readers, intraclass correlation coefficients for the mean and maximum ICA and CCA IMT were 0.74, 0.74, 0.86, and 0.90, respectively.

A subjective estimate of ICA narrowing, graded as 0%, 1–24%, and 25–49%, was made by the sonographer when Doppler-derived peak systolic velocities in the ICA were <150 cm/s. ICA narrowing of hemodynamic significance (≥50%) was defined as present when peak systolic velocities in the ICA were ≥150 cm/s. For these analyses, the degree of stenosis was based on the more diseased ICA. The intrareader reproducibility of carotid stenosis (≥25%) from 159 paired readings on 79 studies was comparable to that reported in other studies (κ value of 0.69).

apoE genotype

Leukocyte DNA was extracted from 5–10 ml of whole blood as previously described (29). *APOE* genotyping was performed as described by Hixson and Vernier (30). A 244 bp sequence of the *APOE* gene including the two polymorphic sites was amplified by PCR in a DNA Thermal Cycler (PTC-100; MJ Research, Watertown, MA), using oligonucleotide primers F4 and F6 (30). Each reaction mixture was heated at 94°C for 2 min and followed by 35 cycles of amplification (94°C for 40 s, 62°C for 30 s, and 72°C for 1 min). The PCR products were digested with 5 units of *Hha*I, and the fragments separated by electrophoresis on an 8% polyacrylamide nondenaturing gel. After electrophoresis, the gel was treated with ethidium bromide for 30 min and DNA fragments were visualized by ultraviolet illumination.

To be consistent with previous publications, we defined three *APOE* genotype groups: *a*) the *APOE2* group includes those subjects carrying the *E2/E2* or *E3/E2* genotype; *b*) the *APOE3* group includes those carrying the *E3/E3* genotype; and *c*) the *APOE4* group includes those carrying the *E4/E3* or *E4/E4* genotype. Participants with the *E4/E2* genotype (n = 49) were excluded from the analyses.

Other clinical variables

Data regarding the medical history and physical examination were derived from the sixth examination cycle (31). Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (A-gen glucose test; Abbot, South Pasadena, CA). Diabetes was defined by fasting glucose ≥ 126 mg/dl or use of insulin or hypoglycemic medication. Plasma total cholesterol, HDL cholesterol, and triglycerides were measured as described previously (1). LDL cholesterol concentrations were estimated with the Friedewald equation. Subjects were classified as current cigarette smokers if they reported having smoked cigarettes during the previous year. Systolic and diastolic blood pressure values were the means of two physician-obtained measurements. The use of medications to control blood pressure was recorded. Height and weight were measured with the individual dressed in an examining gown and wearing no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity was defined as BMI ≥ 30 kg/m^2.

Statistical methods

Chi-square tests to compare proportions across groups and ANOVA to compare means of continuous variables across groups were used. Analysis of covariance was used to determine the carotid IMT mean across *APOE* genotypes, adjusting for covariates, separately for men and women. Logistic regression analysis was conducted to determine the association between genetic variants and the presence of ICA stenosis. Familial correlations were ac-

TABLE 1. Sample characteristics by sex

Variable	Men (n = 1,315)	Women (n = 1,408)
Age	59 (10)	59 (10)
APOE genotype (%)		
E2/E2	0.6	0.2
E3/E2	12.2	14.3
E3/E3	66.5	64.5
E4/E3	18.8	19.7
E4/E4	1.9	1.3
Systolic blood pressure (mm Hg)	130 (17)	128 (20)
Diastolic blood pressure (mm Hg)	77 (9)	74 (20)
Hypertension treatment (%)	31	26
Total cholesterol (mg/dl)	199 (41)	211 (38)
LDL cholesterol (mg/dl)	127 (31)	127 (34)
HDL cholesterol (mg/dl)	43 (12)	58 (16)
Log triglycerides	2.1 (0.2)	2.1 (0.2)
Dyslipidemic treatment (%)	15	11
Smoking (%)	14	16
Diabetes (%)	14	10
BMI (kg/m^2)	28.7 (4.4)	27.4 (5.8)
Postmenopausal (%)	—	78
Estrogen replacement therapy (%)	—	27
CCA IMT (mm)	0.77 (0.19)	0.71 (0.16)
ICA IMT (mm)	0.89 (0.57)	0.73 (0.49)
Carotid stenosis		
≥25%	24.9	15.6
≥50%	3.6	2.6

Data are presented as means (SD) or percentage. BMI, body mass index; CCA IMT, common carotid artery intimal-medial thickness; ICA IMT, internal carotid artery intimal-medial thickness.

