Hum Genet (1996) 97 : 677–684

ORIGINAL INVESTIGATION

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Genotypes with the apolipoprotein ε4 allele are predictors of coronary heart disease mortality in a longitudinal study of elderly Finnish men

Received: 7 June 1995 / Revised: 28 August 1995

Abstract Earlier we reported that allelic variation in the gene coding for apolipoprotein (apoE) is a significant predictor of variation in the risk of coronary heart disease (CHD) death in a longitudinal study of elderly Finnish men. Here we address the question: which of the apoE genotypes confers the risk information in these men, and whether such information persists after other CHD risk factors are considered? We followed two cohorts of elderly Finnish men aged 65 to 84 years, one in Eastern (n = 281) and the other in the Southwestern (n = 344) Finland for 5 years during which 26 (9.3%) of the men from the Eastern cohort and 40 (11.6%) of the men in the Southwestern cohort died from CHD. Baseline high density lipoprotein (HDL) cholesterol and (HDL cholesterol)^2 in the Eastern cohort and age, and total and HDL cholesterol and smoking status in the Southwestern cohort were significant predictors of CHD death ($P < 0.05$). The apoE genotypes were significant predictors in the Southwestern cohort at $P = 0.02$ and in the Eastern cohort at $P = 0.18$. In multivariable models, information about apoE genotypes improved the prediction at $P = 0.10$ level of statistical significance in both cohorts. When genotypes were considered separately, the ε2/4 combined with the ε4/4 in the Eastern cohort (odds ratio = 7.69, 95% CI = 1.67–35.52) and the ε3/4 in the Southwestern cohort (odds ratio = 2.44, 95% CI = 1.16–5.10) had sigificanctly greater odds of CHD death compared to the common ε3/3 genotype. We conclude that apoE genotypes confer risk information about CHD death in two cohorts of elderly Finnish men in a longitudinal study, and this information persists after adjustment for other CHD risk factors. Because different genotypes were predictors in these two cohorts, we further conclude that the utility of a particular genotype as a predictor of CHD death in other populations may depend on the distribution of risk factor profiles at baseline, geographically defined environmental exposures, the CHD mortality history, and the evolutionary history of background genotypes in the population considered.

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Introduction

Epidemiological and clinical studies have revealed the involvement of a large number of biochemical and physiological traits in the initiation, progression, and clinical manifestation of coronary heart disease (CHD). These include quantitative measures of lipid metabolism, glucose tolerance, blood pressure, and hemostasis (Badimon et al. 1993; Dawber 1980; Gordon and Kannel 1982). About 50% of CHD cases can be predicted from knowledge about levels of known risk factor traits (Gordon and Kannel 1982).

Measurements of genetic variation at the DNA and protein-product levels are expected to improve our ability to predict CHD. Genotypes may be indicators of risk because of effects on (1) the phenotypic distributions of multiple biochemical or physiological risk factor traits (Kaprio et al. 1991), some of which may be inaccessible or difficult to measure in vivo, and (2) because utility of risk factor information may be genotype dependent (Reilly et al. 1991, 1994). Furthermore, genotypes also have the potential to predict how individuals will respond to alterations in the environment defined by diet, exedrcise, smoking, gender, and drug therapy (Mänttäri et al. 1991; Nestruck et al. 1987), or to biological changes associated with aging (Davignon et al. 1989; Haviland et al. 1995).

The common alleles, ε2, ε3 and ε4, of the gene coding for apolipoprotein E (apoE) are hypothesized to confer risk information because they are associated with interindividual variation in plasma lipid levels in healthy (Haviland et al. 1995; Kaprio et al. 1991; Xhignesse et al.

1991) and diseased populations (Davignon et al. 1988; Ferrières et al. 1994; Sing and Moll 1989). This hypothesis is supported by findings that the relative ε4 allele frequency is elevated in high risk populations (Davignon et al. 1989; Gerdes et al. 1992) and among those with CHD in most cross-sectional studies (Davignon 1993; Ferrières et al. 1994; Kuusi et al. 1989; Nieminen et al. 1992; Wilson et al. 1994). Recently we have reported that the relative frequency of ε4 was increased among elderly men who died from CHD during a 5-year follow-up (Stengård et al. 1995).

In the study reported here we asked which of the apoE genotypes confers information about risk of CHD death during a 5-year follow-up of two cohorts of Finnish men aged 65 to 84 years, one in Eastern and the other in Southwestern Finland, and whether such information persits after other risk factor traits are considered?

