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Smoking, apolipoprotein E genotypes, and mortality (the LUdwigshafen RIsk and Cardiovascular Health study)

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Aims The genetic polymorphism of apolipoprotein E (*APOE*) has been suggested to modify the effect of smoking on the development of coronary artery disease (CAD) in apparently healthy persons. The interaction of these factors in persons undergoing coronary angiography is not known.

Methods and results We analysed the association between the *APOE-genotype*, smoking, angiographic CAD, and mortality in 3263 participants of the LUdwigshafen RIsk and Cardiovascular Health study. *APOE-genotypes* were associated with CAD [ε22 or ε23: odds ratio (OR) 0.56, 95% confidence interval (CI) 0.43–0.71; ε24 or ε34 or ε44: OR 1.10, 95% CI 0.89–1.37 compared with ε33] and moderately with cardiovascular mortality [ε22 or ε23: hazard ratio (HR) 0.71, 95% CI 0.51–0.99; ε33: HR 0.92, 95% CI 0.75–1.14 compared with ε24 or ε34 or ε44]. HRs for total mortality were 1.39 (95% CI 0.39–0.1.67), 2.29 (95% CI 1.85–2.83), 2.07 (95% CI 1.64–2.62), and 2.95 (95% CI 2.10–4.17) in ex-smokers, current smokers, current smokers without, or current smokers with one ε4 allele, respectively, compared with never-smokers. Carrying ε4 increased mortality in current, but not in ex-smokers (HR 1.66, 95% CI 1.04–2.64 for interaction). These findings applied to cardiovascular mortality, were robust against adjustment for cardiovascular risk factors, and consistent across subgroups. No interaction of smoking and ε4 was seen regarding non-cardiovascular mortality. Smokers with ε4 had reduced average low-density lipoprotein (LDL) diameters, elevated oxidized LDL, and lipoprotein-associated phospholipase A2.

Conclusion In persons undergoing coronary angiography, there is a significant interaction between *APOE-genotype* and smoking. The presence of the ε4 allele in current smokers increases cardiovascular and all-cause mortality.

Keywords C-reactive protein • Apolipoprotein E • Genotype • Inflammation • Coronary artery disease

Introduction

Smoking contributes to the development of cardiovascular disease.[1–3] In men free of symptomatic coronary artery disease (CAD), the risk of atherosclerosis conferred by smoking has been found higher in carriers of at least one ε4 allele at the apolipoprotein E (*APOE*) gene locus.[4–9] A few other studies, however, did not find such an interaction between the ε4 allele and smoking.[10–13] *APOE* is a constituent of triglyceride-rich lipoproteins and high-density lipoproteins (HDL). There are three common alleles at the *APOE* locus (ε2, ε3, and ε4) which give rise to three homozygous (ε22, ε33, ε44) and three heterozygous

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genotypes (ε23, ε24, ε34).[14] The genetic polymorphism of APOE has been suggested to modify the rate of intestinal absorption of sterols,[15,16] the receptor-mediated delivery of sterols to hepatocytes,[14] hepatic sterol production,[4] the expression of low-density lipoprotein (LDL) receptors,[14] and the concentration of C-reactive protein.[17] The *APOE* gene was among the first candidates considered relevant to the development of atherosclerosis.[18] In comparison with the wild-type allele ε3, the ε4 allele increases the risk of CAD, whereas the ε2 allele is protective.[19] Gene-centric analysis confirmed a variant at the *APOE* locus to be related with CAD at genome-wide significance.[20] The impact of smoking and the *APOE-genotype* on vascular disease has mainly been investigated in clinically asymptomatic individuals, but not in patients scheduled for coronary angiography. Further, the question whether the *APOE-genotype* influences the adverse effects of tobacco use on total mortality has not been examined. We studied the role of the *APOE-genotype* as a modifier of the effects of smoking on death from any cause and death from cardiovascular diseases in persons who had undergone coronary angiography.[21]

Methods

Study design and participants

We studied participants of the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study recruited between June 1997 and January 2000. The study design has been described.[21] For more details, please see Supplementary material online.

Laboratory investigations

Laboratory methods and lipoprotein analyses have been described.[21,22,23] The glomerular filtration rate (eGFR) was calculated from creatinine.[24] The average radius of LDL was calculated as described.[25] Oxidized LDL (oxLDL) were measured with a competitive enzyme immunoassay (Mercodia AB, Uppsala, Sweden), lipoprotein-associated phospholipase A_2 ($LpPLA_2$) with the Auto PAF-AH kit (Azwell, Inc., Osaka, Japan), and interleukin 6 (IL-6) with an enzyme immunoassay (R & D Systems, Minneapolis, MN, USA). *APOE* genotyping was performed as described.[26] Ambiguous genotypes (<1%) were simultaneously examined using commercial methodology.[27]

