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Clinical research

The apolipoprotein E polymorphism is associated with circulating C-reactive protein (the Ludwigshafen risk and cardiovascular health study)

Winfried März[a,*], Hubert Scharnagl[a], Michael M. Hoffmann[b], Bernhard O. Boehm[c], Bernhard R. Winkelmann[d]

[a] *Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbrugger Platz 15, A-8036 Graz, Austria*
[b] *Division of Clinical Chemistry, Department of Medicine, University of Freiburg, Germany*
[c] *Division of Endocrinology, Department of Medicine, University Hospital, Ulm, Germany*
[d] *Cooperation Unit of Pharmacogenomics and Applied Genome Research, University of Heidelberg, Germany*

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Background Statins and cholesterol absorption inhibitors lower the concentration of C-reactive protein (CRP). The genetic polymorphism of apolipoprotein (apo) E is a strong endogenous determinant of sterol homeostasis. We therefore examined the relationship of CRP to the apoE polymorphism.
Methods and results We studied 739 and 570 subjects with or without stable angiographic coronary artery disease (CAD), respectively. In carriers of apoE2, apoB was lower ($P < 0.001$) than in apoE3/3 homozygotes; in individuals with apoE3/4 and apoE4/4, it was higher ($P < 0.001$). Both in the presence and absence of CAD, CRP was higher in carriers of apoE2 ($P = 0.002$) and apoE3/3 homozygotes ($P = 0.032$) than in individuals with apoE3/4 or apoE4/4. Fibrinogen and white cell count were not related to the apoE genotype. CRP was associated with CAD. Compared to the lowest tertile, crude odds ratios were 1.87 (95% confidence interval (CI), 1.43–2.45, $P < 0.001$) and 2.24 (95% CI, 1.71–2.94, $P < 0.001$) in the second and third tertile. In carriers of apoE2, the use of tertiles defined in controls with apoE2 only diminished the odds ratios for CAD. In apoE3/4 heterozygotes or apoE4/4 homozygotes, the use of tertiles specific for this group only slightly increased the odds ratios.
Conclusions: The concentration of CRP, but not fibrinogen nor white blood cells is associated with the apoE polymorphism. The activity of the mevalonate pathway in the liver may be related to the metabolism of CRP. The predictive value of CRP for CAD may be modified by the apoE polymorphism.

Introduction

Atherosclerosis shares many characteristics of chronic inflammatory diseases.[1] Acute phase reactants like C-reactive protein (CRP),[2,3] white blood cells,[4] serum

* Corresponding author. Tel.: +43 316 385 3145; fax: +43 316 385 3430.
E-mail address: winfried.maerz@klinikum-graz.at (W. März).

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amyloid A,[5] fibrinogen,[4] and interleukin 6[6] are predictive of future cardiovascular events. Statins have consistently been shown to lower CRP.[7–9] CRP is produced in the liver in response to pro-inflammatory cytokines like interleukin 6, which is released from activated cells at the site of inflammation.[10] We and others [8,11–15] recently observed that the decreases in CRP generated by statins are not accompanied by significant decreases in interleukin 6. Statins may therefore lower CRP by mechanisms not related to systemic inflammation. We hypothesised that such alternative mechanisms may link the production and/or release of CRP to the sterol balance in liver cells.[14]

The common genetic polymorphism of apolipoprotein (apo) E is among the strongest endogenous determinants of hepatic sterol metabolism. In the plasma, apoE is found as a constituent of triglyceride-rich lipoproteins and high density lipoproteins; its major function is to mediate the binding of lipoprotein particles to cell surface receptors. There are three common alleles at the apoE locus, designated *e*2, *e*3, *e*4, giving rise to three homozygous (designated apoE2/2, apoE3/3, apoE4/4) and three heterozygous phenotypes (designated apoE3/2, apoE4/2, apoE4/3).[16,17] The polymorphism of apoE modifies the rate of sterol absorption in the intestine,[18–20] the receptor-mediated delivery of sterols to liver cells,[16,17] hepatic sterol production,[18,21] and the expression of LDL receptors.[16,17]

To investigate further the hypothesis that changes in hepatic sterol homeostasis might specifically affect the expression of CRP, we examined the relationship between the polymorphism of apoE and circulating levels of CRP in patients with and without angiographically documented coronary artery disease (CAD). Fibrinogen and white cell count were included in the study in an attempt to exclude the possibility that the apoE polymorphism itself differentially affects low-grade inflammation.

Materials and methods

Study design and participants

To examine the relationships between the apoE polymorphism and markers of inflammation, we studied white participants of the Ludwigshafen risk and cardiovascular health (LURIC) study. LURIC is an ongoing prospective hospital-based cohort study of white individuals investigating environmental, biochemical and genetic risk factors for CAD.[22] Between June 1997 and January 2000, 3297 German patients who had undergone coronary angiography at the Ludwigshafen General Hospital were included. The study was approved by the institutional review board at the ''Ärztekammer Rheinland-Pfalz''. Informed written consent was obtained from each of the participants. With the exception of acute coronary syndromes, the patients had to present in a stable clinical condition without major concomitant non-cardiovascular disease. CAD was assessed angiographically using the maximum luminal narrowing estimated by visual analysis. In LURIC, clinically relevant CAD was defined as the occurrence of at least one stenosis $\geqslant$20% in at least one of 15 coronary segments.[23] Individuals with stenoses <20% were considered as controls. To examine the impact of other definitions of CAD on the current analysis, we provisionally used the presence of one or more stenoses $\geqslant$50% as a criterion. This did not materially change our results.

