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Original Article

Utility of the predictors of coronary heart disease mortality in a longitudinal study of elderly Finnish men aged 65 to 84 years is dependent on context defined by *Apo E* genotype and area of residence

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A common assumption underlying most genetic studies is that individuals with different genotypes respond similarly to exposure to internal (epigenetic and background genotype effects) and external (ecological) environments. Here we evaluate whether this assumption is true in individuals with different genotypes of the gene coding for the apolipoprotein E (Apo E) molecule, an important determinant of the metabolic fate of plasma lipids and lipoproteins. We addressed whether the utility of known risk factors of coronary heart disease (CHD) in the prediction of CHD death in a 5-year follow-up is the same for the two most common *Apo E* genotypes, ε3/3 and ε4/3, in two cohorts of elderly Finnish men (age at baseline: 65–84 years), one in Eastern and the other in Southwestern Finland. The CHD mortality rate was higher in the ε4/3 than in the ε3/3 genotype in both cohorts (11.1 versus 7.8%, $Pr = 0.281$ in the Eastern cohort and 19.6 versus 8.2%, $Pr = 0.002$ in the Southwestern cohort). In the Eastern cohort, serum high density lipoprotein (HDL) cholesterol level was identified as a strong predictor of CHD death in the ε3/3 genotype ($\beta = -2.155$, $Pr = 0.019$). In the Southwestern cohort, age ($\beta = 0.139$, $Pr = 0.006$), body mass index (BMI) ($\beta = 0.149$, $Pr = 0.016$), and serum total cholesterol level ($\beta = 0.453$, $Pr = 0.051$) were identified as strong predictors in the ε3/3 genotype, as were smoking ($\beta = 0.236$, $Pr = 0.008$) and BMI ($\beta = -0.124$, $Pr = 0.057$) in the ε4/3 genotype. The latter observation indicates that in Southwestern Finland the probability of CHD death decreases with increasing BMI in elderly men with the ε4/3 genotype, while in their counterparts with the ε3/3 genotype the risk increases with increasing BMI. This difference was statistically significant ($Pr = 0.002$). These observations clearly argue against the assumption that individuals with different genotypes respond similarly to exposures to internal and/or external environments. These observations are consistent with a complex pathobiology of CHD involving biochemical and physiological agents that are under the influence of interactions between genetic and environmental factors. Information about these interactions is necessary for developing a more precise risk assessment and ultimately to improve public health and clinical strategies to prevent this devastating disease both at the individual and population levels.

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Key words: Apo E polymorphism – elderly men – known risk factors – prediction of CHD mortality

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There is ample evidence that interindividual differences in levels of biochemical and physiological traits, which are involved in the regulation of lipid metabolism, glucose homeostasis, blood pressure, and hemostasis, contribute significantly to interindividual variation in the initiation, progression, and severity of atherosclerosis (1, 2). Knowledge about levels of these traits is widely used to predict the risk of coronary heart disease (CHD), which is a clinically important manifestation of atherosclerosis. In clinical practice, and in public health programs, individuals with extreme values of the known risk factors are considered to be candidates for lifestyle alterations and therapeutic interventions.

Apolipoprotein E (Apo E), a structural constituent of various plasma lipoprotein particles, functions as an important determinant of the metabolic fate of plasma lipids and lipoproteins (3, 4). Three major Apo E isoforms, ε_2, ε_3, and ε_4 have been described (3, 4). Allelic variations responsible for this protein polymorphism, denoted ε_2, ε_3, and ε_4, are associated with variation in, and covariation between, quantitative measures of lipid and lipoprotein metabolism (4–9) and the presence of CHD (3, 4) in many cross-sectional epidemiological and clinical studies. Previously we reported that the common genotypes of the gene that codes for the three Apo E isoforms contribute to the prediction of CHD death among elderly Finnish men in a 5-year prospective follow-up (10). The influence of the Apo E polymorphism on the levels of plasma lipids and apolipoproteins may depend, however, on the levels of other risk factors such as body size, gender, and smoking status (11). Such dependency suggests that the information about CHD that a risk factor provides may vary among the *Apo E* genotypes. In this study we address this possibility by asking whether the utility of known CHD risk factors in the prediction of CHD death in a 5-year follow-up is the same for the two most common *Apo E* genotypes ε3/3 and ε4/3 in two cohorts of elderly Finnish men, one representing Eastern and the other Southwestern Finland.

Methods

Sample

The Seven Countries Study, which included Finland, was initiated in the late 1950's to study cardiovascular disease mortality and morbidity and related risk factor levels in different populations (12, 13). The original Finnish cohorts consisted of men born between 1900 and 1919 and living in two geographically defined rural areas, one in Eastern (n = 823) and the other in Southwestern Finland (n = 888). Of these 1711 men, 766 (45%) were still alive in 1984, when baseline assays for the current study were performed. Knowledge about both the *Apo E* genotype and risk factor levels was available from 625 (82%) of these men. In this sample, 559 (89%) had either the ε3/3 or the ε4/3 genotype. The Eastern cohort was comprised of 256 men, of whom 193 (75%) had the ε3/3 genotype. The Southwestern cohort was comprised of 303 men, of whom 196 (65%) had the ε3/3 genotype.

At the time of the follow-up survey in 1989, of the 559 men who had either the ε3/3 or the ε4/3 genotype, 387 (183 in the Eastern cohort and 204 in the Southwestern cohort) were still alive and 172 (73 (29%) in the Eastern cohort and 99 (33%) in the Southwestern cohort) had died during the 5-year follow-up period. The vital status of each man was ascertained through personal contacts, except for 11 men whose vital status was ascertained through the Finnish Population Registry. Death certificates and hospital records were obtained for all the deceased men and the cause of death was coded according to fixed criteria (13).

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 (14, 15). All participants fasted for at least 4 h before their visit to the clinic (mean fasting time was 13 h, standard deviation 5 h). 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), a measure of body size used in the current analyses.