Statistics

Clinical characteristics were compared between never-smokers, ex-smokers, and current smokers with or without ε4 by χ^2 contingency table testing, analysis of variance (ANOVA), or logistic regression using co-variables as indicated (*Table 1*). We used the Cox proportional hazards model to examine the association between *APOE-genotype* (*Table 2*), smoking status (*Table 3*), and the interaction of these factors with mortality from all causes (*Table 4*) and with cardiovascular (*Table 5*) and non-cardiovascular (Supplementary material online, *Table S6*) causes of death. The time-to-death random variable was defined as the time period between the date of birth and the date of death or the time to the last follow-up (30 April 2010) for the censored subjects. As indicated by log-minus-log diagnostic plots, the proportional hazards assumption was met. Adjustment was carried out for sex (*Tables 4* and *5*, Model 1) or, in addition, for the use of lipid-lowering agents (>97% statins), risk factors [body mass index, diabetes mellitus, hypertension, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), log-transformed triglycerides, eGFR], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI) (Model 2). When LDL-C was replaced by apolipoprotein B, there was practically no effect on the point estimates for the respective hazard ratios (HRs) (data not shown).

The effect of smoking and the *APOE-genotype* on lipids, oxidative stress, and/or inflammation was investigated by ANOVA with covariables (*Table 6*). The assumption that residuals are normally distributed was confirmed using plots of observed vs. predicted values. We report estimated marginal means of the dependent variables along with their 95% confidence intervals (CIs). The least significant different *t*-test was used for *post hoc* comparisons (smokers with or without ε4). Tests were two-sided; $P < 0.05$ was considered significant. The SPSS 16.0 statistical package (SPSS, Inc.) was used.

Results

Patients characteristics

Current or past smoking was more prevalent among men than among women (*Table 1*). Never-smokers and ex-smokers were older than current smokers. Never-smokers had less CAD. Current smokers were more likely to present with STEMI than ex-smokers or never-smokers. Among ex-smokers, the proportion of persons presenting with stable CAD was higher than among never-smokers and current smokers. Current smokers had, on average, a lower body mass index than never-smokers or ex-smokers. The prevalence of diabetes mellitus was greater in ex-smokers than in never-smokers and current smokers. History of hypertension was not different across the categories. However, current smokers had lower mean systolic and diastolic blood pressures compared with never-smokers or ex-smokers, which may be related to the fact that these subjects more frequently presented with acute myocardial infarction. Among current smokers, non-carriers of ε4 were significantly older than carriers of ε4. No substantial differences were seen with regard to the clinical presentation, body mass index, the prevalence of diabetes mellitus, or hypertension between smokers with or without ε4. Diastolic blood pressure, however, was lower in current smokers with than in those without ε4.

APOE-genotype and mortality

Among the 3263 persons studied, 757 deaths (23.2%) occurred during a median follow-up time of 7.7 years. No relationship was seen between the *APOE-genotype* and mortality from all causes (not shown). Death certificates were available for 3239 individuals. Among these, 474 (14.5%) died due to cardiovascular diseases. When individuals were assigned to three groups according to their *APOE-genotypes* (E4: ε24, ε34, or ε44; E3: ε33; and E2: ε22, ε23), the HR for cardiovascular mortality was significantly lower in persons having either an ε22 or ε23 genotype (*Table 2*). Explaining the absence of any obvious association between total mortality and *APOE-genotypes*, non-cardiovascular mortality was increased in individuals with the genotypes ε33 or ε23 or ε22, compared with carriers of ε4 (*Table 2*). This may have been driven by an increased rate of fatal infectious disease (HR and 95% CI 1.22, 0.65–2.31, non-carriers vs. carriers of ε4) rather than cancer (HR and 95% CI 1.04, 0.65–1.66, non-carriers vs. carriers of ε4).

Table 1 Clinical characteristics of study participants according to smoking habits and *APOE-genotype*

Smoking status	Never-smokers	Ex-smokers	Current-smokers	Current-smokers without ε4	Current-smokers with ε4	*P*-value[a]	*P*-value[b]	*P*-value[c]
n	1152	1345	766	586	180			
Sex [*n* (%)]								
Men	527 (46)	1156 (86)	598 (78)	465 (79)	133 (74)	<0.001[d]	<0.001[d]	0.087[d]
Women	625 (54)	189 (14)	168 (22)	121 (21)	47 (26)			
Age (years)	65 ± 10	64 ± 10	56 ± 11	57 ± 10	55 ± 11	<0.004[e]	<0.001[e]	0.038[e]
Coronary artery disease [*n* (%)]								
None	356 (31)	196 (15)	147 (19)	109 (19)	38 (20)	<0.001[f]	<0.001[f]	0.151[f]
Stable CAD	487 (42)	732 (54)	313 (41)	251 (42)	62 (34)			
UAP (TnT−)	215 (19)	277 (21)	135 (18)	105 (18)	30 (17)			
NSTEMI (TnT+)	33 (3)	55 (4)	26 (3)	20 (3)	6 (3)			
STEMI (TnT+)	61 (5)	85 (6)	145 (19)	101 (17)	44 (24)			
Body mass index (kg/m^2)	27.4 ± 4.2	27.8 ± 3.8	27.0 ± 4.2	27.0 ± 4.2	27.0 ± 4.2	<0.001[g]	<0.001[g]	0.585[g]
Diabetes mellitus [*n* (%)]	355 (31)	478 (36)	208 (27)	163 (28)	45 (25)	0.027[h]	0.054[h]	0.734[h]
Hypertension [*n* (%)]	882 (77)	1007 (75)	485 (63)	378 (65)	107 (59)	<0.287[h]	0.217[h]	0.587[h]
Systolic blood pressure (mmHg) (means ± SD)	144 ± 23	143 ± 23	134 ± 23	135 ± 24	131 ± 22	0.008[i]	0.013[i]	0.254[i]
Diastolic blood pressure (mmHg) (means ± SD)	81 ± 11	82 ± 11	79 ± 11	80 ± 12	77 ± 11	<0.001[i]	<0.001[i]	0.006[i]