Individuals were classified as having diabetes mellitus if plasma glucose was >1.25 g/L in the fasting state or >2.00 g/L two hours after the oral glucose load (performed in all subjects not previously diagnosed as having DM), respectively,[24] or if individuals were receiving oral anti-diabetics or insulin. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mmHg or if there was a significant history of hypertension.

We wished to eliminate confounding by lipid lowering drugs and acute phase reactions which both affect CRP concentrations. Out of 3279 individuals with coronary angiograms, 1040 presented with unstable angina, non-ST-elevation myocardial infarction (Troponin $T \geqslant 0.1$ μg/L), or ST-elevation myocardial infarction (Troponin $T \geqslant 0.1$ μg/L) who were excluded from the current evaluation. Out of the 2239 remaining subjects, 1321 were not using any lipid lowering drugs, and among these lipids, lipoproteins, apolipoproteins, apoE genotype, CRP, fibrinogen, and white cell count were available in 1309 individuals who were finally analysed.

Laboratory procedures

Lipoproteins and apolipoproteins. Fasting blood samples were obtained by venipuncture in the early morning. Lipoproteins were separated by a combined ultracentrifugation—precipitation method (ß-quantification).[22,25] Cholesterol and triglycerides were measured with enzymatic reagents from WAKO (Neuss, Germany), apolipoprotein B (apoB) and apoE by turbidimetry (Greiner, Flacht, Germany) on a WAKO R30 or Olympus AU640 analyser.[22] ApoE genotyping was performed by allele-specific restriction enzyme analysis with *Afl*III and *Hae*II as described,[26] with the exception that the restriction was carried out separately with each enzyme. Ambiguous genotypes (<1%) were simultaneously examined using commercial methodology.[27]

Other methods. 'Sensitive' CRP was measured by immunonephelometry on a Behring Nephelometer II (N High Sensitivity CRP, Dade Behring, Marburg, Germany). In this assay, the limit of detection for CRP is 0.17 mg/L; it is linear up to 500 mg/L. The lowest and the highest CRP concentrations encountered in this study were 0.17 and 142 mg/L, respectively. White cell counts were obtained on a Technicon H-1 or an Advia 120 analyser (Bayer Diagnostics, Leverkusen, Germany). Fibrinogen was measured according to Clauss (Dade Behring, Marburg, Germany). Blood glucose was determined enzymatically using the hexokinase/glucose-6-phosphate dehydrogenase method (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

CRP was transformed logarithmically before being used in parametric statistical procedures. Clinical and anthropometric characteristics were compared between CAD patients and controls by analysis of variance (ANOVA) or logistic regression using gender as a co-variable (Table 1). We also used logistic regression to examine the association between apoE genotypes and angiographic CAD, either by treating each of the genotypes as a single category or after grouping subjects according to the apoE genotype. These groups consisted of carriers of at least one apoE2 allele (apoE2/2, apoE2/3, apoE2/4), the apoE3/3 homozygotes, and the remaining individuals (apoE3/4 or apoE3/3) (Table 2). Multivariable adjustment was carried out in two steps, first for gender and age, and then, in addition, for cardiovascular risk factors (gender, age, body mass index, diabetes mellitus, hypertension, smoking).

