

# Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study

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## Abstract

**Background:** Apolipoprotein (apo) E is a constituent of lipoproteins with considerable variation due to cysteine–arginine exchanges. The apo E4 (Arg112-Cys) polymorphism has been associated with dementia and hypercholesterolemia. We investigated the relation of APOE genotype to cardiovascular disease (CVD) in the Framingham Offspring Study. **Methods and results:** DNA was isolated from 3413 study participants and APOE genotypes were determined utilizing the polymerase chain reaction and restriction isotyping. In the entire group of subjects, 20.7% had apo E4/4 or E3/4 (Group E4); 14.1% had apo E2/2 or E2/3 (Group E2) and 63.9% had the apo E3/3 genotype (Group E3). Subjects with E2/4 (1.3%) were excluded. Period prevalence of CVD between examinations 1 and 5 (1971–1994) (366 events) was related to APOE genotype. Age adjusted period prevalence of CVD in men was 18.6% for Group E4, 18.2% for Group E2 and 12.7% for Group E3 ( $P = 0.004$ ); while in women these rates were 9.9, 4.9, and 6.6%, respectively ( $P = 0.037$ ). After adjustment for non-lipid risk factors the relative odds for CVD in Group E2 men was 1.79 ( $P = 0.0098$ ) and in Group E4 it was 1.63 ( $P = 0.0086$ ) compared with the Group E3; while in Group E4 women it was 1.56 ( $P = 0.054$ ). After adjustment for all CVD risk factors, the relative odds in Group E2 men was 1.94 ( $P = 0.004$ ) and in Group E4 men it was 1.51 ( $P = 0.0262$ ). **Conclusions:** The presence of the apo E2 or apo E4 alleles in men is associated with significantly greater CVD risk. This genotypic information may help to identify individuals at increased risk for CVD events. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Apolipoprotein E; Cardiovascular disease; Lipoproteins; Genetic variation; Population studies

## 1. Introduction

Cardiovascular disease (CVD), including coronary heart disease (CHD) and stroke, is the major cause of death and disability in developed countries. Major CVD risk factors include advancing age, male sex, hypertension, smoking, diabetes, elevated total serum low-density lipoprotein (LDL) cholesterol ( $\geq 160$  mg/dl, 4 mmol/l), and decreased high density lipoprotein (HDL) cholesterol ( $< 35$  mg/dl, 0.9 mmol/l) [1]. An

additional important risk factor is family history of premature coronary disease [1,2].

A variety of familial lipoprotein disorders have been associated with CVD, and some of them, such as lipoprotein(a) excess and familial hypercholesterolemia (FH) have been linked to specific loci [3–6]. However, some of these disorders are rare and have a modest effect over the CHD risk in the population at large. More common mutations in the general population include those at the APOE locus. Numerous population studies have clearly implicated APOE genetic variation as a major modulator of LDL cholesterol [7–12].

Apolipoprotein E is a protein constituent of both triglyceride-rich lipoproteins (TRL) as well as HDL,

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which plays an important role in liver uptake of TRL remnants. APOE has three common alleles known as  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$ . The common  $\epsilon 3$  allele contains a cysteine at residue 112 and an arginine at residue 158 [13]. The variant  $\epsilon 4$  allele differs from  $\epsilon 3$  in that it contains an arginine at residue 112, whereas the variant  $\epsilon 2$  allele differs from  $\epsilon 3$  in that it contains a cysteine at residue 158 [13]. Many studies assessing the role of APOE genetics on plasma lipids have shown that the presence of the  $\epsilon 4$  allele is associated with elevations in LDL cholesterol, while the presence of  $\epsilon 2$  is associated with decreased levels of LDL cholesterol [7–12]. In addition, a meta-analysis has shown that the apo E phenotype is associated with triglyceride levels [14]. Moreover, some studies have reported that the  $\epsilon 4$  allele is associated with CHD [15] although most of these have been carried out in male subjects. Our purpose was to assess the relation between APOE genotype, gender and CVD prevalence in a population based sample: the Framingham Offspring Study.