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TABLE 2. Plasma lipids, carotid IMT, and carotid stenosis across *APOE* genotype groups in men and women

Variable	*APOE3*	*APOE2*	*APOE4*	*P*
Men	n = 874	n = 169	n = 272	
Total cholesterol (mg/dl)	200 (35)	193 (68)	201 (36)	0.083
LDL cholesterol (mg/dl)	129 (20)	113 (33)	129 (32)	<0.001
HDL cholesterol (mg/dl)	44 (12)	43 (13)	42 (12)	0.135
Log triglycerides	2.08 (0.24)	2.13 (0.32)	2.13 (0.22)	0.001
CCA IMT (mm)	0.78 (0.20)	0.77 (0.17)	0.77 (0.20)	0.827
ICA IMT (mm)	0.88 (0.55)	0.93 (0.64)	0.93 (0.60)	0.288
Carotid stenosis				
⩾25%	23.8	26.0	27.6	0.415
⩾50%	3.0	5.9	4.4	0.132
Women	n = 908	n = 204	n = 296	
Total cholesterol (mg/dl)	212 (37)	199 (38)	217 (38)	<0.001
LDL cholesterol (mg/dl)	129 (33)	111 (33)	133 (33)	<0.001
HDL cholesterol (mg/dl)	58 (15)	61 (19)	57 (16)	0.027
Log triglycerides	2.06 (0.23)	2.07 (0.23)	2.07 (0.23)	0.467
CCA IMT (mm)	0.71 (0.16)	0.70 (0.14)	0.72 (0.19)	0.440
ICA IMT (mm)	0.74 (0.49)	0.68 (0.42)	0.74 (0.52)	0.330
Carotid stenosis				
⩾25%	16.0	10.3	17.9	0.052
⩾50%	2.9	0.5	3.4	0.106

Data are presented as means (SD) or percentage.

counted for using Proc Genmod in SAS (version 8.0). A two-tailed $P < 0.05$ was considered statistically significant. To determine the association between *APOE* genotype and carotid atherosclerosis, we first explored the *APOE* genotype global effect, and afterward, all of the possible differences between groups were explored adjusting for multiple comparisons by the Scheffé method.

We also performed a meta-analysis of the data reported in published studies assessing the association between *APOE* genotype and carotid atherosclerosis. We included those studies with a community-based sampling, using the Review Manager program (version 4.2) (32). Random and fixed models were fitted. The meta-analysis was stratified by sex.

RESULTS

Characteristics of the sample stratified by sex are presented in **Table 1**. The relative frequencies of *APOE* alleles were 0.08, 0.80, and 0.12 for alleles *E2*, *E3*, and *E4*, respectively, and the frequencies were consistent with Hardy-Weinberg equilibrium. After excluding the *APOE E4/E2* genotype (n = 49; 1.77%), the frequency of *APOE* genotypes was 0.4, 13.3, 65.4, 19.3, and 1.6% for *E2/E2*, *E3/E2*, *E3/E3*, *E4/E3*, and *E4/E4*, respectively. The mean carotid IMT and the prevalence of carotid stenosis were higher in men.

In both men and women, LDL cholesterol in the *APOE2* group was lower than in the *APOE3* and *APOE4* groups. Moreover, in men, triglycerides were higher in the *APOE4* group than in the *APOE2* group, whereas in women, HDL cholesterol in the *APOE2* group was higher than in the *APOE4* group. No other statistically significant differences were observed. These lipid differences were consistent with previously reported results in the Framingham Study (1).

We analyzed the relation between carotid IMT and carotid stenosis. The Spearman correlation coefficients between carotid stenosis (defined as 0, 1–24, 25–49, or ⩾50%) and CCA IMT or ICA IMT were 0.49 and 0.52 in men and 0.43 and 0.45 in women, respectively. Moreover, the covariate-adjusted ICA IMT means (SEM) in those subjects without and with carotid stenosis ⩾ 25% were 0.74 mm (0.01) and 1.32 mm (0.04) in men and 0.63 mm (0.01) and 1.21 mm (0.05) in women, respectively.

There was no difference in the carotid IMT or in the proportion of participants with carotid stenosis across *APOE* genotype groups (**Table 2**).