Smoking status was assessed using a standard questionnaire (13). Participants were classified into two categories: smokers of cigarettes, cigars or pipes, and non-smokers. Participants who had not smoked for more than 1 year before the survey were classified as non-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 12×33.5 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

Serum total and high density lipoprotein (HDL) cholesterol concentrations were assayed using an enzymatic method (Monotest, Boehringer Mannheim, GmbH, Mannheim, Germany) and an Olli 3000 photometer (Kone Oy, Finland). Total cholesterol was determined from a fresh serum sample (16). HDL cholesterol was measured after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles with dextran-magnesium-chloride (16). Apo E phenotypes were determined using sera stored at -20°C until utilized in 1992. Apo E phenotyping was carried out according to a modification of the method of Havekes et al. (17), which was based on isoelectric focusing of delipidated serum followed by immunoblotting using rabbit anti-human Apo E antiserum. *Apo E* genotypes were inferred from the isoform phenotypes.

Statistical analysis

Baseline differences between the carriers of the ε3/3 and ε4/3 genotypes were tested for statistical significance by the analysis of variance (means) and Bartlett's test (variances) for quantitative risk factors and χ^2-statistics for categorical variables, seperately for the Eastern and the Southwestern cohorts. The cohorts were analyzed separately because there is ample evidence that the natural history of CHD differs between them (12, 13, 18, 19). The following risk factors were considered in the analysis: age, BMI, systolic and diastolic blood pressure, serum total and HDL cholesterol levels, and smoking status.

The Kaplan–Meier product limit method (20) was used to estimate the *Apo E* genotype-specific probability of surviving, or if an individual died, not dying from CHD, as a function of time. A logistic regression analysis (21) was employed to estimate the intragenotypic probability of CHD death in a 5-year follow-up using a model where each proposed CHD risk factor was considered in a univariable model, separately for the carriers of the ε3/3 and ε4/3 genotypes. Each risk factor that was associated with the probability of CHD death within at least one of the two *Apo E* genotype strata at the $Pr \leq 0.20$ level of statistical significance in these analyses was selected for further analyses to assess whether the observed association is homogeneous across the two *Apo E* genotype strata. This relatively high significance level was selected because the use of a more traditional test criterion ($Pr \leq 0.05$) at this point could result in disregarding risk factors that contribute significantly to the prediction of CHD probability of death when considered in a multivariable model (21). This analysis was carried out separately for the Eastern and the Southwestern cohorts.

In order to test homogeneity of the observed *Apo E* genotype-specific associations between the probability of CHD death and a proposed risk factor, we first employed a complete model where the *Apo E* genotype, a proposed CHD risk factor, and the interaction between the effects of the two *Apo E* genotypes and levels of the proposed risk factor were considered. The effects of the two *Apo E* genotypes were included in the model as a binary variable where the ε3/3 genotype was coded as 0 and the ε4/3 was coded as 1. Interaction between the *Apo E* genotypes and levels of a CHD risk factor was modeled as a product of this binary variable and the risk factor considered. The logit under this most complete model was parameterized as follows: logit of the probability of CHD death $= \alpha + \beta_1^*\varepsilon + \beta_2^*X + \beta_3^*\varepsilon^*X$, where ε represents the code of *Apo E* genotype and X represents the level of the risk factor of interest. Under this parameterization the logit of the probability of CHD death is $\alpha + \beta_2^*X$ for the carriers of the ε3/3 genotype ($\varepsilon = 0$) and $(\alpha + \beta_1) + (\beta_2 + \beta_3)^*X$ for the carriers of the ε4/3 genotype ($\varepsilon = 1$).

Second, we considered a reduced model, where the parameter β_3 was constrained to 0 and the remaining parameters of the model were re-estimated. In this reduced model the estimate of the parameter α is an estimate of the intercept for the logit in the carriers of the ε3/3 genotype, and the sum of the estimates of α and β_1 is an estimate of intercept for the logit in the carriers of the ε4/3 genotype. The estimate of the β_2 parameter in this reduced model is an estimate of the slope of the logit shared by both genotype classes. The maximum value of the likelihood of this reduced 'no interaction' model was compared with the maximum value of the likelihood of the complete model, using a likelihood ratio criterion with one degree of freedom to test whether the slopes of the logits were homogeneous among the two genotypes. Rejection of the null hypothesis that a common slope explains these data supports the hypothesis that the ability to predict the probability of CHD death from the level of a risk factor is dependent on the individual's *Apo E* genotype.

All statistical analyses were performed with the SAS statistical software package. Statistical tests

Table 1. Description of the data at baseline

Risk factor	Eastern cohort		Southwestern cohort	
	ε3/3 n = 193	ε4/3 n = 63	ε3/3 n = 196	ε4/3 n = 107
Age				
Mean (SD)	71.5 (4.9)[1]	71.7 (4.0)	73.2 (5.2)	73.0 (5.4)
BMI (kg/m^2)				
Mean (SD)	25.5 (4.1)	25.2 (3.9)	26.1 (4.2)	25.8 (3.8)
Serum total cholesterol (mmol/l)				
Mean (SD)	6.2 (1.3)	6.3 (1.4)	6.1 (1.1)	6.3 (1.4)[2]
Serum HDL cholesterol (mmol/l)				
Mean (SD)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)
Systolic blood pressure (mmHg)				
Mean (SD)	150.8 (23.0)	146.8 (21.0)	156.9 (21.6)	157.0 (22.0)
Diastolic blood pressure (mmHg)				
Mean (SD)	85.3 (10.6)	82.8 (11.2)	89.6 (9.8)	87.9 (12.1)
Smoking status				
Current (%)	20.2	24.0	16.8	20.6

[1] Heterogeneity of variance between the two genotype strata significant at Pr = 0.038.
[2] Heterogeneity of variance between the two genotype strata significant at Pr = 0.008.

that achieved the $Pr \leq 0.05$ level of probability were considered to be statistically significant unless otherwise stated.