Table 1 Clinical and biochemical characteristics of CAD patients and controls

	Controls		CAD		P^*
	Men (n = 311)	Women (n = 260)	Men (n = 549)	Women (n = 189)	
Age (years) means ± SD	55 ± 12	61 ± 11	64 ± 10	67 ± 9	<0.001
Body mass index (kg/m^2) means ± SD	27 ± 4	27 ± 5	28 ± 4	27 ± 5	0.706
Diabetes mellitus	54 (17%)	47 (18%)	198 (36%)	79 (42%)	<0.001
Systemic hypertension	177 (57%)	173 (67%)	434 (79%)	150 (79%)	<0.001
Past smoking	139 (45%)	39 (15%)	323 (59%)	38 (20%)	<0.001
Current smoking	63 (20%)	38 (15%)	101 (18%)	23 (12%)	0.397
Systolic blood pressure (mm Hg) means ± SD	135 ± 21	138 ± 24	148 ± 23	147 ± 25	<0.001**
Diastolic blood pressure (mm Hg) means ± SD	81 ± 12	79 ± 11	84 ± 12	82 ± 11	<0.001**
Fasting blood glucose (mg/dL) means ± SD	106 ± 32	103 ± 21	117 ± 37	122 ± 48	<0.001
LDL cholesterol (mg/dL) means ± SD	119 ± 29	126 ± 30	123 ± 32	132 ± 37	0.011
HDL cholesterol (mg/dL) means ± SD	4011	47 ± 12	37 ± 10	44 ± 11	<0.001
Triglycerides (mg/dL)	144	122	143	140	0.057
Median (25th and 75th percentile)	(102–198)	(90–164)	(105–197)	(112–196)	
C-reactive protein (mg/L)	1.5	2.5	3.1	3.7	<0.001***
Median (25th and 75th percentile)	(0.8–5.0)	(1.2–6.3)	(1.3–7.4)	(1.6–8.7)	
Fibrinogen (mg/dL)	334	346	367	376	0.001***
Median (25th and 75th percentile)	(289–394)	(301–396)	(314–430)	(322–436)	
White cell count (/nL)	6.80	6.10	7.20	6.80	0.013
Median (25th and 75th percentile)	(5.40–7.70)	(5.30–7.50)	(5.60–8.10)	(5.50–7.70)	
ApoE polymorphism					
E2/2	3 (1.0%)	2 (0.8%)	5 (0.9%)	2 (1.1%)	0.855
E2/3	57 (18.3%)	42 (16.2%)	67 (12.2%)	24 (12.7%)	0.454
E2/4	7 (2.3%)	7 (2.7%)	13 (2.4%)	4 (2.1%)	0.858
E3/3	178 (57.2%)	160 (61.5%)	358 (65.2%)	110 (58.2%)	Reference
E3/4	63 (20.3%)	48 (18.5%)	96 (17.5%)	46 (24.3%)	0.752
E4/4	3 (1.0%)	1 (0.4%)	10 (1.8%)	3 (1.6%)	0.186

* ANOVA or logistic regression, respectively, adjusted for gender.
** P < 0.001 additionally adjusted for use of beta-blockers, ACE inhibitors, AT1 receptor antagonists, calcium channel blockers and diuretics.
*** ANOVA of logarithmically transformed values.

Table 2 Odds ratios (OR) for angiographic CAD according to the apoE polymorphism

ApoE genotype		Model 1 OR (95% CI)	P	Model 2 OR (95% CI)	P	Model 3 OR (95% CI)	P
E2/2, E2/3, E2/4	N = 233	0.70 (0.53–0.94)	0.019	0.65 (0.47–0.89)	0.007	0.64 (0.46–0.88)	0.007
E3/3	N = 806	1.0		1.0		1.0	
E3/4, E4/4	N = 270	0.97 (0.74–1.29)	n.s.	1.01 (0.74–1.37)	n.s.	0.98 (0.72–1.34)	n.s.

Model 1, unadjusted.
Model 2, adjusted for gender and age.
Model 3, in addition adjusted for risk factors (body mass index, diabetes mellitus, hypertension, smoking).

We also studied the effect of the angiographic CAD status, gender, age and risk factors (body mass index, diabetes mellitus, hypertension, smoking) on inflammatory markers using an ANOVA model in which we included those factors not under examination as co-variables (Table 3).

To analyse the association of apoE, apoB, CRP, fibrinogen, and white cell count with the apoE genotype, we used ANOVA in which the apoE genotype groups and the CAD status were included as main effects (either allowing or not allowing for interaction); statistical adjustments were made for age and gender, and for cardiovascular risk factors (body mass index, diabetes mellitus, hypertension, smoking) in a first and a second step, respectively (Figs. 1 and 2).

To explore whether the apoE polymorphism affected CRP independent from systemic low-grade inflammation, we generated tertiles of fibrinogen and white cell count, irrespective of CAD status. These tertiles and apoE genotypes were used as main effects in ANOVA models which were either not adjusted or adjusted for CAD status, gender, age and risk factors (Fig. 3). Further, we performed ANOVA with the apoE genotype groups and CAD status as main effects including, as co-variables, either fibrinogen or white cell count, gender and age, and risk factors in three subsequent models.

To determine the consequences of the association between apoE genotype and CRP for the utility of CRP as a marker for CAD, we also formed tertiles of CRP separately within each of the three apoE genotype subgroups (Table 4). We then used logistic regression to calculate odds ratios of CAD in each of these apoE genotype subgroups either using tertiles derived from the entire control group or those defined in controls belonging to the apoE genotype subgroups. As above, multivariable adjustment was made in two steps, first for gender and age and then in addition for cardiovascular risk factors (Fig. 4).

Table 3 Effect of cardiovascular risk factors and angiographic status on CRP, fibrinogen and white cell count