[a]*P*-value for trend: never-smokers, ex-smokers, current smokers.
[b]*P*-value for trend: never-smokers, ex-smokers, current smokers with or without ε4.
[c]*P*-value for the comparison of current smokers with or without ε4, respectively.
[d]Logistic regression, adjusted for age.
[e]ANOVA, adjusted for sex.
[f]Chi^2 test.
[g]ANOVA, adjusted for age and sex.
[h]Logistic regression, adjusted for age and sex.
[i]ANOVA, adjusted for age and sex and additionally adjusted for use of beta-blockers, ACE-inhibitors, AT1 receptor antagonists, calcium channel blockers, and diuretics.

Table 2 *APOE-genotypes* and mortality

APOE-genotype	Model 1 [HR (95% CI)]	*P*-value	Model 2 [HR (95% CI)]	*P*-value
Cardiovascular mortality				
E4 (ε24, ε34, ε44)	1.0[reference]		1.0[reference]	
E3 (ε33)	0.92 (0.75–1.14)	0.444	0.89 (0.72–1.10)	0.286
E2 (ε22, ε23)	0.71 (0.51–0.99)	0.045	0.67 (0.48–0.96)	0.026
Non-E2 (ε33, ε34, ε44)	1.0[reference]		1.0[reference]	
E2 (ε22, ε23, ε24)	0.89 (0.68–1.16)	0.379	0.88 (0.67–1.17)	0.384
Non-E4 (ε22, ε23, ε33)	1.0[reference]		1.0[reference]	
E4 (ε24, ε34, ε44)	1.13 (0.92–1.38)	0.247	1.17 (0.95–1.43)	0.146
Non-cardiovascular mortality				
E4 (ε24, ε34, ε44)	1.0[reference]		1.0[reference]	
E3 (ε33)	1.39 (1.01–1.91)	0.046	1.40 (1.01–1.93)	0.043
E2 (ε22, ε23)	1.60 (1.05–2.44)	0.030	1.50 (0.97–2.32)	0.072
Non-E2 (ε33, ε34, ε44)	1.0[reference]		1.0[reference]	
E2 (ε22, ε23, ε24)	1.20 (0.87–1.66)	0.273	1.14 (0.81–1.59)	0.463
Non-E4 (ε22, ε23, ε33)	1.0[reference]		1.0[reference]	
E4 (ε24, ε34, ε44)	0.70 (0.52–0.96)	0.028	0.71 (0.52–0.97)	0.033

Model 1: adjusted for sex.
Model 2: multifactorially adjusted for sex, use of lipid-lowering drugs (>97% statins), cardiovascular risk factors [body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log-transformed), eGRF], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI).

Table 3 Smoking and mortality

Smoking category	Model 1 [HR (95% CI)]	*P*-value	Model 2 [HR (95% CI)]	*P*-value
Total mortality				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.40 (1.16–1.68)	<0.001	1.35 (1.21–1.62)	0.002
Current smokers	2.29 (1.85–2.83)	<0.001	2.24 (1.80–2.79)	<0.0016
Cardiovascular mortality				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.33 (1.06–1.67)	0.015	1.27 (1.01–1.60)	0.044
Current smokers	1.94 (1.48–2.55)	<0.001	1.92 (1.45–2.54)	<0.001
Non-cardiovascular mortality				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.55 (1.13–2.14)	0.007	1.51 (1.10–2.09)	0.012
Current smokers	2.95 (2.05–4.23)	<0.001	2.82 (1.95–4.09)	0.001
Fatal infection				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	2.41 (1.19–4.87)	0.014	2.16 (1.06–4.41)	0.035
Current smokers	3.25 (1.42–7.46)	0.005	3.51 (1.51–8.18)	0.004
Fatal cancer				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.41 (0.83–2.38)	0.205	1.43 (0.84–2.42)	0.189
Current smokers	3.03 (1.70–5.39)	<0.001	2.73 (1.51–4.92)	0.001
Miscellaneous causes of death				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.34 (0.82–2.20)	0.241	1.32 (0.80–2.17)	0.280
Current smokers	2.71 (1.55–4.74)	<0.001	2.52 (1.41–4.48)	0.002

Model 1: adjusted for sex.
Model 2: multifactorially adjusted for sex, use of lipid-lowering drugs (>97% statins), cardiovascular risk factors [body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log-transformed), eGRF], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI).