Materials and methods

Sample

The Seven Countries Study was initiated in the late 1950s to study variation in cardiovascular disease mortality and morbidity and related risk factor levels among countries, including Finland (Keys 1970; Pekkanen 1987). The original Finnish cohorts (n = 1711) consisted of all men born between 1900 and 1919 in two geographically defined rural areas, one in Eastern and the other in Southwestern Finland. Of these, 823 were from the Eastern cohort and 888 from the Southwestern cohort. In 1984, when baseline assays for the current study were performed, 766 of the 1711 men were 65 to 84 years old and were still alive. Of these eligible men, 716 (93%) participated in the medical examination. The reasons for the nonparticipation of 50 individuals included length of travel distance, recent myocardial infarct, severe rheumatic fever, and refusal to participate. Knowledge about apoE phenotype was available for 666 (87%) men. Of the 50 nonparticipants and 50 participating subjects whose apoE phenotype was not known at baseline, a total of 45 died during the follow-up period.

At the time of the follow-up survey in 1989, of the 666 men whose apoE phenotype was known at baseline, 468 were still alive and 198 had died. Death certificates and hospital records were obtained for all the deceased men and the cause of death was coded by one of the authors (J.P.) according to fixed criteria (Keys et al. 1967). The coding was performed without knowledge about the individual's apoE genotype. All participants gave their informed consent before their inclusion in the study.

Study protocol

Both baseline and follow-up surveys of each geographic region were conducted during the same month of the year, October in the East and November in the Southwest. Complete details of the study protocols of the 1984 baseline survey and the follow-up survey in 1989 are given elsewhere (Nissinen et al. 1986, 1993). All participants fasted at least 4 h before their visit at the clinic (mean fasting time was 13 h, standard deviation 5 h; 75% of the participants fasted 8 h or more). At the clinic, blood samples were drawn from the antecubital vein for laboratory analyses. Body weight was measured to the nearest 0.1 kg in light undergarments. Height was measured only in 1959, when the men were first seen in connection with the Seven Countries Study. This height was used to calculate body mass index (BMI, kg/m^2), an index of body size used in the current analyses.

Smoking status was assessed using a standard questionnaire (Keys et al. 1967). Participants were classified into the following two categories: smokers of cigarettes, cigars or pipes, and nonsmokers. Participants who had quit smoking less than 1 year before the survey were classified as smokers.

Blood pressure was measured by a trained nurse. After at least a 5-min rest at the end of clinical examination, two successive readings were taken from the right arm using a mercury manometer with a 12 cm by 33.5 cm cuff. Readings were taken to the nearest 2 mmHg and complete disappearance of the fifth phase of Korotkoff sounds was recorded as the diastolic pressure. We used the mean values of the two readings for the analyses presented in this study.

Laboratory measurements

Total and HDL cholesterol concentrations were assayed using an enzymatic method (Monotest, new, Boehringer Mannheim, Mannheim, Germany) and an Olli C3000 photometer (Kone Oy, Finland). Total cholesterol was determined from a fresh serum sample (Kostner 1976). HDL cholesterol was measured after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles with dextran magnesium chloride (Kostner 1976). ApoE phenotypes were determined using sera stored at –20° C until utilized in 1992. ApoE phenotyping was carried out according to a modification of the method of Havekes (Ehnholm et al. 1986; Havekes et al. 1987), which was based on isoelectric focusing of delipidated serum followed by immunoblotting using rabbit anti-human apoE antiserum. ApoE genotypes were inferred from the isoform phenotypes.

Statistical analysis

Baseline differences between the men who died from CHD and those who did not die from CHD during the 5-year follow-up period were tested for statistical significance using the analysis of variance for quantitative traits and χ^2 statistics for categorical variables.

A three-stage model-building strategy and hypothesis-testing procedure was used to estimate the nature of the association between variation in CHD death probability and variation in CHD risk factor levels and genotypic variation in apoE. First, a logistic regression analysis was employed to assess whether any of the CHD risk factor traits, or information about the apoE genotype, predicted CHD mortality when they were considered one at a time in the model, separately for the Eastern and the Southwestern cohorts. The CHD risk factor traits considered were age, BMI, systolic and diastolic blood pressure, total and HDL cholesterol levels, and smoking status. The effect of each apoE genotype was modeled by coding design variables in such a way that the ε3/3 genotype represented the reference class. Twice the logarithm of the ratio of the maximum value of the likelihood of a model with (complete model) and without (reduced model) a particular trait was taken to be distributed approximately as a χ^2 for the test of whether its contribution was statistically significant.