		C-reactive protein (mg/L)*	*P*	Fibrinogen (g/L)*	*P*	White cell count (/nL)*	*P**
Gender							
Men	(*n* = 860)	2.52 ± 1.04		3.67 ± 0.03		6.88 ± 0.07	
Women	(*n* = 449)	3.46 ± 1.06	<0.001	3.73 ± 0.04	0.233	6.92 ± 0.10	0.800
Age (years)							
<60	(*n* = 508)	2.35 ± 1.06		3.53 ± 0.04		7.04 ± 0.10	
60—70	(*n* = 468)	2.93 ± 1.06		3.72 ± 0.04		6.90 ± 0.10	
>70	(*n* = 333)	3.48 ± 1.07	0.001	3.90 ± 0.05	<0.001	6.7 ± 0.12	0.071
Body mass index (kg/m^2)							
⩽26 or 27[a]	(*n* = 621)	2.34 ± 1.05		3.60 ± 0.03		6.79 ± 0.08	
>26 or 27[a]	(*n* = 688)	3.31 ± 1.05	<0.001	3.77 ± 0.03	<0.001	6.99 ± 0.08	0.101
Diabetes mellitus							
No	(*n* = 931)	2.63 ± 1.04		3.62 ± 0.03		6.77 ± 0.07	
Yes	(*n* = 378)	3.29 ± 1.07	0.003	3.87 ± 0.05	<0.001	7.20 ± 0.11	0.001
Hypertension							
No	(*n* = 375)	2.98 ± 1.07		3.82 ± 0.05		6.82 ± 0.11	
Yes	(*n* = 934)	2.74 ± 1.04	0.267	3.64 ± 0.03	0.008	6.93 ± 0.07	0.424
Smoking							
Never	(*n* = 545)	2.27 ± 1.05		3.56 ± 0.04		6.53 ± 0.09	
Former	(*n* = 539)	3.07 ± 1.05		3.73 ± 0.04		6.83 ± 0.09	
Current	(*n* = 225)	3.77 ± 1.09	<0.001	3.92 ± 0.06	<0.001	7.95 ± 0.14	<0.001
Coronary artery disease							
No	(*n* = 571)	2.42 ± 1.05		3.60 ± 0.04		6.75 ± 0.09	
Yes	(*n* = 738)	3.15 ± 1.05	<0.001	3.76 ± 0.03	0.002	7.01 ± 0.08	0.039

[a] Thresholds of 26 and 27 kg/m^2 apply to males and females, respectively.
* Estimated marginal means (±SEMs) obtained in a general linear model (ANOVA) adjusted for each of the other factors, whereby body mass index and age were used as continuous rather than categorial co-variables, geometric means are reported in the case of CRP.

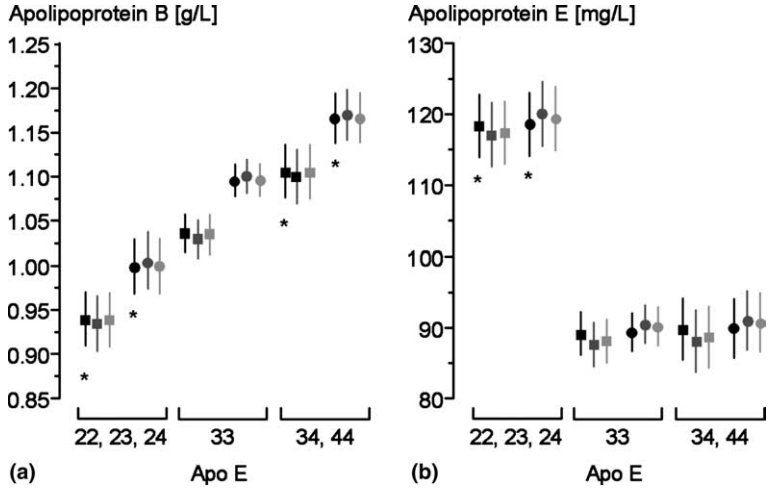

Fig. 1 ApoB (left panel) and ApoE (right panel) by apoE phenotypes. Squares, individuals in whom CAD had been ruled out by angiography; circles, individuals with angiographically proven CAD; black, estimated marginal means (±95% CI) of a linear model (ANOVA) including the apoE phenotype and CAD status as main effects; dark grey, estimated marginal means in addition adjusted for age and gender; light grey, estimated marginal means in addition adjusted for smoking (never, former, current), BMI, diabetes mellitus, and hypertension. ApoB and apoE were significantly associated with the apoE genotype (overall $P < 0.001$ in all models, adjusted and unadjusted). ApoB, but not apoE was higher in CAD patients than in controls (overall $P < 0.001$ in all models). Asterisks indicate statistical significance (*post hoc* pairwise comparisons according to Bonferroni) compared to apoE3/3 homozygotes.

To check the assumption of ANOVA that variances across groups are homogeneous, we used Levene's test of equality of error variances. In none of the models was the null hypothesis (equal error variances) rejected, with the exception of the models including apoE as a dependent variable. To account for the inflation of type I error due to multiple testing, *post hoc* com-