Smoking and mortality

Both previous and current smoking increased the rate of all-cause and cardiovascular and non-cardiovascular deaths. After adjusting for sex (*Table 3*, Model 1) and for other risk factors (*Table 3*, Model 2), ex-smokers were at moderately, but significantly increased risk of death. Current smokers were at higher risk to die than never-smokers and ex-smokers. Among non-cardiovascular deaths, smoking increased the rates of fatal infections and cancer at approximately the same degree (*Table 3*).

Interaction of the *APOE-genotype* and smoking

There was no relationship between the *APOE-genotype* and mortality from all causes or from cardiovascular causes in both never-smokers and ex-smokers (Supplementary material online, *Tables S1* and *S2*). We therefore stratified the cohort into four groups, namely never-smokers, ex-smokers, current smokers with, and current smokers without an ε4 allele. Among current smokers, mortality was higher in those with ε4 (HR 2.95, 95% CI 2.10–4.17) than without (HR 2.07, 95% CI 1.64–2.62, *Table 4*, Model 1A). We further analysed the relationship between the *APOE-genotype*, smoking, and mortality in a Cox model allowing for interaction between the presence of ε4 and smoking (*Table 4*, Model 1B). This demonstrated a statistically significant interaction only between current smoking and the presence of an ε4 allele (HR 1.66, 95% CI 1.04–2.64). Including cardiovascular risk factors and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI) as co-variables only slightly modified these findings (*Table 4*, Models 2A and 2B).

Beyond mortality from all causes, we studied cardiovascular mortality in relation to the *APOE-genotype* and smoking. Compared with never-smokers, HRs for cardiovascular death were 1.32, 1.94, 1.74, and 2.79 in ex-smokers, current smokers, current smokers without ε4, and current smokers with ε4, respectively (*Table 5*, Model 1A). These HRs did not change substantially upon adjustment for major cardiovascular risk factors.

Mortality from all causes and cardiovascular mortality were also higher in smokers with ε4 than in those without ε4 when we considered persons without or with angiographic CAD separately. However, due to smaller numbers of events in each of the subgroups, the smoking and ε4 interaction was not significant in persons without CAD, and it approached (but did not reach)

Table 4 Hazard ratios for death from all causes according to smoking status and *APOE-genotype* in 3263 persons undergoing coronary angiography

Smoking status	Model 1 [HR (95% CI)]	*P*-value	Model 2 [HR (95% CI)]	*P*-value
Model A				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.39 (1.16–1.67)	<0.001	1.34 (1.12–1.62)	0.002
Current smokers[a]	2.29 (1.85–2.83)	<0.001	2.24 (1.80–2.79)	0.002
Current smokers without ε4	2.13 (1.69–2.68)	<0.001	2.07 (1.64–2.63)	<0.001
Current smokers with ε4	2.95 (2.10–4.17)	<0.001	2.97 (2.09–4.21)	<0.001
Model B				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.37 (1.16–1.67)	0.003	1.31 (1.06–1.63)	0.012
Current smokers[a]	2.61 (2.05–3.32)	<0.001	2.54 (1.99–3.25)	<0.001
ε4 allele carriers	1.06 (0.89–1.26)	0.535	1.08 (0.91–1.29)	0.389
Ex-smokers × ε4	0.95 (0.64–1.41)	0.789	0.92 (0.62–1.36)	0.676
Current smokers × ε4	1.66 (1.04–2.64)	0.034	1.64 (1.03–2.61)	0.039

Model 1: adjusted for sex.
Model 2: multifactorially adjusted for sex, use of lipid-lowering drugs (>97% statins), cardiovascular risk factors [body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log-transformed), eGRF], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI).
Model A: no interaction terms.
Model B: including smoking (never, previous, or current) and absence or presence of at least one ε4 allele as main effects and in addition an interaction term smoking × ε4.
[a]Includes both current smokers with and without ε4.

Table 5 Hazard ratios for death from cardiovascular causes according to smoking and *APOE-genotype* in 3250 persons undergoing coronary angiography

Smoking status	Model 1 [HR (95% CI)]	*P*-value	Model 2 [HR (95% CI)]	*P*-value
Model A				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.32 (1.05–1.66)	0.017	1.26 (1.00–1.59)	0.047
Current smokers[a]	1.94 (1.48–2.55)	<0.001	1.92 (1.45–2.54)	<0.001
Current smokers without ε4	1.74 (1.29–2.35)	<0.001	1.72 (1.27–2.33)	0.001
Current smokers with ε4	2.79 (1.82–4.29)	<0.001	2.81 (1.82–4.35)	<0.001
Model B				
Never-smokers	1.0[reference]		1.0[reference]	
Ex-smokers	1.30 (1.01–1.68)	0.046	1.23 (0.95–1.59)	0.112
Current smokers[a]	2.21 (1.64–2.98)	<0.001	2.18 (1.61–2.95)	<0.001
ε4 allele present	1.24 (1.00–1.54)	0.049	1.28 (1.03–1.59)	0.026
Ex-smokers × ε4	0.95 (0.59–1.52)	0.817	0.93 (0.58–1.49)	0.752
Current smokers × ε4	1.74 (0.98–3.09)	0.060	1.69 (0.95–3.01)	0.074