Results

Comparison of the risk factor levels at baseline between the two common *Apo E* genotypes

The *Apo E* genotype-specific means and standard deviations of risk factor levels at baseline are given separately for the Eastern and the Southwestern cohorts in Table 1. No statistically significant heterogeneity was observed for average age, BMI, serum total and HDL cholesterol, systolic and diastolic blood pressure levels, and proportion of current smokers between the two *Apo E* genotypes in either cohort. In contrast, variance of age in the Eastern cohort was significantly larger in the carriers of the ε3/3 genotype than in the carriers of the ε4/3 genotype (Pr = 0.038). The variance of serum total cholesterol in the Southwestern cohort was significantly larger in the carriers of the ε4/3 genotype than in the carriers of the ε3/3 genotype (Pr = 0.008).

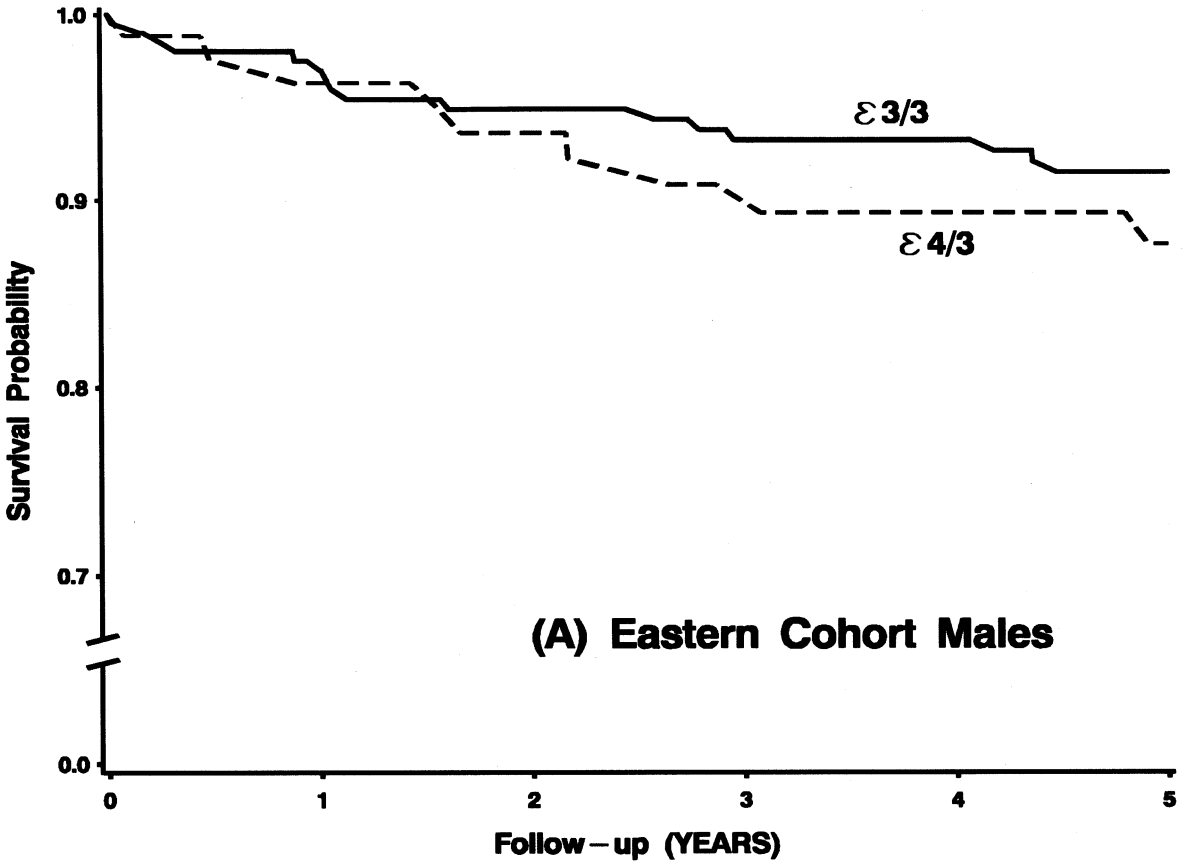
Comparison of CHD death between the two common *Apo E* genotypes

During the 5-year follow-up period, a total of 22 CHD deaths were recorded in the Eastern cohort. Of these, 15 were carriers of the ε3/3 genotype (7.8% of the ε3/3 individuals) and 7 were carriers of the ε4/3 genotype (11.1% of the ε4/3 individuals). In the Southwestern cohort, a total of 37 CHD deaths were recorded. Of these, 16 were carriers of the ε3/3 genotype (8.2% of the ε3/3 individuals) and 21 were carriers of the ε4/3 genotype (19.6% of the ε4/3 individuals). Comparison of the Kaplan–Meier survival probability curve for the carriers of the ε3/3 genotype with the curve for the carriers of the ε4/3 genotype indicates that the observed heterogeneity in CHD mortality rates between the two *Apo E* genotypes in the Eastern cohort was not statistically significant (Pr = 0.281) (Fig. 1A). In contrast, the observed heterogeneity in the CHD mortality rates between genotypes was statistically significant in the Southwestern cohort (Pr = 0.002) (Fig. 1B).

Predictive utility of known risk factors among the carriers of the two common *Apo E* genotypes

In the Eastern cohort, serum HDL cholesterol was identified as a statistically significant predictor of CHD death in the 5-year follow-up in the ε3/3 genotype at the Pr = 0.019 level of probability (Table 2). BMI contributed to the prediction of CHD death at a Pr = 0.104 level of statistical sig-

Fig. 1. Illustrations of the *Apo E* genotype-specific survival probability curves, separately for the Eastern (A) and the Southwestern (B) male cohorts. The Kaplan–Meier product limit method was used to estimate probability of surviving, or if an individual died, not dying from CHD, as a function of time, separately for the ε3/3 and ε4/3 genotypes in each cohort. The null hypothesis that the curves are homogeneous among the genotypes could not be rejected in the Eastern cohort (Pr = 0.281) while in the Southwestern cohort it was rejected (Pr = 0.002).