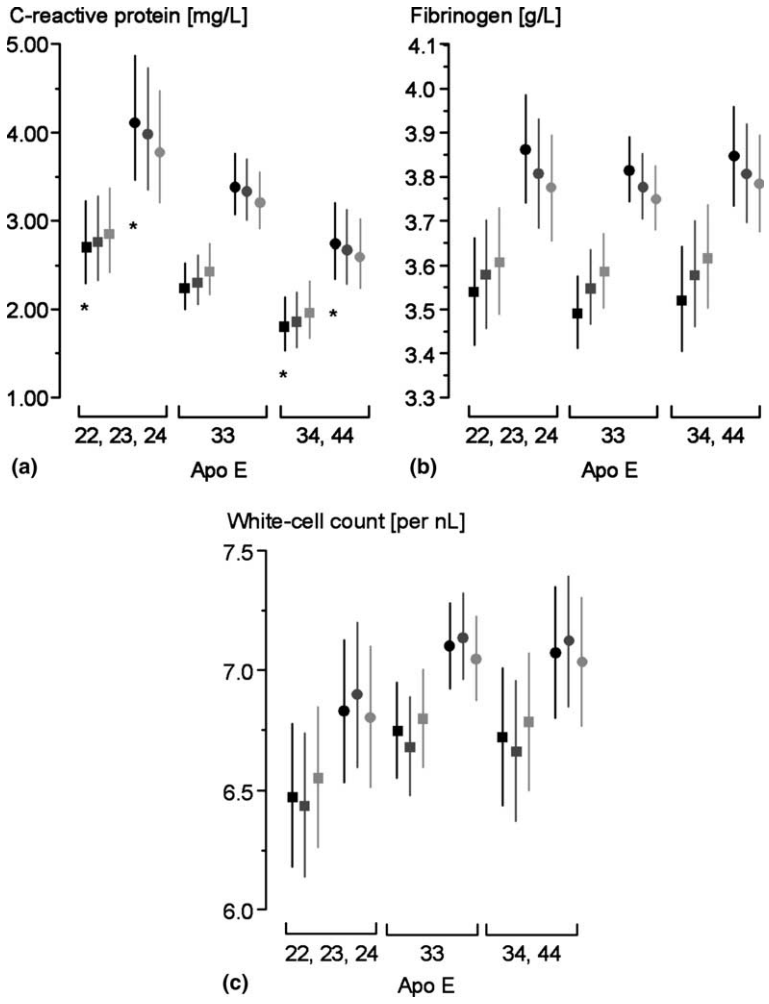

Fig. 2 CRP (panel (a)), fibrinogen (panel (b)) and white cell count (panel (c)) by apoE phenotypes. Squares, individuals in whom CAD had been ruled out by angiography; circles, individuals with angiographically proven CAD; black, estimated marginal means (±95% CI) of a linear model (ANOVA) including the apoE genotype and CAD status as main effects; dark grey, estimated marginal means in addition adjusted for age and gender; light grey, estimated marginal means in addition adjusted for smoking (never, former, current), BMI, diabetes mellitus, and hypertension. Geometric means are reported in the case of CRP. CRP, but neither fibrinogen nor white cell count was significantly associated with the apoE genotype (overall $P < 0.001$ in all models, adjusted and unadjusted). CRP, fibrinogen, and white cell counts were significantly higher in CAD patients than in controls (overall $P < 0.05$). Asterisks indicate statistical significance (*post hoc* pairwise comparisons according to Bonferroni) compared to the group of apoE3/4 heterozygotes or apoE4/4 homozygotes.

parisons of more than two groups were performed in unadjusted models only according to Bonferroni. All statistical tests were two-sided. $P < 0.05$ was considered statistically significant. The SPSS 11.0 statistical package (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

Characteristics of CAD patients and controls

Patients with stable CAD were significantly older than controls (Table 1). Current or past smoking, diabetes mellitus, and hypertension were more prevalent in CAD patients compared to controls. After adjusting for gender, systolic and diastolic blood pressure, fasting glucose, cholesterol, LDL cholesterol, CRP, fibrinogen, and white blood cells were higher in stable CAD patients than in controls; HDL cholesterol was lower. Body mass index and triglycerides were not significantly different.

Association of the apoE polymorphism with angiographic CAD

The prevalences of apoE genotypes among controls were similar to those found in other white populations (Table 1).[28] When each of the genotypes was treated as a single category, there was no significant association with CAD (Table 1). When we formed three groups according to the apoE genotype, the first one including the carriers of at least one apoE2 allele, the second one consisting of apoE3/3 homozygotes, and the third one including