TABLE 3. Age-adjusted and multivariable-adjusted mean carotid IMT across *APOE* genotype groups in men and women

Variable	*APOE3*	*APOE2*	*APOE4*
Men			
CCA IMT			
Model 1	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)
Model 2	0.78 (0.01)	0.77 (0.01)	0.77 (0.01)
Model 3	0.78 (0.01)	0.78 (0.01)	0.77 (0.01)
ICA IMT			
Model 1	0.87 (0.02)	0.95 (0.05)	0.93 (0.04)
Model 2	0.87 (0.02)	0.95 (0.05)	0.92 (0.03)
Model 3	0.88 (0.02)	0.95 (0.05)	0.91 (0.03)
Women			
CCA IMT			
Model 1	0.71 (0.01)	0.69 (0.01)[a]	0.72 (0.01)
Model 2	0.71 (0.01)	0.69 (0.01)[a]	0.72 (0.01)
Model 3	0.71 (0.01)	0.70 (0.01)	0.72 (0.01)
ICA IMT			
Model 1	0.73 (0.02)	0.66 (0.03)[b]	0.74 (0.03)
Model 2	0.73 (0.02)	0.67 (0.03)[b]	0.74 (0.03)
Model 3	0.73 (0.02)	0.68 (0.03)	0.73 (0.03)

Data are presented as mm (SEM). Multivariate models were as follows: model 1, adjusted for age and familial correlation; model 2, further adjusted for systolic blood pressure, hypertension treatment, smoking, diabetes, BMI, menopausal status (for women), and use of estrogen replacement therapy (for women); model 3, further adjusted for HDL and LDL cholesterol, triglycerides, and dyslipidemic treatment.

[a] $P \leqslant 0.01$ compared with *APOE4* genotype.

[b] $P < 0.05$ compared with *APOE3* genotype.

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TABLE 4. Age-adjusted and multivariable-adjusted odds ratios for carotid stenosis (≥25%) by *APOE* genotype in men and women

Variable	Model 1	Model 2	Model 3
Men			
APOE3 (n = 874)	1	1	1
APOE2 (n = 169)	1.24 (0.79, 1.94)	1.22 (0.77, 1.95)	1.22 (0.74, 2.00)
APOE4 (n = 272)	1.25 (0.90, 1.75)	1.26 (0.89, 1.78)	1.15 (0.81, 1.63)
Women			
APOE3 (n = 908)	1	1	1
APOE2 (n = 204)	0.54 (0.33, 0.87)	0.49 (0.30, 0.81)	0.50 (0.30, 0.86)
APOE4 (n = 296)	1.26 (0.87, 1.83)	1.24 (0.84, 1.83)	1.18 (0.80, 1.76)

Multivariate models were as follows: model 1, adjusted for age and familial correlation; model 2, further adjusted for systolic blood pressure, hypertension treatment, smoking, diabetes, BMI, menopausal status (for women), and use of estrogen replacement therapy (for women); model 3, further adjusted for HDL and LDL cholesterol, triglycerides, and dyslipidemic treatment.

We examined means of the different measurements of carotid IMT across *APOE* groups by sex after adjustment for age, familial correlation, and other covariates (**Table 3**). In men, no differences were observed in CCA or ICA IMT across *APOE* groups. In women, the CCA and ICA IMT in the *APOE2* group were lower than those of the *APOE4* and *APOE3* groups. This difference was statistically significant between *APOE2* and *APOE4* for CCA IMT and between *APOE2* and *APOE3* for ICA IMT. When we included plasma lipids in the statistical model, the associations were no longer statistically significant, suggesting that the effects were, at least in part, mediated through plasma lipids (Table 3, model 3).

Adjusted odds ratios of *APOE* genotype groups for ICA stenosis ⩾ 25% are shown in **Table 4**. In men, no statistically significant association between *APOE* genotype and carotid stenosis was observed. In women, the *APOE2* group was associated with a lower prevalence of carotid stenosis compared with the *APOE3* and *APOE4* groups. This association remained statistically significant after adjusting for lipid levels. The interaction between *APOE2* and sex was statistically significant ($P = 0.007$). The same analyses were performed using a cutoff point of 50% for carotid stenosis; odds ratios were of similar magnitude with wider confidence intervals (data not shown).