1.0
0.9
0.8
0.7
0.0
Survival Probability
ε3/3
ε4/3
(A) Eastern Cohort Males
0
1
2
3
4
5
Follow – up (YEARS)

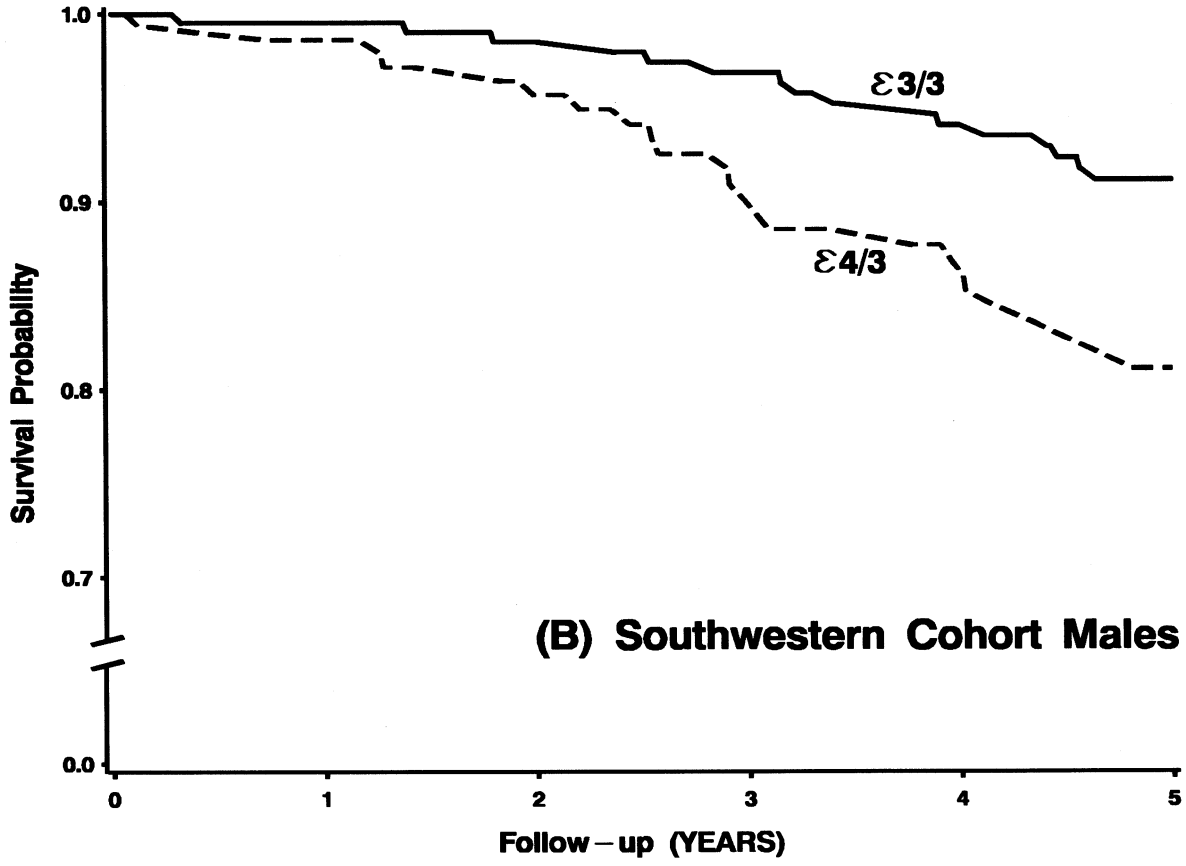

1.0
0.9
0.8
0.7
0.0
Survival Probability
ε3/3
ε4/3
(B) Southwestern Cohort Males
0
1
2
3
4
5
Follow – up (YEARS)

Table 2. Predictive utility of known risk factors among the carriers of the two common *Apo E* genotypes, separately for the two cohorts

		Eastern cohort			Southwestern cohort		
Risk factor	Parameters	Estimate	χ^2	Pr	Estimate	χ^2	Pr
Age							
ε3/3	β2	0.054	1.050	0.306	0.139	7.435	0.006
ε4/3	β2+β3	0.069	0.449	0.503	0.031	0.466	0.495
Interaction	β3	*	*	*	−0.108	2.469	0.116
BMI (kg/m^2)							
ε3/3	β2	0.107	2.640	0.104	0.149	5.819	0.016
ε4/3	β2+β3	0.064	0.397	0.529	−0.124	3.633	0.057
Interaction	β3	−0.044	0.134	0.714	−0.272	9.278	0.002
Serum total cholesterol (mmol/l)							
ε3/3	β2	−0.135	0.394	0.530	0.453	3.805	0.051
ε4/3	β2+β3	0.354	1.897	0.168	0.294	3.004	0.083
Interaction	β3	0.489	2.147	0.142	−0.159	0.300	0.584
Serum HDL cholesterol (mmol/l)							
ε3/3	β2	−2.155	5.511	0.019	−0.895	0.901	0.343
ε4/3	β2+β3	0.159	0.013	0.908	−1.357	2.588	0.108
Interaction	β3	2.314	1.782	0.182	−0.462	0.124	0.725
Systolic blood pressure (mmHg)							
ε3/3	β2	−0.009	0.545	0.466	0.012	1.024	0.312
ε4/3	β2+β3	−0.018	0.794	0.373	0.003	0.085	0.770
Interaction	β3	*	*	*	*	*	*
Diastolic blood pressure (mmHg)							
ε3/3	β2	−0.016	0.373	0.542	0.028	1.146	0.285
ε4/3	β2+β3	−0.004	0.014	0.904	−0.014	0.492	0.483
Interaction	β3	*	*	*	*	*	*
Smoking status							
ε3/3	β2	−1.335	2.313	0.128	0.551	0.751	0.386
ε4/3	β2+β3	1.012	1.405	0.236	1.438	6.995	0.008
Interaction	β3	2.347	3.567	0.059	0.887	1.229	0.268

* No test was carried out when Pr > 0.200 for each genotype.

nificance and smoking status at the Pr = 0.128 level of statistical significance. In contrast, among the carriers of the ε4/3 genotype, no risk factor was detected as a predictor of CHD death at the Pr ≤ 0.05 level of statistical significance. Serum total cholesterol was the only risk factor that contributed to the prediction of CHD death at the Pr ≤ 0.200 level of statistical significance in this genotype class.