The antilogarithm of the difference between the estimated logits computed at two different levels of a risk factor trait gives an estimate of the ratio of the odds of dying from CHD for individuals at the two levels of the trait. In this study the odds ratio for each quantitative trait was calculated for a one standard deviation increase above its mean value, separately for the Eastern and the Southwestern cohorts.

In the second stage, a parsimonious subset of the risk factor traits, ignoring the apoE genotype, was selected, separately for the Eastern and the Southwestern cohorts. Initially any trait whose log-likelihood ratio test in the first stage gave a type I error probability less than 0.25 was considered in the model in the cohort where this result was found. This relatively high probability level was selected because use of a more traditional test criterion (such as 0.05) at this point often fails to identify traits that can become important predictors of outcome only when they are considered in a multivariable prediction model (Hosmer and Lemeshow 1989). The importance of each variable, its second-order term, or any of

their pairwise interactions in the multivariable model, was verified by the stepwise backward elimination procedure. Removal of a variable was based on the value of the residual χ^2. The level of statistical significance was taken to be $P < 0.05$ for the elimination of a variable from the final model. A Hosmer-Lemeshow approach, which partitions the distribution of predicted probabilities into deciles, and subsequently sums the difference between the observed proportion of the men who died from CHD and the average predicted probability for subjects in each decile (Hosmer and Lemeshow 1989), was used to assess goodness-of-fit of the final model. This sum, denoted here as $\acute{C}_{HL}$, is distributed approximately as a χ^2 with 8 degrees of freedom *(df)*. Hence, $\acute{C}_{HL}$ greater than 15.508 was taken as an indication of lack-of-fit at the $P < 0.05$ level of statistical significance. The evaluation for goodness-of-fit was informative because the log-likelihood ratio statistic used in the model building process is based on the relative comparison of two fitted models, not on the absolute comparison of the probabilities predicted by the proposed model with the observed outcomes. Somer's *D* was also employed to explore effectiveness of the proposed model to discriminate between the men who died from CHD and those who did not die from CHD. The value of Somer's *D* varies between 0 and 1, where a value of 1 indicated perfect ability of the proposed model to discriminate between the men who died from CHD and those who did not die from CHD.

In the third stage, a multivariable logistic regression analysis was employed to assess whether addition of the apoE genotype information to the model established in the second stage improved the prediction, separately for the Eastern and the Southwestern cohort. A log-likelihood ratio test, which compares the maximum likelihood of a multivariable model that includes both information about apoE genotype and the parsimonious subset of other risk factor traits (complete model) and maximum likelihood of the model that includes only the other traits (reduced model), was used to judge whether the improvement in prediction attributable to the apoE genotype was statistically significant. The degrees of freedom for the χ^2 statistic associated with this test are one less than the number of apoE genotypes considered in the analysis.

All statistical analyses were performed with the SAS statistical software package (SAS 1985). Statistical significance was taken to be $P < 0.05$ unless otherwise noted.

Results

Description of the cohorts

The Eastern cohort included 281 men and the Southwestern cohort 344 men for whom knowledge about both apoE genotype and all risk factor trait levels were available. Of them, 26 (9.3% of the sample) in the Eastern cohort and 40 (11.6% of the sample) in the Southwestern cohort died from CHD during the 5-year follow-up period. Heterogeneity of the relative 5-year CHD mortality between the Eastern and the Southwestern cohorts was not statistically significant $\chi^2 = 0.38$, $df = 1$, $P = 0.54$).