A statistically significant modifying effect of diabetes was noted in the association between *APOE* genotype and ICA IMT in men ($P = 0.044$) but not in women. The ICA IMT across *APOE* groups in men stratified by diabetes is presented in **Fig. 1**. No differences across groups were observed in nondiabetic men. However, in diabetic men, the ICA IMT of the *APOE4* group was statistically significantly higher than that of the *APOE3* and *APOE2* groups (Fig. 1). After adjusting for lipids, the interaction was marginally not statistically significant ($P = 0.079$), although the ICA IMT of the *APOE4* group (1.18 mm) was still significantly higher than that of the *APOE3* group (0.89 mm) ($P = 0.049$). Diabetes was associated with higher IMT only in *APOE4* men ($P = 0.011$), whereas in women, the association between diabetes and carotid IMT was significant ($P = 0.009$) independently of the *APOE* genotype.

There was no evidence for a significant interaction of smoking, hypertension, or obesity on the association between *APOE* genotype and either carotid IMT or stenosis. For all of the above analyses, we observed similar results when we used the mean of the mean IMT instead of the mean of the maximum IMT.

DISCUSSION

In this community-based study, we observed that associations between *APOE* genotype and carotid atherosclerosis measures are modified by diabetes and by sex. In women, the *E2* allele was associated with lower CCA and ICA IMT and lower prevalence of carotid stenosis. The association with IMT was mainly mediated through plasma lipids, but the association with ICA stenosis was still significant even after adjusting for lipid levels. The *E4* allele was associated with higher ICA IMT in diabetic men.

The inverse association between the *E2* allele and carotid atherosclerosis measures observed in women is consistent with previous research in the Framingham Heart Study showing a protective association between the *E2* al-

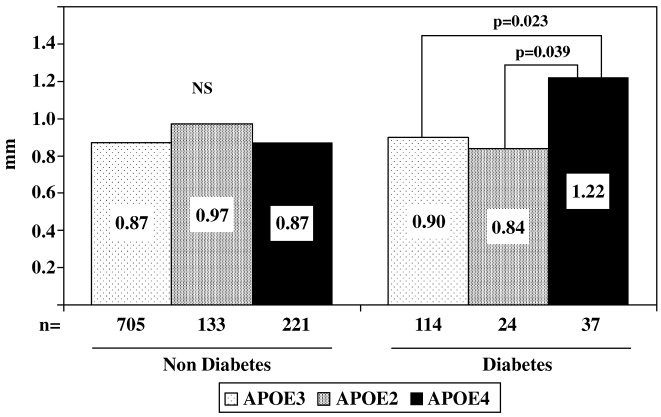

Fig. 1. Multivariate adjusted (age, familial correlation, systolic blood pressure, hypertension treatment, smoking, and body mass index) mean internal carotid artery (ICA) intimal medial thickness across *APOE* genotype groups in diabetic and nondiabetic men. The *P* value for the diabetes-*APOE* genotype interaction in ICA intimal medial thickness = 0.044.

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TABLE 5. *APOE2* and *APOE4* odds ratios and their 95% CIs for carotid atherosclerosis in different studies (the *APOE3* group is the reference group), and overall association estimated by meta-analysis using random effects models, excluding (Overall-a) and including (Overall-b) the results of this study

	APOE2 vs. *APOE3*			*APOE4* vs. *APOE3*		
Study	Odds Ratio (95% CI)	Overall Effect *P*	Heterogeneity *P*	Odds Ratio (95% CI)	Overall Effect *P*	Heterogeneity *P*
Men						
Slooter et al. (15)	1.13 (0.85. 1.51)			1.03 (0.82, 1.28)		
Beilby et al. (11)	1.08 (0.61, 1.92)			1.18 (0.77, 1.81)		
Overall-a	1.12 (0.86, 1.45)	0.39	0.89	1.06 (0.87, 1.29)	0.59	0.58
Framingham	1.13 (0.77, 1.64)			1.22 (0.90, 1.66)		
Overall-b	1.12 (0.91, 1.39)	0.29	0.99	1.10 (0.93, 1.30)	0.26	0.64
Women						
Slooter et al. (15)	1.03 (0.82, 1.29)			0.97 (0.80, 1.17)		
Beilby et al. (11)	0.50 (0.25, 1.00)			0.82 (0.51, 1.32)		
Overall-a	0.77 (0.39, 1.54)	0.47	0.05	0.95 (0.79, 1.13)	0.54	0.52
Framingham	0.60 (0.37, 0.98)			1.15 (0.81, 1.62)		
Overall-b	0.73 (0.45, 1.15)	0.19	0.04	0.98 (0.84, 1.15)	0.85	0.50

CI, confidence interval. Carotid atherosclerosis definitions are as follows: Slooter et al. (15), focal widening relative to adjacent segments with protrusion into the lumen; Beilby et al. (11), clearly identified area of focal increased thickness (>1 mm); Framingham, carotid stenosis ⩾ 25%.

lele and prevalent CVD in women (1). We performed a meta-analysis to assess the consistency of our results with previously published data. In the meta-analysis of data from women, there was no significant overall association between *APOE* genotype and carotid atherosclerosis determined as either the continuous measure of CCA IMT or a dichotomous variable (**Fig. 2**, **Table 5**).