In the Southwestern cohort, age, BMI, and serum total cholesterol level were identified as significant predictors of CHD death among the carriers of the ε3/3 genotype at the Pr = 0.05 level of probability (Table 2). In those with the ε4/3 genotype, BMI (Pr = 0.057), serum total cholesterol (Pr = 0.083), serum HDL cholesterol (Pr = 0.108), and smoking status (Pr = 0.008) each contributed to the prediction of CHD death.

Heterogeneity of the predictive utility of different risk factors among the carriers of the two common *Apo E* genotypes

In the Eastern cohort, the null hypothesis that the slopes of the logits of CHD death are homogeneous across the two common *Apo E* genotypes was rejected (Pr = 0.059) when an association between the probability of CHD death and smoking status was tested in a bivariable model (Table 2). In this case, the estimate of the slope was negative for the carriers of the ε3/3 genotype and positive for the carriers of the ε4/3 genotype. No statistically significant heterogeneity (Pr ≤ 0.05) of the slopes of the logits could be detected in this cohort when BMI and/or serum total and/or HDL cholesterol level were considered as predictors of CHD death.

In the Southwestern cohort, the null hypothesis of the homogeneity of the slopes of the logits of CHD death was rejected (Pr = 0.002) in a bivariable model when BMI was considered as a predictor (Table 2). In this case, the estimate of the slope was positive for the carriers of the ε3/3 genotype and negative for the carriers of the ε4/3 genotype. The probability of CHD death as a function of BMI based on these estimates in the Southwestern cohort is given in Fig. 2B, separately for the carriers of the ε3/3 and ε4/3 genotypes. For comparison, CHD death probabili-

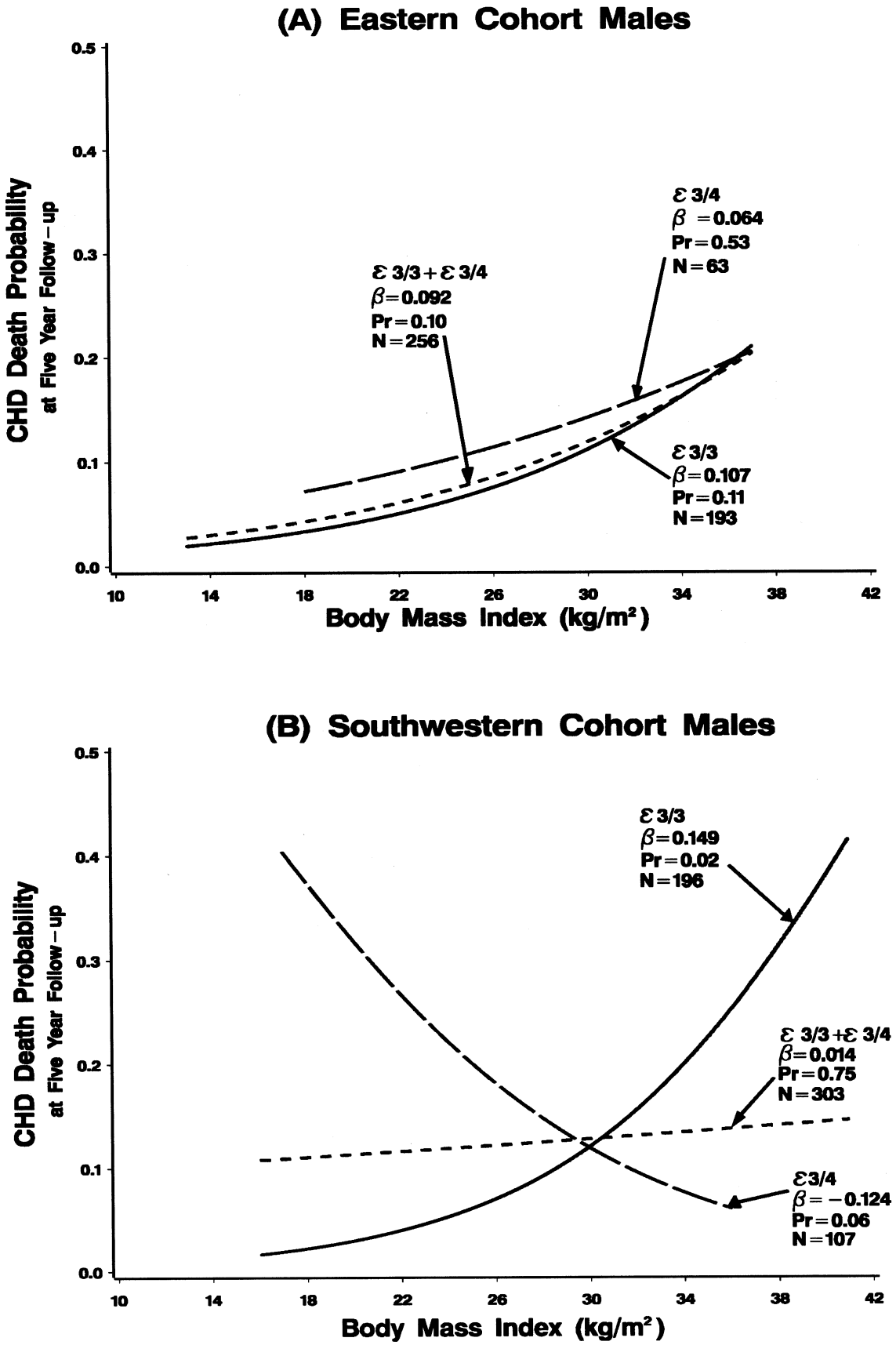


Fig. 2. Illustrations of the *Apo E* genotype-specific probabilities of CHD death as a function of BMI (kg/m^2), separately for the Eastern (A) and the Southwestern (B) male cohorts. A logistic regression analysis was employed to estimate these probabilities.

ties as a function of BMI, based on the estimates obtained in the Eastern cohort, are given in Fig. 2A. No other statistically significant heterogeneity ($Pr \leq 0.05$) of the slopes of the logits could be detected in the Southwestern cohort when the other predictors of CHD death (age, serum total and HDL cholesterol levels, and smoking status) were considered separately.