## 2. Methods

### 2.1. Subjects

The details of the design and methods of the Framingham Heart Study have been presented elsewhere [16]. Starting in 1948, 5209 subjects between the ages of 28 and 62 were enrolled in the cohort study, and beginning in 1971, a total of 5124 of their children and the children's spouses were enrolled [17]. Blood samples for DNA were collected between 1987 and 1991. In the Framingham Offspring Study, lipid profiles, information on CVD risk factors and APOE genotypes were available for 1671 men (age range, 11–65 years; mean age  $\pm$  S.D. at the first examination (1971–1978),  $35.6 \pm 10.3$  years) and 1742 women (age range, 9–62 years; mean age  $\pm$  S.D.,  $35.6 \pm 10.0$  years). Nearly all subjects were Caucasian. Data on smoking, blood pressure, height, weight, and diabetes were obtained on these subjects as previously described [16,17]. CVD included the presence of CHD, stroke, peripheral vascular disease, and congestive heart failure. CHD included the presence of myocardial infarction, angina pectoris, coronary insufficiency and coronary death. The diagnosis of CVD was based on clinical information obtained at the time of Framingham Heart Study visits, records from personal physicians, and hospitalizations, as previously described [16,17]. All suspected CVD events were reviewed by a panel of three physicians to ascertain the presence of CVD. The study was approved by all the institutional review committees and the subjects gave informed consent.

### 2.2. Apolipoprotein E genotyping

Leucocyte DNA was extracted from 5–10 ml of whole blood as previously described [18]. APOE genotype was performed as described by Hixson and Vernier [19]. A 244-bp sequence of the APOE gene including the two polymorphic sites was amplified by polymerase chain reaction (PCR) in a DNA Thermal Cycler (PTC-100, MJ Research, Watertown, MA), using oligonucleotide primers F4 and F6 [19]. 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 U 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 UV illumination.

### 2.3. Other covariate measures

Height and weight for each participant were measured with the individual dressed in an examining gown and wearing no shoes. Body mass index (BMI) was expressed as weight (kg) divided by the square of height ( $m^2$ ). Physician determined blood pressure was measured in the left arm after the individual had been seated for at least 5 min. Cigarette use was based on smoking status in the year prior to the examination and included assessment of the number of cigarettes smoked per day. Individuals were considered to have diabetes if they met one of the following criteria: fasting glucose  $\geq 140$  mg/dl at the current examination, or current use of oral hypoglycemic agent or insulin preparations.

Plasma lipids were measured after a 12- to 14-h overnight fast, using blood collected in tubes containing 0.1% EDTA. Plasma was subjected to ultracentrifugation at density 1.006 g/ml for 18 h [20]. Plasma HDL-cholesterol was measured after precipitation of plasma apo B-containing lipoproteins as previously described [20]. Plasma total cholesterol, 1.006 g/ml infranatant cholesterol, HDL cholesterol and triglyceride levels were measured as previously described [20]. LDL cholesterol was calculated by difference (1.006 g/ml infranatant cholesterol minus HDL cholesterol) using standard Lipid Research Clinic procedures [21]. Coefficients of variation between runs for all lipid assays were less than 5%.

### 2.4. Statistical analyses

Period prevalence for vascular disease was estimated for subjects free of disease at examination 1 (1971–1978). Events occurring during the subsequent 15- to 20-year period up to examination 5 (1991–1994) were used for period prevalence. We focus on period preva-

lence since only those subjects who had APOE genotype determined at exams 4 and 5 were included in this analysis. To evaluate the effect of APOE genotype, subjects were categorized into three groups: (1) 3/3 (2) 2/3 or 2/2 and (3) 3/4 or 4/4. Subjects with the 2/4 genotype (1.3%) were excluded a priori from our analyses. Two endpoints were considered: CVD and CHD. Separate analyses were conducted for men and women.