In men, we observed a direct but nonsignificant association between the *E2* allele and carotid stenosis. The *E2* allele was not significantly associated with carotid IMT, al-

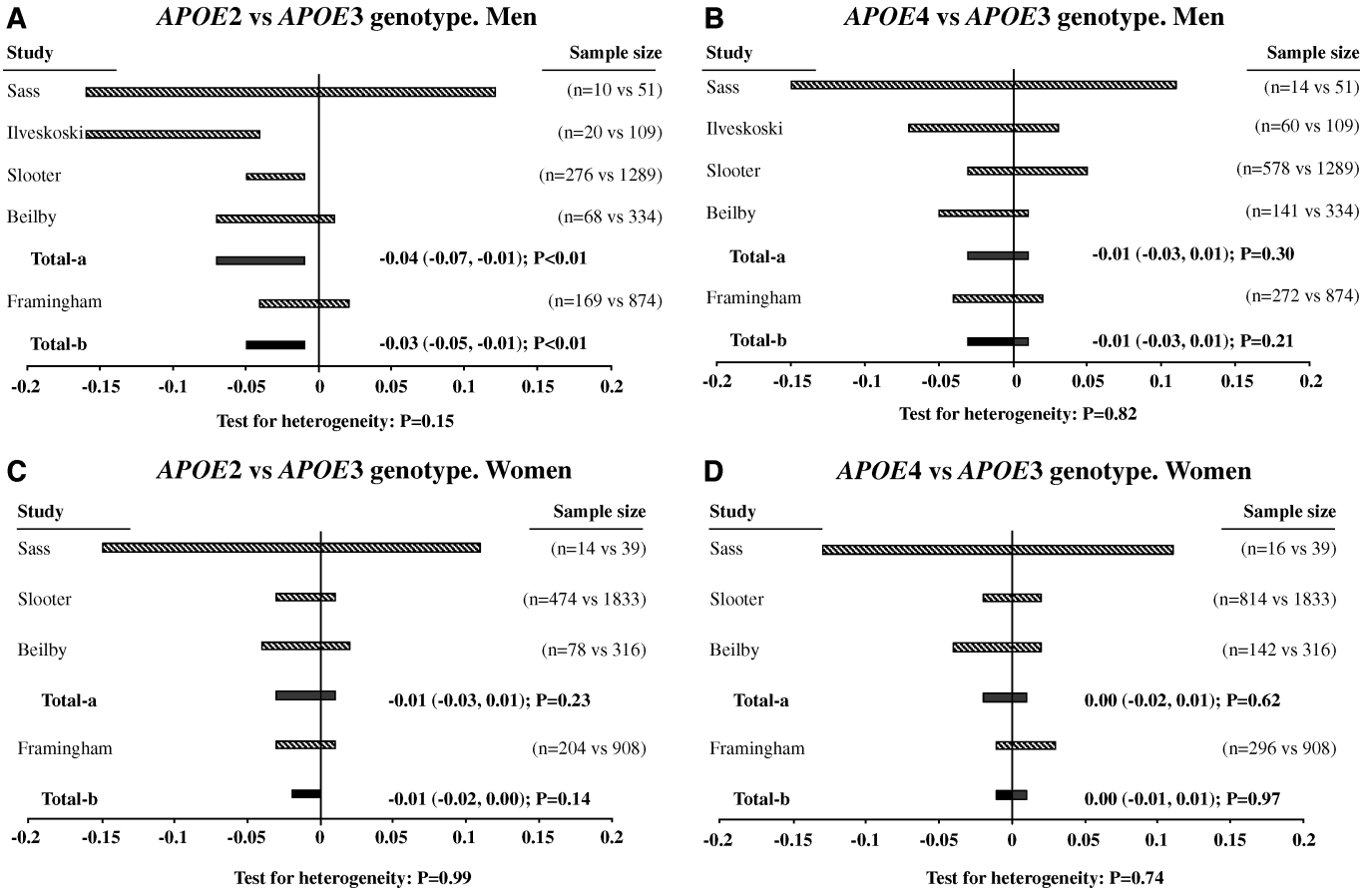

Fig. 2. Common carotid artery intimal medial thickness weighted mean difference between *APOE* genotype groups (*APOE2* and *APOE4* vs. *APOE3*) in men and women. Meta-analysis results using random effect models.