The following observations were statistically significant at the $P < 0.05$ level of probability: the men who died from CHD in the Eastern cohort were older, had lower average HDL cholesterol levels, and used antihypertensive drugs more often than those who did not die from CHD (Table 1). In the Southwestern cohort the men who died from CHD were significantly older, had significantly higher average total cholesterol levels, had significantly

Table 1 Baseline characteristics by cohort and coronary heart disease (CHD) death status after 5 years follow-up

Trait	Eastern cohort and CHD death		Southwestern cohort and CHD death	
	Yes ($n = 26$)	No ($n = 255$)	Yes ($n = 40$)	No ($n = 304$)
Age (years)				
Mean (SD)	73.7 (4.7)*	71.5 (4.8)	75.2 (5.8)*	73.0 (5.2)
Anthropometry				
BMI (kg/m^2)				
Mean (SD)	26.3 (4.2)	25.3 (4.0)	26.4 (3.9)	25.9 (4.2)
Obese (%)	36	34	45	39
Serum cholesterol				
Total (mmol/l)				
Mean (SD)	6.1 (1.6)	6.2 (1.3)	6.5 (1.7)*	6.0 (1.2)
HDL (mmol/l)				
Mean (SD)	1.1 (0.4)*	1.2 (0.3)	1.1 (0.3)*	1.2 (0.3)
Blood pressure				
Systolic (mmHg)				
Mean (SD)	142 (25)	150 (22)	159 (24)	157 (22)
Diastolic (mmHg)				
Mean (SD)	83 (12)	85 (11)	89 (10)	89 (11)
Antihypertensive drugs (%)	54*	31	38	33
Hypertensive (%)	64	52	69	66
Smoking status				
Current (%)	19	23	31*	17

* $P < 0.05$, compared subjects who had died from CHD and those who had not died from CHD within a sample

Table 2 The ApoE genotypes by cohort and CHD death status after 5 years follow-up[a]

[a] Fisher's exact test was employed because expected cell sizes were less than five in more than 25% of the cells. The relative frequencies of genotypes were heterogeneous between the men who had died from CHD and those who had not died from CHD in the Eastern sample at $P = 0.104$ and in the Southwestern sample at $P = 0.014$

	Eastern cohort and CHD death, number (%)				Southwestern cohort and CHD death, number (%)			
	Yes		No		Yes		No	
Genotype								
ε2/2	0	(0)	0	(0)	0	(0)	0	(0)
ε2/3	1	(3.9)	15	(5.9)	1	(2.5)	26	(8.5)
ε3/3	15	(57.7)	178	(69.8)	16	(40.0)	180	(59.2)
ε3/4	7	(26.9)	56	(21.9)	21	(52.5)	86	(28.3)
ε2/4	2	(7.7)	4	(1.6)	2	(5.0)	12	(4.0)
ε4/4	1	(3.8)	2	(0.8)	0	(0)	0	(0)
All	26	(100.0)	255	(100.0)	40	(100.0)	304	(100.0)

Table 3 Predictors of CHD death among elderly Finnish men in a univariable logistic regression analysis, separately for the Eastern and the Southwestern cohort (χ^2_{LHR} = Log-likelihood ratio χ^2, $df = 1$ for all tests, *95% CI* = 95% confidence interval, *BMI* = body mass index (kg/m^2), *HDL* = high density lipoprotein cholesterol; *Reg coef* = regression coefficient; *SE* = standard error)

Trait	Eastern cohort						Southwestern cohort					
	Reg coef	SE	χ^2_{LHR}	*P*	Odds ratio	95% CI	Reg coef	SE	χ^2_{LHR}	*P*	Odds ratio	95% CI
Age	0.08	0.04	3.96	0.05	1.47	(1.00, 2.14)	0.08	0.03	6.02	0.01	1.53	(1.12, 2.08)
Anthropometry												
BMI	0.06	0.05	1.55	0.21	1.27	(0.86, 1.89)	0.03	0.04	0.49	0.48	1.13	(0.82; 1.55)
Serum cholesterol												
Total	0.03	0.16	0.03	0.86	1.04	(0.69, 1.57)	0.37	0.13	7.82	0.01	1.56	(1.14, 2.12)
HDL	–1.28	0.71	3.61	0.06	1.54	(0.96, 2.46)	–1.42	0.61	5.52	0.01	1.57	(1.07, 2.29)
Blood pressure												
Systolic	–0.01	0.01	1.43	0.23	0.80	(0.52, 1.24)	0.01	0.01	0.45	0.50	1.24	(0.81, 1.91)
Diastolic	–0.01	0.02	0.36	0.55	0.90	(0.59, 1.37)	–0.01	0.02	0.04	0.84	0.90	(0.59, 1.37)
Smoking status												
No	0	–	0.17	0.68	1	–	0	–	5.08	0.02	1	–
Yes	–0.21	0.52	–	–	0.81	(0.29, 2.25)	0.87	0.37	–	–	2.39	(1.15, 4.95)
Apo E genotype												
ε3/3	0	–	4.96	0.18	1	–	0	–	10.46	0.02	1	–
ε2/3	–0.23	1.07	–	–	0.79	(0.10, 6.40)	–0.84	1.05	–	–	0.43	(0.06, 3.40)
ε2/4[a]	1.78	0.76	–	–	5.93	(1.35, 26.1)	0.63	0.81	–	–	1.88	(0.39, 9.12)
ε3/4	0.39	0.48	–	–	1.48	(0.58, 3.82)	1.01	0.36	–	–	2.75	(1.37, 5.53)