Model 1: adjusted for sex.
Model 2: multifactorially adjusted for sex, use of lipid-lowering drugs (>97%), cardiovascular risk factors [body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log transformed), eGRF], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI).
Model A: no interaction terms.
Model B: including smoking (never, previous, or current) and absence or presence of at least one ε4 allele as main effects and in addition an interaction term smoking × ε4.
[a] Includes both current smokers with and without ε4.

statistical significance in the CAD group (Supplementary material online, *Tables S3* and *S4*). We also examined whether individual causes of cardiovascular deaths were different in smokers with ε4 compared with smokers without ε4. This was true for death from CAD, stroke, congestive heart failure, but not for death from sudden cardiac death (Supplementary material online, *Table S5*).

We also examined whether the presence of an ε4 allele amplified the risk to die from non-cardiovascular causes in current smokers. As shown in Supplementary material online, *Table S6*, ex-smokers and even more so current smokers were at increased risk of mortality. However, presence or absence of an ε4 allele did obviously not essentially modify the risk conferred by current

Table 6 Estimated marginal means of biological markers according to smoking habits and apo E genotype[a]

Smoking status	Never-smokers	Ex-smokers	Current smokers without ε4	Current smokers with ε4	*P*-value[b]	*P*-value[c]
LDL-C (g/L)	1.16 (1.14–1.18)	1.16 (1.14–1.18)	1.18 (1.15–1.21)	1.21 (1.16–1.26)	0.214	0.358
HDL-C (g/L)	0.39 (0.39–0.40)	0.39 (0.39–0.40)	0.37 (0.37–0.38)	0.36 (0.35–0.38)	<0.001	0.174
Triglycerides[d] (g/L)	1.45 (1.41–1.49)	1.53 (1.50–1.57)	1.57 (1.51–1.63)	1.67 (1.55–1.79)	<0.001	0.128
Apo B (g/L)	1.03 (1.01–1.04)	1.04 (1.03–1.05)	1.06 (1.04–1.08)	1.12 (0.1.09–1.17)	<0.001	0.002
Apo AI (g/L)	1.30 (1.29–1.31)	1.31 (1.30–1.32)	1.26 (1.24–1.28)	1.24 (1.21–1.28)	<0.001	0.282
Apo E (mg/L)	89 (87–91)	90 (88–92)	93 (90–96)	85 (80–91)	0.034	0.011
Apo CIII (mg/L)	142 (139–145)	148 (145–151)	148 (145–151)	151 (144–159)	0.014	0.150
LDL–apo B (g/L)	0.84 (0.83–0.85)	0.85 (0.84–0.86)	0.86 (0.85–0.88)	0.90 (0.87–0.94)	0.002	0.029
LDL–apo B/LDL-C	0.74 (0.73–0.74)	0.74 (0.74–0.75)	0.74 (0.73–0.75)	0.78 (0.76–0.79)	<0.001	<0.001
LDL particle radius (nm)	8.28 (8.26–8.29)	8.27 (8.26–8.29)	8.31 (8.30–8.33)	8.23 (8.20–8.26)	<0.001	<0.001
oxLDL (U/L)	73 (70–74)	75 (73–77)	76 (74–79)	82 (78–87)	<0.001	0.020
$LpPLA_2$ activity (U/L)	471 (464–478)	469 (462–475)	486 (476–496)	517 (499–535)	0.009	0.002
Homocysteine (μmol/L)	13 (13–13)	13 (13–13)	14 (14–15)	15 (14–16)	<0.001	0.105
IL-6 (ng/L)	4.62 (4.27–4.97)	5.11 (4.79–5.43)	6.15 (5.67–6.63)	7.11 (6.21–8.00)	<0.001	0.057

[a]Estimated marginal means and 95% CIs obtained in a general linear model (ANOVA) adjusted for age and sex, use of lipid-lowering drugs (>97% statins), cardiovascular risk factors [body mass index, diabetes mellitus, hypertension, LDL-C, HDL-C, triglycerides (log-transformed), eGFR], and clinical presentation (no CAD, stable CAD, UAP, NSTEMI, or STEMI).
[b]Overall *P*-values.
[c]*P*-values for the comparison between current smokers without or with ε4, respectively.
[d]The estimated marginal means and confidence intervals of logarithmically transformed triglycerides have been back-transformed to the original scale.

smoking. Looking at individual categories among non-cardiovascular deaths revealed that the presence of an ε4 allele increased the risk of both death from cancer and from infection, but this was outweighed by the fact that other non-cardiovascular deaths were less frequent in current smokers with ε4 than in those without ε4.