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though it tended to be associated with higher ICA IMT and with lower CCA IMT. In the meta-analysis for men, there was an inverse and significant association between the *E2* allele and CCA IMT (Fig. 2) and a direct and nonsignificant association between the *E2* allele and carotid atherosclerosis as a dichotomous variable (Table 5). These paradoxical results are consistent with previous observations that *APOE* genotype (24) and classic risk factors (33) are associated with a segment-specific carotid IMT and support the hypothesis that different pathophysiologic mechanisms may explain the observed differences between ICA and CCA. Turbulent blood flow in regions of arterial bifurcation, such as the ICA, may predispose to lipid deposition and thrombosis (34). Laminar blood flow, as seen in the CCA, is mainly related to wall shear stress. If the wall shear stress decreases, the time that blood comes in contact with endothelial cells increases and may enhance the delivery of atherogenic particles to the arterial wall (35). These different mechanisms may underlie the observed disparity in the associations between *APOE* genotype and CCA and ICA IMT.

The modifying effect of sex on the association between *APOE2* and carotid atherosclerosis is not well understood, although some plausible explanations exist. Carriers of the *E2* allele present a higher concentration of atherogenic triglyceride-rich lipoprotein (TRL) (36). In men, the atherogenic potential associated with TRL may be expressed in subjects with lower HDL cholesterol, whereas in women, this atherogenic effect may be counterbalanced by their higher HDL cholesterol levels. The higher postprandial TRL response present in men compared with women (37) may also play a role in this sex difference. Additionally, estrogens upregulate APOE gene expression, resulting in higher apoE production and a more efficient remnant clearance (38).

We did not observe a significant association between the *E4* allele and carotid atherosclerosis. The association between the *E4* allele and higher carotid IMT has been observed in several studies (13, 22–24), although others have failed to find such an association (11, 15–22). In the meta-analysis, the *E4* allele was not associated with either carotid IMT or carotid atherosclerosis (Fig. 2B–D, Table 5).

In our study, although it was associated with a nonsignificant increase in ICA IMT in men, the *E4* allele was significantly associated with higher carotid IMT only in diabetic men. This observation has been previously reported (39) and suggested in patients with peripheral arterial disease (40), for exercise-induced silent myocardial ischemia (41), and for cognitive decline (42). The combination of the *E4* allele and diabetes could increase the concentration of small, dense LDLs and inflammatory biomarkers, thereby increasing the risk of carotid atherosclerosis. We have recently documented in the same population an interaction between *APOE* genotype and obesity on fasting insulin and glucose levels in men (43). Taken together, these data suggest that the *E4* allele risk may be much higher in men with the metabolic syndrome.

Although we examined a priori for plausible interactions, it is possible that our observed interaction is falsely positive, given the multiple comparisons examined during our analyses.

There are several potential explanations for the differences in the presence and magnitude of associations between *APOE* genotype and carotid atherosclerosis measurements across studies (44). Differences in the study population characteristics, methods of carotid atherosclerosis measurement (CCA or ICA IMT, carotid plaque, carotid stenosis), statistical analyses (sex stratification, interaction terms analyzed), and small sample sizes and differing prevalences of diabetes may contribute to the reported variability. Diet (45) and exercise (46) also modify the association between *APOE* genotype and plasma lipid levels. Our study is conducted in men and women from a community-based cohort with a typical distribution of risk factor profiles and prevalence of diabetes. Differences with other populations in these environmental factors may explain some of the observed variation.

Some limitations of our study exist. Misclassification bias may have occurred in the subjective evaluation of carotid stenosis, although we consider that this misclassification would be random and lead to a conservative bias.

Conclusion

APOE2 genotype was associated with lower carotid atherosclerosis in women, supporting the previous observation of the protective role of this genotype on CVD only in women (1). The mechanisms of this sex interaction remain to be determined. *APOE4* genotype was significantly associated with a higher ICA IMT only in diabetic men. Although this observation should be considered with caution, the different association between *APOE* genotype and atherosclerosis in diabetic and nondiabetic patients could have prognostic and therapeutic implications.

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