Discussion

It is widely accepted that the relative importance of risk factors in the prediction of CHD endpoints may vary among populations (1, 2, 13). There is also accumulating evidence that within a population (level A, Fig. 3) there may be subgroups of individuals (levels B–D, Fig. 3) which have different risk profiles. Recently subgroups differing in age have attracted particular attention because the predictive power of many risk factors may diminish in the elderly (22–25). Here we further considered the subdivision of the population by genotype. Our analysis considers the relative impact of area of residence, age stratification, and the *Apo E* genotype on the influence of variation in the levels of risk factors on the variation in risk of CHD death. There are both an historical and a practical rationale for considering these hierarchically defined subgroups. Initial observations in the 1950's established that both the average levels of most risk factors and CHD morbidity and mortality rates are different in Eastern Finland and Southwestern Finland (12, 13, 18, 19). Because the knowledge about the Apo E status was available only from the elderly men, age was considered in the hierarchy prior to the *Apo E* genotype. The rationale for performing a stratified evaluation of CHD risk is a response to clinical and public health needs to identify individuals (or patients) (level E, Fig. 3) who have a similar prognosis and/or who are candidates for similar therapeutic interventions.

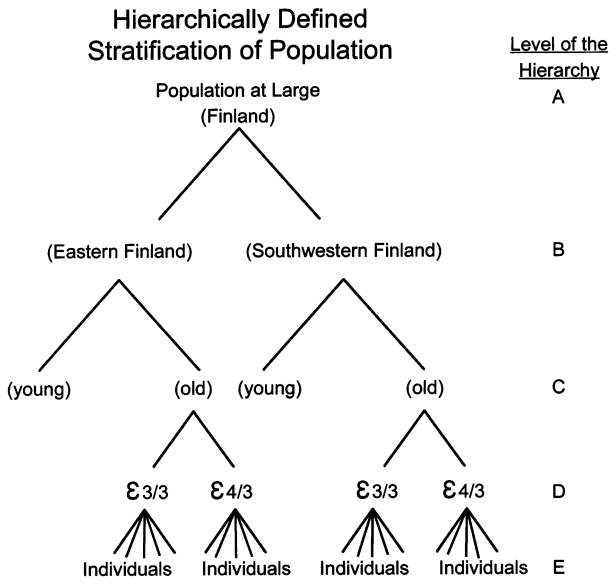


Fig. 3. In this paper we have performed a stratified risk assessment. Here we illustrate how different strata are defined. The purpose of the graph is to help visualize how the information obtained in this study can be employed in public health (level A) and in clinical (level E) practice. The letters B, C, and D refer to the levels where the splits are made in area of residence, age, and the *Apo E* genotype, respectively.

Geographic and age-specific differences in the contributions of known risk factors to the prediction of CHD death

This study, together with other studies (10, 22, 26–28), demonstrates that the risk factors, which are initially identified as predictors of CHD in middle-aged men, are also predictors in elderly men. The combinations of risk factors that were identified as predictors of CHD death in a 5-year follow-up in Eastern and Southwestern Finland in this study and in our earlier study (10) differ, however, from the combinations reported to be present in these cohorts in the 1950's, when the men were middle-aged (18, 19). In the latter studies, serum total cholesterol level, smoking, and blood pressure were major predictors of CHD endpoints in both cohorts. In the elderly survivors of these cohorts studied here, none of these risk factors was identified as a predictor of CHD death in Eastern Finland and only serum total cholesterol level and smoking status were predictors in Southwestern Finland (10) at a $Pr \leq 0.05$ level of statistical significance. A fraction of the observed age and geography-related differences in the predictive power of the CHD risk factors may reflect a difference in the age-specific death rates in the two subpopulations. In the Eastern cohort there has been an excess of individuals who have had extreme high risk factor values and who have died of CHD in the past (12). Survivors with high risk factor values may be less susceptible to atherogenesis. An alternative explanation is that the age-related changes in the predictive power of CHD risk factors may reflect biochemical and physiological changes, which are associated with the aging process *per se*, that alter an individuals atherogenic potential. On average, these age-dependent changes may differ between these subpopulations.

There is also evidence that variation in the genetic susceptibility of CHD reflected by measures of variation in the *Apo E* gene may vary among different subgroups of individuals. In our earlier

study we found that different *Apo E* genotypes were predictors of CHD death in elderly Finnish men both in Eastern Finland and in Southwestern Finland (10). The odds of CHD death during a 5-year follow-up in the carriers of the high risk ε4/3 genotype were approximately 2.5 higher than in the carriers of the ε3/3 genotype in the Southwestern cohort while in the Eastern cohort the respective ratio was 1.5. In contrast, in the Eastern cohort the odds of CHD death was approximately 7.5 times higher in the carriers of two rare ε4/2 and ε4/4 genotypes when compared with carriers of the ε3/3 genotype. Consistent with these earlier observations, comparisons of the disease-specific survival curves in the present study demonstrate an excess of CHD mortality among the carriers of the ε4/3 genotype as compared with carriers of the ε3/3 genotype. The observed difference was statistically significant in the Southwestern cohort but not in the Eastern cohort. Our earlier observation that the relative frequency of the ε4 allele was lower in the Eastern cohort than in the Southwestern cohort (29) suggests that a fraction of the geographic differences in the predictive power of the ε4/3 genotype in this study may reflect a higher selective mortality against the ε4 allele in the past in the Eastern cohort than in the Southwestern cohort.

Carriers of the ε4 allele are hypothesized to be more prone to CHD than individuals who do not carry this allele because on average they have higher serum total cholesterol levels (3, 4). Consistent with this hypothesis both the average serum total cholesterol level and its intragenotypic variation were higher in the carriers of the ε4/3 genotype than in the carriers of the ε3/3 genotype in our study in both cohorts. The difference in the interindividual variance of serum total cholesterol level between genotypes was statistically significant in the Southwestern cohort. Our observation that the CHD mortality rate was higher in the ε4/3 genotype than in the ε3/3 genotype in this cohort may thus be a consequence, at least in part, of a larger proportion of individuals with hypercholesterolemia among those with the ε4/3 genotype, which is evident from the significantly greater dispersion of serum total cholesterol level in the Southwestern cohort.