Smoking, *APOE-genotype*, and markers of lipid metabolism, inflammation, and oxidative stress

We used ANOVA to investigate the effects of *APOE-genotypes* on markers of lipid metabolism, inflammation, and oxidative stress. LDL-C, HDL-C, triglycerides, apoliproteins AI and CIII, and homocysteine were not significantly different between current smokers with ε4 allele and without ε4 allele (*Table 6*). However, current smokers with ε4 allele had, on average, higher concentrations of apo B, oxLDL, $LpPLA_2$ activity, and IL-6 ($P = 0.057$), whereas the concentration of apo E and LDL particle radius was lower.

Discussion

We completed the first study of the interaction between the *APOE-genotype* and smoking in relation to cardiovascular risk and mortality in persons undergoing coronary angiography. The key result is that the presence of at least one ε4 allele increases the risk of mortality from any cause and mortality due to cardiovascular diseases in individuals who smoke. No such clear interaction was found between ε4 and smoking as risk factors of non-cardiovascular mortality.

The *APOE-genotype* has for almost 30 years been implicated in the development of atherosclerosis.[18,19] Mortality from all causes was not linked to any of the less frequent *APOE* alleles. When we subsequently analysed individual categories of death, the ε2 allele was associated with lower cardiovascular mortality, while there was a (non-significant) tendency towards higher cardiovascular mortality in carriers of ε4. Of interest, the opposite was true for non-cardiovascular mortality: there appeared to be a higher incidence rate of deaths in the absence of ε4, which may have been driven by fatal infectious diseases rather than by fatal cancer. Owing to a limited number of deaths due to non-cardiovascular causes, the tendency for increased death from infection was not statistically significant. We are also not able to decide whether this finding reflects an increased risk for a competing cause of death or a specific protection from infection by the ε4 allele. At least, a previously established role of *APOE* in immune modulation would support the latter,[28] and speculations exist that the ε4 may have conferred an advantage during evolution by mediating resistance to infectious diseases.[29]

Previous and more so current smoking was independently associated with total mortality, cardiovascular mortality, and non-cardiovascular mortality (fatal infection and death from cancer). We have particularly been interested in the hypothesis that the ε4 allele modifies the adverse effects of smoking. We found a strong and more than additive interaction of current smoking and possession of at least one ε4 allele; the latter attains significant prognostic importance in current smokers only, but not in never-smokers and ex-smokers. On the other hand, both previous and more so current smoking remain strong predictors of death even in the absence of ε4.

The effects of smoking and the *APOE-genotype* have so far been studied in population-based studies such as the Northwick Park Heart Study,[5] the Framingham Offspring Study,[6,9] and the National Heart, Lung, and Blood Institute Family Heart Study[8] showing that adverse cardiovascular effects of ε4 particularly occur in current smokers. Our study confirms and extends this work to persons undergoing coronary angiography who are at an intermediate-to-high risk of future cardiovascular events. It further demonstrates for the first time that smoking and the *APOE-genotype* relevantly interact in predicting total mortality.

We sought to evaluate mechanisms underlying the smoking and ε4 interaction. We therefore compared lipoproteins, along with markers of inflammation and oxidative stress in current smokers with or without ε4. Although LDL-C did not significantly differ between the two groups, current smokers carrying ε4 had significantly higher concentrations of apo B and LDL-associated apo B. The ratio of apo B to cholesterol in LDL was greater and the calculated diameter of LDL was significantly lower in smoking carriers of ε4, indicating increasing proportions of small and dense LDL in carriers of ε4.[30] Decreased lipoprotein lipase activity as a consequence of smoking is unlikely to contribute to this finding, because the largest difference in LDL particle diameter occurred between current smokers carrying or not carrying an ε4 allele and because LDL particle diameters presented have been adjusted for triglycerides. Small and dense LDL appear to be cleared from the plasma at a decreased rate,[31] possibly by cell-surface sites different from the LDL receptor.[32,33] As a consequence of their prolonged time of residence in the circulation and their decreased content of antioxidants (e.g. vitamin E or ubiquinone), small, dense LDL are more susceptible to oxidation.[34–37] Evidently, as long as oxidative stress is low, this may leave LDL unaffected. However, once small, dense LDL are exposed to pro-oxidants in smokers, appreciable amounts of oxLDL (and foam cells) may be generated. It is in line with these considerations that we found higher concentrations of oxLDL and higher activities of $LpPLA_2$, a marker of unstable plaques, in current smokers with ε4 compared with those without ε4. A report by Talmud *et al.*[9] points in the same direction. They found elevated oxLDL in the plasma of smokers with ε4. It is yet an open question whether this is due to a direct effect of the *APOE-genotype* on the antioxidant capacity of the plasma. At least theoretically, such a possibility exists. Apo E2 contains two and apo E3 contains one free thiol groups per molecule, although no free thiol group is available in apo E4. Apo E4 may therefore have completely lost any anti-oxidative capacity.[38,39] However, we believe that this is hardly relevant *in vivo*: Only one out of five LDL particles contains one molecule of *APOE*[40]; *APOE*-containing particles are rapidly cleared,[41] and apo E4 preferentially associates with HDL, but less with LDL. Thus, predominance of small, dense LDL caused by ε4 may largely explain the observed ε4 × smoking interaction.