For each sex, age-adjusted period prevalences for CVD and CHD were calculated for each genotype group by the direct method. For these calculations, subjects were free of CVD at examination 1 for the CVD endpoint (and free of CHD for the CHD endpoint). The differences in the age-adjusted period prevalences among the genotype groups were evaluated by the Mantel–Haenszel statistic, stratified for age at examination 1 (5–14, 15–24, 25–34, 35–44, 45–54, 55+). To evaluate the effect of APOE genotype independent of other cardiovascular risk factors, logistic regression models were applied to obtain odds ratios for the genotype groups. Crude and multivariate-adjusted odds ratios were calculated. To adjust for potential confounders, the mean of the measures from examinations 1–5 were used for systolic blood pressure, number of cigarettes smoked per day, BMI, HDL cholesterol and LDL cholesterol. If a subject developed CVD or CHD, the mean of these measures up to the examination of the event was used. For dichotomous measures (diabetes, left ventricular hypertrophy, and hormone usage in women), the presence of the trait at any time over the follow up period (to examination 5 or to a CVD event) was used. For example, subjects who were diabetic at any point over the follow up period were classified as diabetic. All logistic regression analyses were adjusted for age at examination 1, diabetes status, number of cigarettes, left ventricular hypertrophy, systolic blood pressure, body mass index and

hormone usage in women. Logistic regression models were also applied, which adjusted for LDL cholesterol and HDL cholesterol in addition to the preceding list of covariates in order to evaluate the extent to which these potential confounding variables affected the associations.

Population attributable risk was calculated using the odds ratios from the logistic regression models and the prevalence of APOE genotypes.

### 3. Results

For the entire population the percentage of subjects with the various APOE genotypes were: 3/3, 63.9%; 3/4, 19.0%; 2/3, 13.7%; 4/4, 1.7%; 2/4, 1.3% and 2/2, 0.4%. The allele frequencies were  $\epsilon$ 3, 0.802;  $\epsilon$ 4, 0.119; and  $\epsilon$ 2, 0.079. No significant differences between men and women were observed with regard to either genotype or allele frequencies. The relative frequencies of  $\epsilon$ 2,  $\epsilon$ 3 and  $\epsilon$ 4 alleles were in Hardy–Weinberg equilibrium.

For the purpose of statistical analysis, this population was subdivided into three APOE genotype subgroups: (A) subjects carrying the  $\epsilon$ 2/ $\epsilon$ 2 or the  $\epsilon$ 2/ $\epsilon$ 3 genotypes (APOE2 group; 14.1%), (B)  $\epsilon$ 3/ $\epsilon$ 3 subjects (63.9%; APOE3 group) and (C) subjects with genotypes  $\epsilon$ 3/ $\epsilon$ 4 or  $\epsilon$ 4/ $\epsilon$ 4 (20.7%; APOE4 group). Due to their low frequency and the opposite effects on lipid levels of the  $\epsilon$ 2 and the  $\epsilon$ 4 alleles, subjects with the  $\epsilon$ 2/ $\epsilon$ 4 genotype (1.3%) were excluded from these groups and were not considered in the analyses.

Data on prevalence of CVD between examinations 1 and 5 unadjusted and adjusted for age are provided in Table 1 by APOE group for both men and women. In men the age adjusted CVD prevalence for the APOE2 (182.1/1000) and APOE4 (186.3/1000) groups were significantly ( $P = 0.004$ ) higher than in men within the

Table 1  
Period prevalence of cardiovascular disease by apo E group (examinations 2–5)

	Number of subjects	Total events	Mean age $\pm$ S.D. at first CVD event	Cumulative prevalence per 1000	Age adjusted cumulative prevalence per 1000
<i>Men</i> (1668)					
E2 Group <sup>a</sup>	216	40	55.1 $\pm$ 7.9	185.2	182.1
E3 Group <sup>b</sup>	1097	139	55.3 $\pm$ 10.5	126.7	127.4
E4 Group <sup>c</sup>	355	66	56.2 $\pm$ 9.3	185.9	186.3
<i>P</i> -value*			N.S.	0.005	0.004
<i>Women</i> (1739)					
E2 Group	253	13	53.8 $\pm$ 6.4	51.4	49.2
E3 Group	1112	74	53.6 $\pm$ 9.8	66.5	65.6
E4 Group	374	34	54.4 $\pm$ 7.5	90.9	99.4
<i>P</i> -value*			N.S.	0.130	0.037

<sup>a</sup> E2 group includes E2/2 and E2/3 subjects.

<sup>b</sup> E3 group includes E3/3 subjects.

<sup>c</sup> E4 includes E3/4 and E4/4 subjects.

\* *P*-values correspond to the differences in prevalence by apo E group for cardiovascular disease; Mantel–Haenzel statistics.