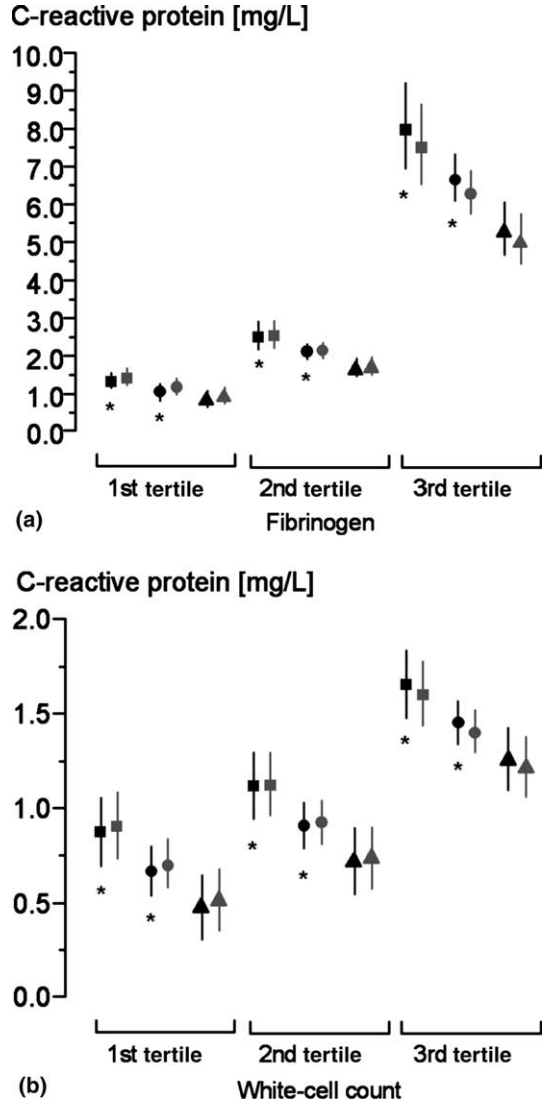

Fig. 3 Association of CRP with apoE genotypes by tertiles of fibrinogen (panel (a)) and white cell count (panel (b)). Squares, apoE genotypes 2/2, 2/3, and 2/4; circles, apoE genotype 3/3; triangles, apoE genotypes 3/4 and 4/4; black, geometric estimated marginal means (±95% CI) of a linear model (ANOVA) including the apoE genotype and the respective tertiles of either fibrinogen (panel (a)) or white cell count (panel (b)) as main effects; grey, same model adjusted for CAD status, age, gender, smoking (never, former, current), BMI, diabetes mellitus, and hypertension. CRP was significantly associated with the tertiles of fibrinogen and white cell count (overall $P < 0.001$ in adjusted and unadjusted models) and the apoE genotype (overall $P < 0.002$ in adjusted and unadjusted models). Asterisks indicate statistical significance (*post hoc* pairwise comparisons according to Bonferroni) compared to the group of apoE3/4 heterozygotes or apoE4/4 homozygotes.

the remaining individuals, logistic regression revealed a significantly lower prevalence of CAD among carriers of apoE2 (Table 2), a finding which was robust against multivariable adjustment.

Association of cardiovascular risk factors with CRP, fibrinogen, and white cell count

We examined the effect of gender, age, and risk factors (body mass index, diabetes mellitus, hypertension, smoking) and of the angiographic status on inflammatory markers in a general linear model in which we included those factors not under examination as covariables. CRP, but neither fibrinogen nor white cell count was higher in women than in men. Both CRP and fibrinogen, but not white cell count increased significantly in parallel to age and body mass index. The three inflammatory markers were elevated in patients with diabetes mellitus and in smokers. Fibrinogen, but neither CRP nor white cell count was elevated in patients with hypertension compared to normotensives (Table 3). Finally, CRP, fibrinogen, and white cell count were significantly higher in CAD patients than in individuals without CAD.

Effect of the apoE polymorphism on apoB and apoE concentrations

The apoE genotype was significantly associated with the concentrations of both apoB and apoE (overall $P < 0.001$). In carriers of at least one apoE2 allele, unadjusted apoB was lower ($P < 0.001$ according to Bonferroni) than in apoE3/3 homozygotes, whereas it was higher in individuals with the genotypes apoE3/4 and apoE4/4 ($P < 0.001$ according to Bonferroni). ApoE was significantly higher in carriers of at least one apoE2 allele compared to two other genotype groups ($P < 0.001$ according to Bonferroni). There was no difference in apoE concentrations between apoE3/3 homozygotes and apoE3/4 heterozygotes or apoE4/4 homozygotes. As expected, CAD patients had significantly higher concentrations of apoB than individuals without angiographic CAD (overall $P < 0.001$). ApoE did not differ between CAD patients and controls. The effects of the apoE genotype and CAD status on apoB and apoE were robust against adjustment for gender, age, and risk factors (overall $P < 0.001$ throughout). Further, we obtained consistent results in all statistical models when we allowed for an interaction of the apoE genotype and CAD status, indicating that the

Table 4 Tertiles of CRP by apoE phenotypes in subjects without coronary artery disease

		1st tertile	2nd tertile	3rd tertile
All individuals	(n = 571)	(n = 191) <1.18	(n = 189) 1.18—3.80	(n = 191) >3.80
ApoE22, E23, E24	(n = 118)	(n = 39) <1.40	(n = 40) 1.40—5.39	(n = 39) >5.39
ApoE33	(n = 338)	(n = 113) <1.23	(n = 112) 1.23—3.71	(n = 113) >3.71
ApoE34, E44	(n = 115)	(n = 38) <0.83	(n = 39) 0.83—2.55	(n = 38) >4.18