[a] ε4/4 was combined with ε2/4 in the Eastern cohort; ε4/4 was not observed in the Southwestern cohort

lower average HDL cholesterol levels, and were current smokers significantly more often than the men of this cohort who did not die from CHD (Table 1).

The relative apoE genotype frequencies in the Eastern cohort were heterogeneous between the men who died from CHD and those who did not die from CHD at the $P = 0.10$ level of probability. The ε3/3 was the most frequent genotype and the ε3/4 was the next in both of these strata. Relative frequencies of the ε3/4 genotype and the other ε4 allele-containing genotypes, ε2/4 and ε4/4, were higher among the men who died from CHD (26.9%, 7.7%, and 3.8%, respectively) than among those who did not die from CHD (21.9%, 1.6%, and 0.8%, respectively) whereas relative frequency of the ε3/3 genotype was lower among those who died from CHD (57.7%) than among those who did not die from CHD (69.8%). The least frequent ε4/4 and ε2/4 genotypes were, however, recorded only in three men who died from CHD and six men who did not die from CHD in the Eastern cohort.

The relative apoE genotype frequencies in the Southwestern cohort were heterogeneous between the men who died from CHD and those who did not die from CHD at the $P = 0.01$ level of probability (Table 2). The ε3/4 was the most frequent genotype (52.5%) and the ε3/3 the next most frequent genotype (40.0%) among the men who died from CHD, whereas in the men who did not die from the CHD the ε3/3 was the most frequent genotype (59.2%) and the ε3/4 the next most frequent genotype (28.3%). The two least frequent genotypes in the Southwestern cohort were ε2/3 and ε2/4. These genotypes were recorded in less than 5% of the men who died from CHD and less than 10% of the men who did not die from CHD. No individuals with the ε4/4 genotype were recorded in the Southwestern cohort. The ε2/2 genotype was not recorded in either cohort.

Univariable analysis

Age and HDL cholesterol were the only traits in the Eastern cohort that were significant predictors of CHD death at the $P < 0.06$ level of probability when considered separately in the logistic regression model (Table 3). Furthermore, BMI and systolic blood pressure fall below the $P < 0.25$ level of significance, indicating that these traits should be considered as candidates in the subsequent multivariable logistic regression analysis in the Eastern cohort. In the Southwestern cohort, age, total and HDL cholesterol levels, and smoking status were each significant predictors of CHD death at the $P < 0.05$ level of statistical significance. No other predictors reach the $P < 0.25$ level of probability in the Southwestern cohort.

Variation among the observed apoE genotypes contributed to the prediction of the CHD death at the $P = 0.18$ level of statistical significance in the Eastern cohort when variation in other risk factor traits was ignored. In the Southwestern cohort the contribution of genotypic variation was significant at the $P = 0.02$ level of probability. Because the ε4/4 genotype was recorded only in three participants in the Eastern cohort, the carriers of the ε4/4 were combined with the carriers of the ε2/4 genotypes for the logistic regression analysis in the Eastern cohort.

The odds of CHD death for those with the rare ε2/4 and the ε4/4 genotypes in the Eastern cohort was about six times higher than odds of CHD death among the carriers of the reference ε3/3 genotype (Table 3). The odds of CHD death for those with the ε3/4 genotype was about three times higher than the odds of the carriers of the reference ε3/3 genotype in the Southwestern cohort.