Table 2  
Estimated odds ratios for cardiovascular disease by apo E genotype group<sup>a</sup>

	Age-adjusted	Age and non-lipid CVD risk-adjusted <sup>b</sup>	Age and all CVD-risk-adjusted <sup>c</sup>
<i>Men</i> (1668)			
Apo E2 O.R. (95% CI)	1.65 (1.10, 2.50)	1.79 (1.15, 2.77)	1.94 (1.23, 3.04)
<i>P</i> -value	0.0165	0.0098	0.004
Apo E4 O.R. (95% CI)	1.61 (1.15, 2.27)	1.63 (1.13, 2.34)	1.51 (1.05, 2.18)
<i>P</i> -value	0.0061	0.0086	0.0262
<i>Women</i> (1739)			
Apo E2 O.R. (95% CI)	0.75 (0.40, 1.38)	0.79 (0.42, 1.48)	0.91 (0.47, 1.74)
<i>P</i> -value	0.3544	0.4560	0.7807
Apo E4 O.R. (95% CI)	1.54 (1.00, 2.38)	1.56 (0.99, 2.45)	1.48 (0.93, 2.34)
<i>P</i> -value	0.0521	0.0538	0.0944

<sup>a</sup> The apo E3 group was used as reference. E2 includes E2/2 and E2/3 subjects; E3 includes E3/3 subjects, and E4 includes E3/4 and E4/4 subjects; O.R., odds ratio; CVD, cardiovascular disease.

<sup>b</sup> Non-lipid CVD risk factors: age (at examination 1); diabetes, smoking, systolic blood pressure, body mass index and left ventricular hypertrophy, and in women use of estrogens.

<sup>c</sup> Includes in addition to non-lipid risk factors, mean LDL-C and HDL-C.

Table 3  
Period prevalence of coronary heart disease by apo E group (examinations 2–5)

	Number of subjects	Total events	Cumulative prevalence per 1000	Age adjusted cumulative prevalence per 1000
<i>Men</i> (1671)				
E2 Group <sup>a</sup>	218	29	133.0	131.2
E3 Group <sup>b</sup>	1098	111	101.1	101.6
E4 Group <sup>c</sup>	355	52	146.5	145.7
<i>P</i> -value*			0.044	0.045
<i>Women</i> (1742)				
E2 Group	254	10	39.4	37.4
E3 Group	1114	51	45.8	44.8
E4 Group	374	21	56.1	62.5
<i>P</i> -value*			0.587	0.302

\* *P*-values correspond to the differences in prevalence by apo E; Mantel–Haenszel statistics.

<sup>a</sup> E2 group includes E2/2 and E2/3 subjects.

<sup>b</sup> E3 group includes E3/3 subjects.

<sup>c</sup> E4 includes E3/4 and E4/4 subjects.

APOE3 group (127.4/1000). In women, the age adjusted prevalence in the APOE4 group was higher (99.4/1000) than in the APOE3 group (65.6/1000); whereas the CVD prevalence of those in the APOE2 group was lower (49.2/1000).

The relative odds for CVD, adjusted for age (at examination 1), and non-lipid risk factors [diabetes, smoking, systolic blood pressure, left ventricular hypertrophy, body mass index and in women use of estrogens], and age and all risk factors (including mean LDL-C and HDL-C) are shown in Table 2 for men and women for APOE2 and APOE4 groups as compared to the APOE3 group. The odds of CVD for men within the APOE2 group was 1.94-fold greater ( $P = 0.004$ ) than that for men within the APOE3 group after adjustment for all risk factors. For men within the APOE4 group, the odds of CVD compared to the

APOE3 group was 1.63 ( $P = 0.0086$ ) after age and non-lipid risk factor adjustment, and 1.51 ( $P = 0.0262$ ) after adjustment for all risk factors.

For women in the APOE2 group (Table 2), the odds ratios for CVD even with adjustments was consistently lower at 0.75, 0.79, and 0.91 as compared to the APOE3 group. However, these odds ratios did not differ significantly from 1.00. These patterns are different from those observed in men where the presence of the  $\epsilon 2$  allele was associated with higher estimated odds ratios. However, for the APOE4 group in women the pattern observed was similar to that seen in men with odds ratios of 1.56 ( $P = 0.0538$ ) and 1.48 ( $P = 0.0944$ ) with adjustment for various risk factors. The overall data indicate higher odds ratios for men with the  $\epsilon 2$  and  $\epsilon 4$  alleles and for women with the  $\epsilon 4$  allele than individuals within the APOE3 group.