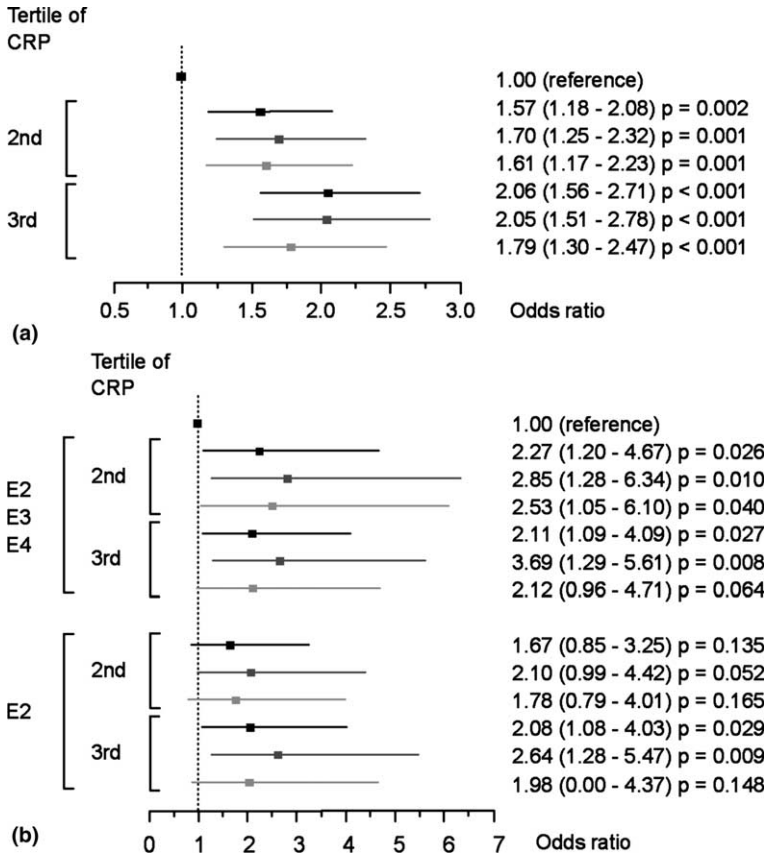

Fig. 4 Odds ratio for angiographic CAD according to tertiles of CRP established in controls across all apoE genotypes or within apoE genotype subgroups. Panel (a) Tertiles of CRP were generated in patients without CAD across all apoE genotypes and odds ratios for CAD were calculated by logistic regression. Panels (b)—(d) Odds ratios for CAD were calculated among subgroups of apoE genotypes (panel (b): apoE2/2, apoE2/3, apoE2/4; panel (c): apoE3/3; panel (d): apoE3/4, apoE4/4) according to tertiles of CRP either generated in controls across all apoE genotypes (E2, E3, E4) or according to tertiles derived from controls within each apoE genotype subgroup (E2 in panel (b), E3 in panel (c), E4 in panel (d)). Black, unadjusted odds ratios; dark grey, odds ratios adjusted for age and gender; light grey, odds ratios in addition adjusted for smoking (never, former, current), BMI, diabetes mellitus, and hypertension.

effects of the apoE genotype on apoB and apoE were the same in CAD patients and controls.

Effect of the apoE polymorphism on CRP, fibrinogen, and white cell count

In adjusted and unadjusted models including both apoE genotype and CAD status as main effects, the apoE genotype was significantly associated with CRP (overall $P = 0.001$ throughout, Fig. 2). *Post hoc* comparisons according to Bonferroni indicated that both carriers of at least one apoE2 allele ($P = 0.002$) and apoE3/3 homozygotes ($P = 0.032$) had significantly higher CRP than individuals with the genotypes apoE3/4 and apoE4/4; the difference between carriers of apoE2 and apoE3/3 homozygotes did not reach statistical significance ($P = 0.263$ according to Bonferroni). Adjusted and unadjusted fibrinogen and white cell count were not related to the apoE genotype (Fig. 2). Again, we obtained identical results throughout when we allowed for an interaction of the apoE genotype and CAD status.

To examine whether the effect of the apoE polymorphism on CRP occurred independent from the levels of systemic low-grade inflammation, we generated tertiles of fibrinogen and white cell count, irrespective of CAD status. In unadjusted models containing either tertiles of fibrinogen or white cell count on the one hand and the apoE genotype on the other hand as main effects, both the apoE genotype was a significant predictor of CRP (overall $P < 0.002$, Fig. 3). As expected, CRP increased in parallel to increasing tertiles of fibrinogen or white cell count (overall $P < 0.001$). Adjustment for CAD status, gender, age and risk factors (body mass index, diabetes mellitus, hypertension, smoking) did not affect these associations. In *post hoc* comparisons, carriers of apoE2 ($P < 0.002$ according to Bonferroni) and apoE3/3 homozygotes ($P < 0.03$ according to Bonferroni) had higher CRP concentrations than individuals with the apoE genotypes 3/4 and 4/4.

We also conducted ANOVA with the apoE genotype groups and CAD status as main effects, including as covariables either fibrinogen or white cell count, gender and age, and risk factors in three subsequent models.