Multivariable models ignoring apoE genotypes

When all putative risk factor traits, other than the apoE genotypes, that reached the $P < 0.25$ test criterion in univariable analyses were considered, the stepwise backward elimination process identified HDL cholesterol and (HDL cholesterol)^2 but not age, BMI, nor systolic blood pressure as the statistically significant predictors of CHD mortality at the $P < 0.05$ level of probability in the Eastern cohort. In contrast, in the Southwestern cohort all the risk factor traits that were significant predictors in univariable models were also detected as significant predictors in a multivariable model of the data. No statistically significant interactions between any of the risk factor traits were detected in either cohort at the $P < 0.05$ level of probability (results not shown). Hence, the final multivariable logistic regression model, when apoE genotypic information was ignored, was: CHD death probability = 2.74–7.17 (HDL cholesterol) + 2.27 (HDL cholesterol)^2 in the Eastern cohort and CHD death probability = –11.25 + 0.11 (age) + 0.47 (total cholesterol) – 1.52 (HDL cholesterol) + 0.87 (smoking status) in the Southwestern cohort. In both cohorts the $\acute{C}_{HL}$ value of the Hosmer-Lemeshow goodness-of-fit statistics was below the critical level (2.00 vs 11.07 in the Eastern cohort and 9.33 vs 15.51 in the Southwestern cohort), indicating that the models fit the observed outcomes acceptably well. Even so, relatively low values of the Somer's *D* (0.38 in the Eastern cohort and 0.47 in the Southwestern cohort) indicate only moderate ability of the models to discriminate between the men who died from CHD and those who did not die from CHD during the 5-year follow-up.

Multivariable models including apoE genotypes

When information about the apoE genotypic variation was entered into a model that already included the selected set of other CHD risk factor traits, it resulted in a marginally significant improvement in the ability to predict CHD death both in the Eastern cohort ($\chi^2_{LHR} = 6.13$,

Table 4 Predictors of CHD death among elderly Finnish men in a multivariable logistic regression analysis when both Apo E genotypic information and the parsimonious set of other CHD risk factor traits are considered, separately for the Eastern and the Southwestern cohort

Trait	Eastern cohort				Southwestern cohort			
	Partial reg coef	SE	Partial odds ratio	95% CI	Partial reg coef	SE	Partial odds ratio	95% CI
Age	–	–	–	–	0.11	0.04	1.79	(1.17, 2.72)
Serum cholesterol								
Total	–	–	–	–	0.42	0.15	1.66	(1.16, 2.34)
HDL	–8.06	2.77	–	–	–1.39	0.65	1.55	(1.03, 2.34)
(HDL)^2	2.56	0.98	2.16	–	–	–	–	–
Smoking status								
No	–	–	–	–	0	–	1	–
Yes	–	–	–	–	0.84	0.40	2.29	(1.05, 5.00)
Apo E genotype								
ε3/3	0	–	1	–	0	–	1	–
ε2/3	–0.30	1.10	0.75	(0.09, 6.49)	–0.42	1.08	0.66	(0.08, 1.70)
ε2/4[a]	2.04	0.78	7.69	(1.67, 35.52)	0.37	0.86	1.45	(0.28, 7.85)
ε3/4	0.46	0.50	1.58	(0.59, 4.22)	0.89	0.38	2.44	(1.16, 5.10)

[a] ε4/4 was combined with ε2/4 in the Eastern cohort; ε4/4 was not observed in the Southwestern cohort

df = 3, *P* = 0.10) and in the Southwestern cohort (χ^2_{LHR} = 6.25, *df* = 3, *P* = 0.10). The $\acute{C}_{HL}$ value of the Hosmer-Lemeshow goodness-of-fit statistics increased from 2.00 to 2.81 in the Eastern cohort and decreased from 9.33 to 7.47 in the Southwestern cohort. The value of the Somer's *D* increased slightly in both cohorts (from 0.38 to 0.46 in the Eastern cohort and from 0.47 to 0.50 in the Southwestern cohort).

The estimates of partial regression coefficients obtained in the multivariable logistic regression analysis where both the selected CHD risk factor traits and the apoE genotypes were considered are given in Table 4, separately for the Eastern and the Southwestern cohorts. In both cohorts, addition of the apoE genotype information to the multivariable logistic regression model resulted in only minor changes in the estimates of the regression coefficients when compared to the respective estimates in the model where apoE genotypic information was ignored. Similarly, there were only minor changes in the estimates of partial regression coefficients for different apoE genotpyes in the multivariable model when compared with the univariable model where apoE genotypic information was the only variable considered.