Period prevalence for CHD (myocardial infarction, angina, coronary insufficiency and coronary death) between examinations 1 and 5 by APOE group are presented in Table 3. The same trends were observed as for CVD. Men within the APOE4 and APOE2 groups had higher age-adjusted CHD prevalence at 14.6 and 13.1% as compared to the APOE3 group at 10.2%. These differences were statistically significant ( $P = 0.045$ ). Women in the APOE4 group had CHD prevalence of 6.3% compared to 3.7 and 4.5% in the APOE2 and APOE3 groups, respectively. These differences did not reach statistical significance ( $P = 0.302$ ).

The odds ratios for CHD, adjusted for age (at examination 1) and non-lipid risk factors (diabetes, smoking, systolic blood pressure, left ventricular hypertrophy, body mass index and use of estrogens), and age and all risk factors (including mean LDL-C and HDL-C) are shown in Table 4 for men and women for APOE2 and APOE4 groups as compared with the APOE3 group. The odds ratio for CHD for men within the APOE2 group was 1.61 ( $P = 0.0577$ ) compared with men within the APOE3 group after adjustment for all risk factors. For men within the APOE4 group, the odds ratio for CHD compared to the APOE3 group was 1.55 ( $P = 0.0280$ ) after age and non-lipid risk factor adjustment, and 1.46 ( $P = 0.0584$ ) after adjustment for all risk factors.

For women in the APOE2 group (Table 4) the odds ratios for CHD were 0.85, 0.91, and 1.11 for age-adjusted, age and non-lipid CHD risk and all CHD risk-adjusted, respectively, as compared to the APOE3 group. These odds ratios did not differ significantly from 1.00. These trends are different from those observed in men where the presence of the APOE2 allele was associated with higher estimated relative odds. However, for the APOE4 group in women the pattern observed was similar to that seen in men with odds

ratios of 1.35 ( $P = 0.2815$ ), and 1.36 ( $P = 0.2802$ ) with adjustment for various risk factors. The overall data suggest a higher relative odds for men with the  $\epsilon 2$  and  $\epsilon 4$  alleles and in women with the  $\epsilon 4$  allele compared with the apo E3/3 genotype.

The population attributable risk (PAR%) was calculated using the following equation:

$$\text{PAR}\% = \text{Prev}(\text{OR} - 1) / [\text{prev}(\text{OR} - 1) + 1]$$

where Prev indicates prevalence, and OR is odds ratio. The calculated %PAR in men, after lipid adjustments, were 9.5% for the APOE4 group and 11.7% for the APOE2 group.

#### 4. Discussion

CVD accounts for a major proportion of the death rate and disability in our society. Family history of premature CHD, defined as CHD prior to age 55 in a male first-degree relative, or prior to age 65 in a female first-degree relative is an important CHD risk factor. Common familial disorders associated with premature heart disease include familial Lp(a) excess, familial combined hyperlipidemia, and familial dyslipidemia [1]. Additional disorders include familial hypoalphalipoproteinemia and familial hypercholesterolemia [1]. A subset of subjects with the latter disorder have clearly been found to have mutations at the LDL receptor locus, resulting in markedly delayed clearance of LDL particles from the bloodstream, with two- to- threefold elevations in LDL cholesterol in the plasma, and at least a 20-fold increased risk of age-adjusted CHD risk [5,6]. The incidence of individuals heterozygous for familial hypercholesterolemia is estimated to be one in 500 in the general population, yielding a population attributable CHD risk of about 5%. Familial combined

Table 4  
Estimated odds ratios for coronary heart disease by apo E genotype group<sup>a</sup>