Are there clinical consequences of the current results? Advice to quit smoking should be given to any individual, regardless of the *APOE-genotype*. This in particular applies to persons at elevated risk of cardiovascular events as they participated in the LURIC study. Good reasons might exist, however, to obtain *APOE-*

genotypes in persons who fail to quit smoking, because their risk is still amplified considerably. First, it has been demonstrated that awareness of individual genetic susceptibility factors could potentially enhance interventions aiming at smoking cessation.[42] Second, because the effects of ε4 may be attributable to the accumulation of small dense LDL, lipid-lowering treatment might be further intensified following detection of ε4 in smokers.

This is the first investigation to demonstrate a significant interaction between the ε4 allele at the *APOE* locus and current smoking as risk factors predicting cardiovascular and all-cause mortality. Future research will have to address whether or not the assessment of the *APOE-genotype* possibly along with other susceptibility genes will support efforts to prevent CAD by behavioural changes.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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References

1. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004;**364**:937–952.
2. Doll R, Peto R, Boreham J, Sutherland I. Mortality in relation to smoking: 50 years' observations on male British doctors. *BMJ* 2004;**328**:1519.
3. Raupach T, Schäfer K, Konstantinides S, Andreas S. Secondhand smoke as an acute threat for the cardiovascular system: a change in paradigm. *Eur Heart J* 2006;**27**:386–392.
4. Miettinen TA, Gylling H, Vanhanen H, Ollus A. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arterioscler Thromb* 1992;**12**:1044–1052.
5. Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet* 2001;**358**:115–119.
6. Lahoz C, Schaefer EJ, Cupples LA, Wilson PW, Levy D, Osgood D, Parpos S, Pedro-Botet J, Daly JA, Ordovas JM. Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. *Atherosclerosis* 2001;**154**:529–537.
7. Karvonen J, Kauma H, Kervinen K, Ukkola O, Rantala M, Paivansalo M, Savolainen MJ, Kesaniemi YA. Apolipoprotein E polymorphism affects carotid artery atherosclerosis in smoking hypertensive men. *J Hypertens* 2002;**20**:2371–2378.
8. Djousse L, Myers RH, Province MA, Hunt SC, Eckfeldt JH, Evans G, Peacock JM, Ellison RC. Influence of apolipoprotein E, smoking, and alcohol intake on carotid atherosclerosis: National Heart, Lung, and Blood Institute Family Heart Study. *Stroke* 2002;**33**:1357–1361.
9. Talmud PJ, Stephens JW, Hawe E, Demissie S, Cupples LA, Hurel SJ, Humphries SE, Ordovas JM. The significant increase in cardiovascular disease risk in APOEepsilon4 carriers is evident only in men who smoke: potential relationship between reduced antioxidant status and ApoE4. *Ann Hum Genet* 2005;**69**:613–622.
10. Scuteri A, Bos AJ, Zonderman AB, Brant LJ, Lakatta EG, Fleg JL. Is the apoE4 allele an independent predictor of coronary events? *Am J Med* 2001;**110**:28–32.
11. Volcik KA, Barkley RA, Hutchinson RG, Mosley TH, Heiss G, Sharrett AR, Ballantyne CM, Boerwinkle E. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC study participants. *Am J Epidemiol* 2006;**164**:342–348.
12. Wilson PW, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study. *JAMA* 1994;**272**:1666–1671.
13. Ward H, Mitrou PN, Bowman R, Luben R, Wareham NJ, Khaw KT, Bingham S. APOE-genotype, lipids, and coronary heart disease risk: a prospective population study. *Arch Intern Med* 2009;**169**:1424–1429.
14. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;**1**:507–537.
15. Uusitupa MI, Miettinen TA, Sarkkinen ES, Ruuskanen E, Kervinen K, Kesaniemi YA. Lathosterol and other non-cholesterol sterols during treatment of hypercholesterolaemia with beta-glucan-rich oat bran. *Eur J Clin Nutr* 1997;**51**:607–611.
16. Tammi A, Ronnemaa T, Rask-Nissila L, Miettinen TA, Gylling H, Valsta L, Viikari J, Valimaki I, Simell O. Apolipoprotein E phenotype regulates cholesterol absorption in healthy 13-month-old children—The STRIP Study. *Pediatr Res* 2001;**50**:688–691.
17. März W, Scharnagl H, Hoffmann MM, Boehm BO, Winkelmann BR. The apolipoprotein E polymorphism is associated with circulating C-reactive protein (the Ludwigshafen Risk and Cardiovascular Health Study). *Eur Heart J* 2004;**25**:2109–2119.
18. Menzel HJ, Kladetzky RG, Assmann G. Apolipoprotein E polymorphism and coronary artery disease. *Arteriosclerosis* 1983;**3**:310–315.
19. Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J. Association of apolipoprotein E genotypes with lipid levels and coronary risk. *JAMA* 2007;**298**:1300–1311.
20. IBC 50k CAD consortium. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS Genet* **7**:e1002260.
21. Winkelmann BR, März W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J. Rationale and design of the LURIC study—a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics* 2001;**2**(1 Suppl. 1):S1–S73.
22. Wanner C, Hörl WH, Luley C, Wieland H. Effects of HMG-CoA reductase inhibitors in hypercholesterolemic patients on hemodialysis. *Kidney Int* 1991;**31**:754–760.
23. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2006;**29**(Suppl. 1):S43–S48.
24. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;**130**:461–470.
25. Baumstark MW, Kreutz W, Berg A, Frey I, Keul J. Structure of human low-density lipoprotein subfractions, determined by X-ray small-angle scattering. *Biochim Biophys Acta* 1990;**1037**:48–57.
26. Zivelin A, Rosenberg N, Peretz H, Amit Y, Kornbrot N, Seligsohn U. Improved method for genotyping apolipoprotein E polymorphisms by a PCR-based assay simultaneously utilizing two distinct restriction enzymes. *Clin Chem* 1997;**43**:1657–1659.
27. Nauck M, Hoffmann MM, Wieland H, März W. Evaluation of the apo E genotyping kit on the LightCycler. *Clin Chem* 2000;**46**:722–724.
28. Zhang H, Wu LM, Wu J. Cross-talk between apolipoprotein E and cytokines. *Mediators Inflamm* 2011;**2011**:949072.
29. Finch CE, Morgan TE. Systemic inflammation, infection, ApoE alleles, and Alzheimer disease: a position paper. *Curr Alzheimer Res* 2007;**4**:185–189.
30. Skoglund-Andersson C, Ehrenborg E, Fisher RM, Olivecrona G, Hamsten A, Karpe F. Influence of common variants in the CETP, LPL, HL and APO E genes on LDL heterogeneity in healthy, middle-aged men. *Atherosclerosis* 2003;**167**:311–317.