Discussion

Coronary heart disease accounted for 30% to 40% of the deaths recorded during the 5-year follow-up period in the two cohorts of elderly Finnish men considered in this study. Of the known CHD risk factor traits, age and HDL cholesterol were predictors of CHD death in both cohorts. However, age was a significant predictor only in the Southwestern cohort after effects of other risk factor traits were considered. Similarly, total cholesterol and smoking status were predictors only in the Southwestern cohort. The ranges of variability of these risk factor traits at baseline were approximately the same as the ranges reported in other studies of elderly men (Barrett-Connor et al. 1984; Jacobsen et al. 1992; Stavenow et al. 1990). These findings are consistent with earlier reports that CHD risk factor traits that are identified as predictors in middle-aged men are predictors also in elderly men (Assmann and Schulte 1989; Barrett-Connor et al. 1984; Harris et al. 1988). The combinations of the CHD risk factors that were identified as predictors in the Eastern and Southwestern cohorts in this study differ, however, from the combinations reported to be present in these cohorts 20 years earlier (Heliövaara et al. 1981; Karvonen et al. 1970). In the earlier studies, when the men were in their middle ages, total cholesterol, blood pressure and smoking status were significant predictors of CHD events in both cohorts (Heliövaara et al. 1981; Karvonen et al. 1970). This age related change in the predictive power of the CHD risk factor traits may reflect (1) biochemical and physiological changes that are associated with the aging process per se that lead to an altered atherogenic risk factor profile, (2) selective mortality against high risk individuals in their early adulthood, or (3) age-related changes in the environment defined by physical activity, diet, other diseases, and drug therapy.

Baseline distributions of the apoE genotypes differed significantly between the Eastern and Southwestern cohorts (χ^2_{HLR} = 14.93, *df* = 4, *P* = 0.005). An excess of the ε2/3, the ε3/4, and the ε2/4 genotypes was recorded in the Southwestern cohort compared with the Eastern cohort (Stengård et al. 1995). This heterogeneity of the apoE genotypic distribution may reflect selective mortality against the high risk ε4 allele containing genotypes in the Eastern cohort in the past. An earlier observation that apoE genotype frequencies did not differ significantly between children born in Eastern and Southwestern Finland (Lehtimäki et al. 1990) supports the selective mortality hypothesis.

Our longitudinal 5-year follow-up study demonstrates that apoE genotypic variation is a statistically significant predictor of variation in risk of CHD death in the Southwestern cohort when contribution of other risk factor traits are ignored. The ε3/4 genotype was, however, the only genotype in this cohort that was associated with significantly increased odds of CHD death (95% confidence interval did not include one) as compared with odds for the reference ε3/3 genotype. In the Eastern cohort the contribution of apoE ganotypic variation to prediction of variation in risk of CHD death was only marginally significant (*P* = 0.18). The odds of CHD death among carriers of the combined groups of the rare ε2/4 and the ε4/4 genotypes in the Eastern cohort was, however, significantly higher than the odds for the carriers of the reference ε3/3 genotype. The latter finding suggests that individual genotypes may confer risk information that is not detected by the global test of the contribution of all the observed genotypes. In this context we emphasize that we did not record the ε2/2 genotype in either cohort and the ε4/4 was recorded only in the Eastern cohort. Inferences based on samples with all the apoE genotypes represented may differ from the inferences suggested by the results obtained in this study. Furthermore, differences between the genotypes that were identified as predictors in the Eastern and the Southwestern cohorts emphasize that genotypic information may be expected to be context dependent where context is defined by at least (1) the mortality history of the population, (2) geographically defined environmental factors, or (3) the evolutionary history of the background genotypes that influence risk of CHD.

The odds of CHD death among the carriers of the ε2/3 genotype tended to be 30% to 50% lower than the odds of the carriers of the reference genotype ε3/3 in both cohorts. This finding is consistent with a hypothesis that the ε2 allele confers some protection against CHD (Davignon 1993). On the other hand, our finding is inconsistent with observations that the ε2 allele confers atherogenic potential in some populations (Davignon 1993; Andrade et al. 1995). The lowered estimates of the odds were not statistically significant in either cohort in this study. Any inferences about the atherogenic or antiatherogenic potential of the ε2/3 genotype based on our results must, however, be tentative because only 3 (1 in the Eastern cohort and 2

in the Southwestern cohort) CHD deaths were recorded for this genotype class during the 5-year follow-up, i.e., the result may be a type II error.