	Age-adjusted	Age, and non-lipid CVD risk-adjusted <sup>b</sup>	Age and all CVD risk-adjusted <sup>c</sup>
<i>Men</i> (1671)			
Apo E2 O.R. (95% CI)	1.36 (0.86, 2.14)	1.50 (0.92, 2.42)	1.61 (0.99, 2.63)
<i>P</i> -value	0.1887	0.1003	0.0577
Apo E4 O.R. (95% CI)	1.54 (1.06, 2.22)	1.55 (1.05, 2.28)	1.46 (0.99, 2.15)
<i>P</i> -value	0.0221	0.0280	0.0584
<i>Women</i> (1742)			
Apo E2 O.R. (95% CI)	0.85 (0.42, 1.71)	0.91 (0.44, 1.86)	1.11 (0.53, 2.33)
<i>P</i> -value	0.6431	0.7925	0.7767
Apo E4 O.R. (95% CI)	1.38 (0.81, 2.36)	1.35 (0.78, 2.35)	1.36 (0.78, 2.38)
<i>P</i> -value	0.2363	0.2815	0.2802

<sup>a</sup> The apo E3 group was used as reference. E2 includes E2/2 and E2/3 subjects; E3 includes E3/3 subjects, and E4 includes E3/4 and E4/4 subjects; O.R., odds ratio; CVD, cardiovascular disease.

<sup>b</sup> Non-lipid coronary heart disease risk factors: age (at examination 1); diabetes, smoking, systolic blood pressure, body mass index and left ventricular hypertrophy, and in women use of estrogens.

<sup>c</sup> Includes in addition to non-lipid risk factors, mean LDL-C and HDL-C.

hyperlipidemia is a much more common cause of hypercholesterolemia; however, no clear mutations have been associated with this disorder. There is some evidence that some patients with this latter disorder may have various forms of heterozygous lipoprotein lipase deficiency [22]. All these inherited lipid abnormalities have a minimal effect over the lipid levels in populations. Conversely, variation at the APOE gene locus has been shown to affect levels of total cholesterol and LDL cholesterol in the general population. Population studies indicate that subjects with the  $\epsilon 2$  allele have lower LDL cholesterol levels than subjects with  $\epsilon 3/\epsilon 3$  genotype, while those with the  $\epsilon 4$  allele have higher levels [7,12]. It has been suggested that the presence of the  $\epsilon 2$  allele results in decreased LDL cholesterol because of delayed clearance of chylomicron remnants by the liver and upregulation of LDL receptor activity, while the  $\epsilon 4$  allele results in elevated levels of LDL cholesterol because of enhanced uptake of chylomicron remnants and down-regulation of the LDL receptor. A meta analysis by Dallongeville et al. indicated that subjects with the  $\epsilon 2$  and  $\epsilon 4$  alleles had higher triglyceride levels than subjects with the  $\epsilon 3$  allele [14]. These associations affecting well-known lipid-related CHD risk factors suggest that variation at this locus could be a major determinant of CHD risk in the general population.

Multiple studies have examined the association between CHD and APOE polymorphisms. Most investigators have used a case-control design [23–35], but others have used other experimental approaches, including age of onset in MI survivors [36–38], parental history of CHD [39,40], arteriographic imaging analysis [41–49] and autopsy studies [50–52]. In our previous meta analysis including those studies published up to 1995, the CHD odds ratio associated with the  $\epsilon 2$  allele was 0.98, with a confidence interval of 0.85–1.14, and for the  $\epsilon 4$  allele was 1.26 with a confidence interval of 1.13–1.41 ( $P < 0.05$ ) [15]. Such associations were observed both in men and in women and indicate that the presence of the  $\epsilon 4$  confers an increased risk of heart disease. However, the potential protective effect associated with the  $\epsilon 2$  allele is controversial and some studies have suggested that the  $\epsilon 2$  allele may be associated with higher CHD risk in an age and gender-dependent manner and could be also influenced by behavioral factors [36,38,39,47,53].