31. Packard CJ, Demant T, Stewart JP, Bedford D, Caslake MJ, Schwertfeger G, Bedynek A, Shepherd J, Seidel D. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J Lipid Res* 2000;**41**:305–318.
32. Shepherd J, Caslake MJ, Lorimer AR, Vallance BD, Packard CJ. Fenofibrate reduces low density lipoprotein catabolism in hypertriglyceridemic subjects. *Arteriosclerosis* 1985;**5**:162–168.
33. Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *J Lipid Res* 1998;**39**:1263–1273.
34. deGraaf J, Hak-Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, Stalenhoef AFH. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction. *Arterioscler Thromb* 1991;**11**: 298–306.
35. Chait A, Brazg RL, Tribble D, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 1993;**94**:350–355.
36. Goulinet S, Chapman MJ. Plasma LDL and HDL subspecies are heterogenous in particle content of tocopherols and oxygenated and hydrocarbon carotenoids. Relevance to oxidative resistance and atherogenesis. *Arterioscler Thromb Vasc Biol* 1997;**17**:786–796.
37. Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am J Med* 2001;**110**:103–110.
38. Smith JD, Miyata M, Poulin SE, Neveux LM, Craig WY. The relationship between apolipoprotein E and serum oxidation-related variables is apolipoprotein E phenotype dependent. *Int J Clin Lab Res* 1998;**28**:116–121.
39. Jolivalt C, Leininger-Muller B, Bertrand P, Herber R, Christen Y, Siest G. Differential oxidation of apolipoprotein E isoforms and interaction with phospholipids. *Free Radic Biol Med* 2000;**28**:129–140.
40. März W, Baumstark MW, Scharnagl H, Ruzicka V, Buxbaum S, Herwig J, Pohl T, Russ A, Schaaf L, Berg A, Böhles H-J, Usadel KH, Groß W. Accumulation of 'small dense' low density lipoproteins in a homozygous patient with familial defective apolipoprotein B-100 results from heterogenous interaction of LDL-subfractions with the LDL receptor. *J Clin Invest* 1993;**92**:2922–2933.
41. Schäfer J, Scharnagl H, Baumstark MW, Schweer H, Zech LA, Seyberth H, Winkler K, Steinmetz A, März W. Homozygous familial defective apolipoprotein B-100. Enhanced removal of apolipoprotein E-containing VLDLs and decreased production of LDLs. *Arterioscler Thromb Vasc Biol* 1997;**17**:348–353.
42. Sanderson SC, Michie S. Genetic testing for heart disease susceptibility: potential impact on motivation to quit smoking. *Clin Genet* 2007;**71**:501–510.