Influence of the two most common *Apo E* genotypes on predictive utility of known risk factors within population and age-defined strata

Most genetic studies assume that individuals with different genotypes respond similarly to exposures to internal (epigenetic and background genotype effects) and external (ecological) environments (30). The goal of such studies is to identify genetic polymorphisms that have an independent influence on interindividual variation in the risk of disease. Only a few studies have been carried out with the intention of characterizing the contribution of genetic variation to interindividual differences in the relationships between various risk factors and of differences in the responses to exposures to internal and/or external environments. The biological fact that the contribution of any genetic or environmental variation to the variation in the risk of CHD must be translated through variation in, and covariation between, various biochemical and physiological risk factors suggests, however, that knowledge about the other aspects of the genetic architecture of CHD may be as important for risk assessment as is the knowledge about the average genotypic differences in the risk factor levels and in the risk of the disease. This reality is demonstrated by the influence of the Apo E polymorphism on covariation between lipid and lipoprotein traits (9). The proportion of the individuals that exceed a threshold for high risk determined by multiple traits is different in different genotype groups even when the average levels of the risk factors may not differ. Recent evidence that the association between the amount of coronary calcification (an index of atherosclerosis) and plasma lipid levels may be different in different *Apo E* genotypes (31) documents the importance of the *Apo E* genotypes in determining the biological context in which the atherosclerotic process is occurring. Our study further documents that the statistical relationship between the risk of CHD death and established risk factors such as age, BMI, serum total and HDL cholesterol level, and tobacco smoking in elderly men may be different in different *Apo E* genotypes. However, since the influences of the *Apo E* genotypes on this relationship for a particular risk factor (e.g., BMI) were different in the Eastern and the Southwestern cohorts, our study suggests statistical interactions between genotype variation and variation in measures of risk that are indexed by area of residence.

Clinical and public health implications of our observations

In clinical practice, knowledge about risk factor levels is widely used to identify individuals who are at increased risk. High risk individuals are considered to be candidates for lifestyle alterations and related therapeutic interventions. The utility of genetic information both at the clinical level (level E, Fig. 3) and at the public health level (level A, Fig. 3) depends on whether it improves risk assessment.

Our study suggests that from the risk assessment point of view an important benefit of genetic knowledge is that a trait may be a significant predictor of a disease outcome only in individuals with a particular geno(me)type, even though it is not identified as a predictor in studies of the population at large. For instance, BMI is a well-known risk factor that has been positively associated with the risk of CHD in some populations (1, 2, 25) but not in others (1, 2). We did not identify BMI as a predictor of CHD death in elderly Finnish men either in the Eastern or the Southwestern cohort in this study nor in our earlier study (10). However, in the present study variation in BMI was significantly associated with variation in the risk of CHD death both in the ε3/3 ($Pr = 0.016$) and the ε4/3 ($Pr = 0.057$) genotype groups in the Southwestern cohort. The estimate of the slope was positive in the former genotype group and negative in the latter genotype group, indicating that in Southwestern Finland the risk of CHD death increases with increasing BMI in elderly men with the ε3/3 genotype, while the relative risk declines with increasing BMI in their counterparts with the ε4/3 genotype. Since weight reduction is expected to lower the risk of CHD in obese individuals (32, 33), a therapeutic implication of our result is that obese elderly men in the ε3/3 genotype group in Southwestern Finland are targets for intensified weight reduction intervention(s).

A statistically significant decline in the risk of CHD death with advancing BMI (e.g., in elderly men with the ε4/3 genotype in Southwestern Finland) is an unexpected observation that has no obvious explanation. One possibility is that a combination of obesity and the ε4/3 genotype, both of which are associated with a highly atherogenic risk factor profile, has been extremely deleterious for middle-aged men in the Southwestern cohort where the CHD mortality rate has otherwise been low in the past. Alternatively, there would have to be other, unmeasured factors indexed by low BMI, which together with the ε4/3 genotype increase the risk of CHD death in elderly men, and which have persisted in the Southwestern cohort where the CHD mortality rate has been low in the past but not in the Eastern cohort where the CHD mortality rate has been high. In any case, these findings serve as a document to why we failed to identify BMI as a predictor of CHD death in our earlier studies of the same cohort when the contribution of the *Apo E* genotypes was not considered.

In conclusion, a major finding of this study is that in elderly Finnish men the two common *Apo E* genotypes, together with the area of residence, serve to define contexts in which known risk factors such as age, BMI, serum total and HDL cholesterol, and tobacco smoking are operating in a different manner to determine the risk of CHD death. Although the first-order interactions reported here can reflect only a fraction of the complex pathobiology of CHD, they are consistent with the fact that CHD death is an emergent property of complex interactions between many genetic and environmental factors. Our observations make clear that to understand this complex pathobiology it is incumbent that we focus on the interactions between agents involved in causality rather than seeking their independent invariant properties. Stratified risk assessment serves as a strategy that takes advantage of the prognostic importance of these interactions. Identification of prognostically important strata, such as those defined by area of residence, age, and the *Apo E* genotype in this study, can result in a more precise risk assessment and ultimately improve clinical and public health strategies to prevent the disease both at individual and population levels.

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References

1. Goldberg RJ. Coronary heart disease: epidemiology and risk factors. In: Ockene IS, Ockene J, eds. Prevention of Coronary Heart Disease. New York: Little, Brown and Co, 1992: 3–39.
2. McGill HC Jr. Major risk factors and primary prevention, Part 1. In: Ross VFR, Topol EJ, eds. Atherosclerosis and Coronary Artery Disease. Philadelphia, PA: Lippincott-Raven, 1996: 25–38.
3. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988: 8: 1–21.
4. Davignon J. Apolipoprotein E polymorphism and arteriosclerosis. In: Born GVR, Schwartz CJ, eds. New Horizons in Coronary Heart Disease. London: Current Science, 1993: 5.1–5.21.
5. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am J Hum Genet 1985: 37: 268–285.
6. Kaprio J, Ferrell RE, Kottke BA, Kamboh MI, Sing CF. Effects of polymorphisms in apolipoproteins E, A-IV, and H on quantitative traits related to risk for cardiovascular disease. Arterioscler Thromb 1991: 11: 1330–1348.
7. Haviland MB, Lussier-Cacan S, Davignon J, Sing CF. Impact of apolipoprotein E genotype variation on means variances, and correlations of plasma lipid, lipoprotein, and apolipoprotein traits in octogenarians. Am J Med Genet 1995: 58: 315–331.
8. Reilly SL, Ferrell RE, Kottke BA, Kamboh MI, Sing CF. The gender-specific apolipoprotein E genotype influence on the distribution of lipids and apolipoproteins in the population of Rochester, MN. I. Pleiotropic effects on means and variances. Am J Hum Genet 1991: 49: 1155–1166.

