

## Short Report

# Apolipoprotein E polymorphism and preclinical carotid artery disease in untreated hypertensive men

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**Methods** The apolipoprotein E (apoE) polymorphism was evaluated alone, and in interaction with smoking status, in predicting carotid intima–medial thickness and plaque among 182 untreated, hypertensive, white men (mean age  $56 \pm 8.9$  years).

**Results** After covariate adjustment (age, education, smoking, body mass index, systolic and diastolic blood pressure, glucose, total:high-density lipoprotein), apoE genotype (no  $\epsilon 4$  versus any  $\epsilon 4$ ) predicted mean ( $\beta = -0.139$ ,  $P = 0.023$ ) and maximum ( $\beta = -0.138$ ,  $P = 0.028$ ) intima–medial thickness, as well as plaque ( $\beta = -0.744$ ;  $P = 0.072$ ). Tests of a genotype-x-smoking interaction were non-significant.

**Conclusions** Mean intima–medial thickness was greater in men with the 3/3 than  $\epsilon 4$  genotypes (2/4 3/4 4/4), suggesting genetic risk for carotid atherosclerosis may be conferred by the  $\epsilon 3$ , rather than the  $\epsilon 4$ , allele. *Eur J Cardiovasc Prev Rehabil* 13:98–100 © 2006 The European Society of Cardiology

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## Introduction

The apolipoprotein E (apoE) polymorphism putatively promotes atherogenesis through its role in lipid transport and metabolism, as well as immune-mediated processes affecting the arterial wall [1]. The  $\epsilon 4$  allele predicts risk for coronary artery disease [2], though study findings of its association with carotid artery atherosclerotic disease (CAAD) are mixed. Greater CAAD has been observed both in individuals possessing at least one  $\epsilon 4$  allele [3–6] and those homozygous for the  $\epsilon 3$  allele [5,7–9] compared to persons of other genotypes. Moreover, other reports

implicate the  $\epsilon 2$  allele [10,11], or show no associations at all [12,13].

Inconsistent results may be explained by the interaction of the apoE  $\epsilon 4$  allele with other cardiovascular risk factors. Djousse *et al.* [14] reported that current smokers with at least one  $\epsilon 4$  allele have greater CAAD than subjects with either risk factor alone. Interestingly, in Karvonen *et al.* [15], the apoE  $\epsilon 4$  allele, in interaction with smoking status, predicted CAAD, but only among men who were also hypertensive. Here, we evaluated the relationship of the apoE polymorphism to CAAD [intima–medial thickening (IMT) and plaque] among hypertensive men who were untreated, thus mitigating potential confounding due to antihypertensive interventions. We also tested for potential interactive effects of apoE genotype and smoking status on CAAD.

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## Methods

### Participants

One hundred and eighty-two white men (ages 40–70 years) were derived from the University of Pittsburgh Reactivity and Cardiovascular Risk Trial (REACT). All were untreated hypertensive men, confirmed by two resting blood pressure measurements averaging 140–180 mmHg systolic blood pressure (SBP) or 90–110 mmHg diastolic blood pressure (DBP) on each of two evaluations; 87.5% had no history of antihypertensive medication and median treatment length was 4 months among the remainder. See Muldoon *et al.* [16] for sample details. The IRB approved the study and informed written consent was obtained from all participants.

### Carotid atherosclerosis

B-mode ultrasonography (Toshiba SSA-270 scanner; Nasu, Japan) was used to assess mean and maximum IMT and plaque score. Measurements were derived from digitized images of the right and left common carotid artery, carotid bifurcation, and the first centimeter of the internal carotid artery [16]. For analysis, average and maximum IMT values were normalized by reciprocal transformations ( $1/Y$ ) and plaque scores dichotomized (0 versus 1+ plaque).

### Genotyping

The apoE gene locus (three common alleles:  $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) was genotyped using polymerase chain reaction [17]. The proportion of alleles was  $\epsilon 2 = 7.14\%$ ,  $\epsilon 3 = 80.22\%$ , and  $\epsilon 4 = 12.64\%$ . Frequencies by genotype were  $2/2 = 1$ ,  $2/3 = 22$ ,  $2/4 = 2$ ,  $3/3 = 120$ ,  $3/4 = 30$ , and  $4/4 = 7$ ; distributions conformed to Hardy–Weinberg equilibrium ( $\chi^2(5) = 7.67$ , NS).