The contribution of any genotypic variation to the initiation, progression, and clinical manifestation of atherosclerotic process must be translated through variation in and covariation between biochemical and physiological traits that determine variation in risk of disease (Ferrannini 1991; Sing et al. 1994). The complex pathophysiology of atherosclerosis dictates that the contribution of genotypic variation to prediction of any CHD outcome, such as CHD death in this study, may not persist when variation in all traits involved in linking genome variation with variation in risk of disease are considered. Our finding that addition of the information on apoE genotypic variation to models that already include a selected subset of other risk factor traits improved the prediction of CHD death at the $P = 0.10$ level of statistical significance suggests that this null hypothesis does not hold when apoE genotypic variation is considered in these two cohorts of elderly Finnish men. Furthermore, the carriers of the rare ε2/4 and the ε4/4 genotypes in the Eastern cohort and the carriers of the ε3/4 genotype in the Southwestern cohort were associated with an odds of CHD death significantly higher than the odds for the reference ε3/3 genotype in this multivariable model. This latter observation indicates that although genotypic variation of apoE improves the ability in the prediction only marginally, individual apoE genotypes can confer risk information that remains highly significant after other risk factor traits are considered.

Importance of a risk factor, such as a high-risk apoE genotype, to the public health in the population at large is related to both the relative risk and the proportion of the population exposed to this particular risk factor (Kahn 1983). Attributable risk is an epidemiological concept that reflects the fraction of all cases that are associated with the proposed risk factor. An estimate of attributable risk of the CHD death for the rare high risk ε2/4 and ε4/4 genotypes was 0.16 in the Eastern cohort, while the attributable risk for the ε3/4 genotype in Southwestern cohort was 0.32. Estimates of the odds ratio and proportion of the carriers for each genotype were used for the attributable risk computations. These findings suggest that 16% of the CHD deaths in the Eastern cohort and 32% in the Southwestern cohort were associated with these high risk genotypes. In this context we want to emphasize, however, that attributable risk only summarizes the proportion of CHD deaths *associated* with the risk factor.

A relatively small fraction (20% to 30%) of the carriers of the high-risk genotypes, the rare ε2/4 and ε4/4 genotypes in the Eastern cohort and the ε3/4 genotype in the Southwestern cohort, died from CHD during the 5-year follow-up period. A considerable proportion of the men who died from CHD did not carry these high-risk genotypes. Of the non-high-risk genotypes, the common ε3/3 genotype accounted for 60% of the CHD deaths in the Eastern cohort and 40% in the Southwestern cohort. These findings are consistent with the complex pathophysiology of CHD (Ferrannini 1991; Sing et al. 1994). A consequence of this complex pathophysiology is that the mapping function between variation in a single gene, or variation in any single environmental factor, and variation in diesease risk is not simple (Ferrannini 1991; Sing et al. 1994; Wahlsten 1990; Weiss 1993). From the genetic point of view the implication is that an individual's genome type determines the way he or she reacts to a particular history of exposures to environmental factors (Cooper and Rotimi 1994; Lewontin 1974; Wahlsten 1990). Our study serves to further convince that none of the apoE genotypes cause disease; genotypic variation in apoE can only contribute to interindividual variation in susceptibility to CHD.

In conclusion, the results of this 5-year follow-up study demonstrate that conventional CHD risk factor traits such as age, total and HDL cholesterol and smoking status, and variations in the apoE gene, confer information about risk of CHD death in elderly Finnish men. The risk information from apoE genotypes persisted after variation in other risk factor traits was considered. Utility of a particular risk factor trait or a particular apoE genotype as a predictor of future CHD death varied, however, among subdivisions of the Finnish population defined by geographical area of residence. This finding is consistent with earlier findings that have demonstrated the complexity of the mapping function between variation in risk factor levels and variation in risk of CHD. Heterogeneity of predictors among subdivisions of the elderly Finnish population brings into question the applicability of the inferences from this study to other subdivisions of the Finnish population defined by age and gender, and to other populations with different risk factor profiles, with exposures to different environmental histories or with different evolutionary histories for the genetic background. It is most likely that each population represents a different combination of genetic and environmental factors that interact to determine susceptibility to CHD.

Acknowledgements This study was supported by Academy of Finland, Medical Research Council, and by National Institutes of Health grants NIH EDC-1, 1-RO1AG08762-01A1, and NIH HL-39 107.

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