9. Reilly SL, Ferrell RE, Sing CF. The gender-specific apolipoprotein E genotype influence on the distribution of plasma lipids and apolipoproteins in the population of Rochester, MN. III. Correlations and covariances. Am J Hum Genet 1994: 55: 1001–1018.
10. Stengård JH, Pekkanen J, Ehnolm C, Nissinen A, Sing CF. Genotypes with the apolipoprotein ε4 allele are predictors of coronary heart disease in mortality in a longitudinal study of elderly Finnish men. Hum Genet 1996: 97: 677–684.
11. Reilly SL, Kottke BA, Ferrell RE, Sing CF. The gender-specific apolipoprotein E genotype influence on the distribution of plasma lipids and apolipoproteins in the population of Rochester, MN. II. Regression relationships with concomitants. Am J Hum Genet 1992: 51: 1311–1324.
12. Pekkanen J. Coronary heart disease during a 25-year follow-up. Risk factors and their secular trends in the Finnish cohorts of the Seven Countries Study. (thesis, University of Helsinki, Finland). 1987.
13. Keys A, Aravanis C, Blackburn HW, van Buchem FSP, Buzina R, Djordjevic BS. Epidemiological studies related to coronary heart disease: characteristics of men aged 40–59 in seven countries. Acta Med Scand 1967: 460: 1–392.
14. Nissinen A, Tervahauta M, Pekkanen J, Kostiainen E, Piippo H. Levels of some biological risk indicators among elderly men in Finland. Age Ageing 1986: 15: 203–211.
15. Nissinen A, Tervahauta M, Pekkanen J, Kivinen P, Stengård J, Kaarsalo E, Kivelä S-L, Väisänen S, Salonen JT, Tuomilehto J. Prevalence and change of cardiovascular risk factors among men born 1900–1919: the Finnish cohorts of the Seven Countries Study. Age Ageing 1993: 22: 365–376.
16. Kostner GM. Enzymatic determination of cholesterol in HDL fractions prepared by polyanion precipitation. Clin Chem 1976: 22: 695.
17. Havekes LM, de Knijff P, Beisiegel U, Havinga J, Smit M, Klasen E. A rapid micromethod for apolipoprotein E phenotyping directly in serum. J Lipid Res 1987: 28: 455–463.
18. Karvonen MJ, Orma E, Punsar S, Kallio V, Arstila M, Luomanmäki K. VI. Five-year experience in Finland. Circulation 1970: XLI & XLII: I-52–I-62.
19. Heliövaara M, Karvonen MJ, Punsar S, Rautanen Y, Haapakoski J. Serum thiocyanate concentration and cigarette smoking in relation to overall mortality and to deaths from coronary heart disease and lung cancer. J Chronic Dis 1981: 34: 305–311.
20. Everitt BS. Statistical Methods in Medical Investigations. New York, NY: Halsted Press, 1994.
21. Hosmer DW, Lemeshow S. Applied Logistic Regression. Toronto: Wiley, 1989.
22. Harris T, Cook EF, Kannel WB, Goldman L. Proportional hazards analysis of risk factors for coronary heart disease in individuals aged 65 or older. J Am Geriatr Soc 1988: 36: 1023–1028.
23. Denke MA, Winker MA. Cholesterol and coronary heart disease in older adults; No easy answers. JAMA 1995: 274: 575–577.
24. Corti M-C, Guralnik JM, Salive ME, Harris T, Field TS, Wallace RB, Berkman LF, Seeman TE, Glynn RJ, Hennekens CH, Havlik RJ. HDL cholesterol predicts coronary heart disease mortality in older persons. JAMA 1995: 274: 539–544.
25. Stevens J, Cai J, Pamuk ER, Williamson DF, Thun MJ, Wood JL. The effect of age on the association between body-mass index and mortality. New Engl J Med 1998: 338: 1–7.
26. Assmann G, Schulte H. Diabetes mellitus and hypertension in the elderly: concomitant hyperlipidemia and coronary heart disease risk. Am J Cardiol 1989: 63: 33H–37H.
27. Barrett-Connor E, Suarez L, Khaw K-T, Criqui MH, Wingard DL. Ischemic heart disease risk factors after age 50. J Chronic Dis 1984: 37: 903–908.
28. Tervahauta M, Pekkanen J, Nissinen A. Risk factors of coronary heart disease and total mortality among elderly men with and without preexisting coronary heart disease. Finnish cohorts of the Seven Countries Study. J Am Coll Cardiol 1995: 26 (7): 1623–1629.
29. Stengård JH, Zerba KE, Pekkanen J, Ehnholm C, Nissinen A, Sing CF. Apolipoprotein E polymorphism predicts death from coronary heart disease in a longitudinal study of elderly Finnish men. Circulation 1995: 91: 265–269.
30. Falconer D, MacKay TFC. Introduction to Quantitative Genetics. Redwood City, CA: Addison-Wesley, 1996.
31. Kardia SLR, Haviland MB, Sing CF. Correlates of family history of coronary artery disease in children. J Clin Epidemiol 1998: 51: 473–486.
32. Ockene IS. The rationale for intervention. In: Ockene IS, Ockene J, eds. Prevention of Coronary Heart Disease. New York: Little, Brown and Co, 1992: 103–122.
33. Grundy SM. Lipids, nutrition, and coronary heart disease. In: Foster V, Ross R, Topol EJ, eds. Atherosclerosis and Coronary Artery Disease. Philadelphia: Lippinocott-Raven, 1996.