In the present study, we have documented that the  $\epsilon 4$  allele results in a significantly increased odds for CVD in men. With adjustment for age only, this estimated increase in odds is about 61%. After adjustment for both age and other risk factors, this increased odds is about 63%, and after adjustment for all risk factors including lipids, the increased odds is 51%. In women this value is 48%; however, due to the smaller number of events this increase did not reach statistical significance. These data suggest that at least some of the risk associated with  $\epsilon 4$  is due to the moderately higher LDL cholesterol levels

observed in this group. In men, in contrast to women, the  $\epsilon 2$  allele significantly increases the risk for CVD. This association remains significant after adjusting for age and all non-lipid risk factors and after adjusting for all risk factors including lipids. Since the  $\epsilon 2$  allele has been associated with elevated postprandial lipemia, the influence of the  $\epsilon 2$  allele on CVD risk could be due to the higher concentration of atherogenic triglyceride-rich lipoprotein (TRL) present in some carriers of the  $\epsilon 2$  allele [54,55]. However, no associations of the  $\epsilon 2$  allele with increased CVD risk were observed in women. One plausible explanation for these findings is that the atherogenic potential associated with TRL may be primarily expressed within the context of the lower HDL-C levels present in male subjects, whereas in women, the atherogenic effect of elevated TRL may be counterbalanced by their higher HDL-C levels. Another potential mechanism involves the relation between insulin resistance, postprandial lipemia and estrogen levels. It has been shown [56] that fasting insulin levels were similar in male CHD cases and controls bearing the APOE3/3 and the APOE3/4 genotypes; however, on APOE2/3 subjects, cases had significantly elevated insulin levels as compared with controls. Therefore, we could speculate that in APOE2/3 subjects, the insulin resistance state is associated with impaired remnant clearance and postprandial increases of atherogenic TRLs [57]. Conversely, in women, estrogens have been shown to improve insulin sensitivity, which may compensate for the effect observed in APOE2 males [58,59]. Moreover, it has been shown that estrogens up-regulate apo E gene expression by increasing levels of apo E mRNA. This increase in apo E expression could translate into higher secretion of apo E and a more efficient remnant clearance in premenopausal women [60]. Further support of this notion comes from elegant studies in apolipoprotein E2 rabbits [61]. In this animal model, high plasma levels of human apo E2 result in both more severe hyperlipidemia and more extensive atherosclerosis in male than in female animals. Estrogen treatment of male transgenic rabbits normalized the lipid profile. Conversely, ovariectomy in female transgenic rabbits significantly increased all atherogenic lipoproteins. Thus estrogen status appears to be responsible for a high proportion of the gender differences in lipid phenotypes and atherosclerosis observed in this animal model. Overall, we can hypothesize the existence of a complex interaction between gender, APOE, hormonal status and remnant clearance that could be responsible for our reported results.

The variability in the reported associations between APOE genotype and CHD risk may be due to different environmental exposures affecting the association between APOE alleles and plasma lipid levels. We, and others, have reported significant interactions between APOE genotype and plasma lipid response to both diet

and pharmacological therapies (for reviews see Refs. [62,63]). These interactions could explain the fact that the contribution of the  $\epsilon 4$  allele to elevated LDL-C levels is higher in the presence of an atherogenic diet [62]. Similar interactions with APOE have also been reported for blood pressure and diet therapy [64]. Moreover, several authors [65–67] suggest that a low fat diet can suppress the deleterious effects of the  $\epsilon 4$  allele on the plasma lipid profile. Along these lines, a study carried out in a Native American rural population following its traditional lifestyle reported no differences in LDL-C concentrations between  $\epsilon 3/\epsilon 3$  and  $\epsilon 3/\epsilon 4$  subjects [65]. Kamboh et al. [66] reported similar results in Maya Indians living in Mexico. The authors hypothesized that the Mayans are exposed to an ancestral diet and to non-occidental lifestyles that may interact with the genetic susceptibility of the APOE locus to determining plasma lipid levels. Similar gene–environment interactions have been reported in several other populations [67,68]. These data provide evidence suggesting that the APOE gene is a prototypical susceptibility gene.

In summary, the results presented in this study show that variation in the APOE gene may play a gender-specific role in the risk of CVD, with an increased burden of disease being observed in men with the  $\epsilon 2$  and  $\epsilon 4$  alleles, compared to subjects with the  $\epsilon 3/\epsilon 3$  genotype. While familial hypercholesterolemia due to LDL receptor mutations may only raise the population attributable risk of CVD by 5%, common mutations at the APOE gene locus, in our view, can raise the risk of cardiovascular and CHD by approximately 50–60%, and carry a population attributable risk of 10–12% in men.

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