## Results

### Sample characteristics

On average, subjects were  $56 \pm 8.9$  years old with  $15.2 \pm 2.7$  years of education. Body mass index averaged  $28.3 \pm 3.1$  and the majority (58.2%) endorsed current/past smoking. SBP averaged  $149 \pm 10.3$  mmHg and DBP  $92 \pm 7.4$  mmHg. Mean and maximum IMT were  $0.91 \pm 0.16$  and  $1.20 \pm 0.25$  mm, respectively, with 68.7% having plaque scores of  $\geq 1$ . Participants with an  $\epsilon 4$  allele (versus no  $\epsilon 4$  allele) had higher DBP and total:high-density lipoprotein (HDL) ratio ( $P = 0.041$ ,  $P = 0.095$ , respectively) but lower mean and maximum IMT ( $P = 0.050$ ,  $P = 0.104$ , respectively), as well as fewer plaque occurrences scored  $\geq 1$  ( $P = 0.062$ ).

### Multivariate analysis

The relationship of apoE genotype (0 = no  $\epsilon 4$ , 1 = any  $\epsilon 4$  allele) to mean and maximum IMT and plaque score (0 = no plaque, 1 = plaque score of 1+) was assessed by linear and logistic multiple regression, respectively. Covariates [age, education, BMI, SBP, DBP, smoking, (0 = never, 1 = past/current), total:high-density lipoprotein HDL ratio, and fasting glucose] were entered

simultaneously on step one and apoE genotype on step two of each regression equation.

In linear multiple regression, covariates accounted for 37.6 and 34.6% of the variance in mean and maximum IMT, respectively. Having no  $\epsilon 4$  allele predicted greater mean ( $\beta = -0.139$ ,  $P = 0.023$ ) and maximum IMT ( $\beta = -0.13$ ,  $P = 0.028$ ), accounting for an additional 1.9 and 1.8% of the variance, respectively. Unstandardized regression coefficients (b) show the absence of the  $\epsilon 4$  allele to correspond to an increase of 0.063 mm in mean and 0.054 mm in maximum IMT. In multiple logistic regression, having no  $\epsilon 4$  allele also predicted plaque score, albeit marginally ( $P = 0.072$ ). The odds ratio indicates that in the absence of an  $\epsilon 4$  allele, the likelihood of exhibiting a plaque score of 1 or higher is increased by 48%. The interaction of apoE genotype and smoking was non-significant ( $P > 0.05$ ).

### Secondary analysis

Analysis of covariance showed a main effect of apoE genotype [coded:  $\epsilon 2$  (2/2 2/3);  $\epsilon 3$  (3/3);  $\epsilon 4$  (2/4 3/4 4/4)] for mean ( $F = 3.30$ ,  $P = 0.039$ ) and maximum IMT ( $F = 2.57$ ,  $P = 0.080$ ). Bonferroni-adjusted pairwise comparisons showed significant differences between  $\epsilon 3$  ( $M = 0.932$  mm) and  $\epsilon 4$  ( $M = 0.875$  mm), but not  $\epsilon 2$  ( $M = 0.890$  mm) genotypes; results were similar for maximum IMT. Plaque did not differ across genotypes ( $\chi^2(2) = 3.55$ ,  $P = 0.17$ ).

## Discussion

Evidence is mixed regarding which allele of the apoE polymorphism may enhance CAAD risk [3–13]. Here, the absence, rather than the presence, of the  $\epsilon 4$  allele was associated with mean and maximum IMT significantly and with plaque score marginally, after covariate adjustment. Follow-up comparisons showed subjects with the 3/3 genotype to have the greatest carotid IMT and to differ significantly from subjects possessing any  $\epsilon 4$  allele. In contrast to Karvonen *et al.* [15], the relationship of apoE genotype to CAAD did not vary by smoking status.

The potential role of the  $\epsilon 3$  allele in CAAD risk was unanticipated. The  $\epsilon 4$  allele has been most consistently related to higher total and low-density lipoprotein cholesterol [18], as well as coronary artery disease [2], and was considered the best candidate for association with CAAD here. Although cholesterol was elevated minimally among subjects with any  $\epsilon 4$  (versus no  $\epsilon 4$ ) allele, carotid IMT was greater in the latter group, suggesting effects of the apoE polymorphism on lesion development may differ between the coronary and carotid arteries. Emergence of the  $\epsilon 3$  allele, however, is not entirely anomalous, as others have also shown the 3/3 genotype associated with greater carotid IMT, compared with subjects with  $\epsilon 2$  and  $\epsilon 4$  alleles [7,9].

Results also failed to confirm the hypothesis that other cardiovascular risk factors (i.e. hypertension, smoking) promote CAAD as a function of allelic variation in the apoE polymorphism. Notably, because our participants were untreated for their hypertension, this sample may differ from previously studied groups, in which unspecified sample characteristics (e.g. antihypertensive medication) may modify the relationship between the apoE polymorphism and CAAD. In sum, the current study offers further support for an association of the apoE polymorphism with CAAD, but suggests consideration of the  $\epsilon 3$ , rather than the  $\epsilon 4$ , as the 'risk' allele.

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