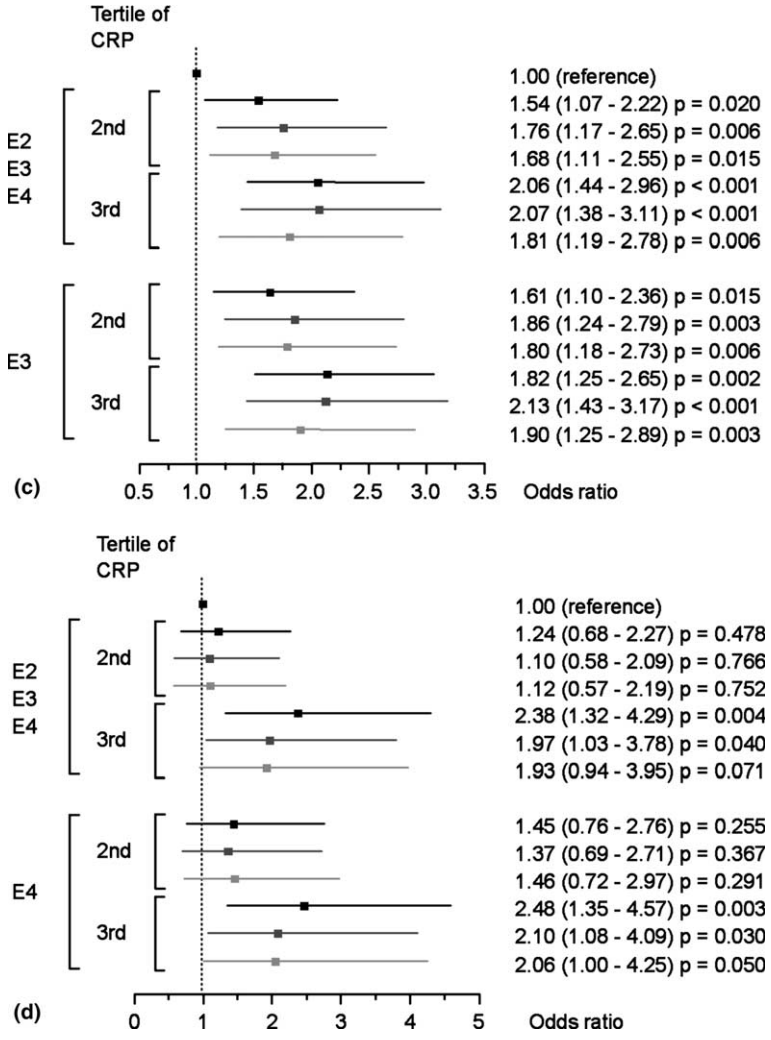

Fig. 4 *(continued)*

In each of the resulting six models, the overall effect of the apoE genotype on CRP remained statistically significant (overall $P < 0.001$ throughout).

Influence of the apoE polymorphism on the predictive value of CRP for CAD

CRP was significantly and independently associated with angiographic CAD. Unadjusted prevalences of angiographic CAD increased in a stepwise fashion across increasing tertiles of CRP. Compared to the lowest tertile, the odds ratios for CAD were 1.57 (95% CI, 1.18—2.08, $P = 0.002$) and 2.06 (95% CI, 1.56—2.97, $P < 0.001$) in the second and third tertile, respectively (Fig. 4(a)). Adjustment for age, gender and cardiovascular risk factors (body mass index, diabetes mellitus, hypertension, smoking) attenuated this association, but CRP remained a significant predictor after any of the adjustments.

We also explored how the relationship between the apoE polymorphism and CRP influenced the usefulness of CRP as a marker of angiographic CAD. We established tertiles of CRP separately within each of the three subgroups of controls defined by their apoE genotype (Table 4). As expected, the inter-tertile range was broader in controls with apoE2 (apoE2/2, apoE2/3, apoE2/4) than in apoE3/3 homozygotes, whereas it was narrower in the remaining controls (apoE3/4 or apoE3/3). We calculated odds ratios of CAD in each of the apoE genotype subgroups either using tertiles derived from the entire control group or using tertiles generated within controls having specific apoE genotypes. Among the apoE3/3 homozygotes, adjusted and unadjusted odds ratios remained virtually unchanged when tertiles were derived from the apoE3/3 homozygotes compared to tertiles derived from the pooled controls (Fig. 4(c)). In carriers of apoE2, the use of tertiles defined in controls carrying apoE2 only diminished the unadjusted and adjusted odds ratios in the second tertile from 2.27 ($P = 0.026$) to 1.67 ($P = 0.135$) and from 2.53 ($P = 0.040$) to 1.78 ($P = 0.165$), respectively (Fig. 4(b)). An opposite, but weaker effect was found in the group including the apoE3/4 heterozy-

gotes and the apoE4/4 homozygotes in which the use of group-specific tertiles slightly increased adjusted and unadjusted odds ratios (Fig. 4(d)).

Discussion

The major and novel finding of the current study is that the common genetic polymorphism of apoE is significantly associated with circulating levels of CRP in both patients with and without angiographic CAD. Apparently, this effect is not related to inflammation for two reasons: First, it was independent of fibrinogen and white cell count, two well recognized markers of systemic inflammation and predictors of CAD,[4,29] and second, both fibrinogen and white cell count themselves were not related to the apoE genotype.

The apoE polymorphism is one of the best characterized endogenous effectors of cholesterol metabolism. Carriers of apoE4 absorb cholesterol more effectively than subjects with other apoE genotypes;[18–20] further, apoE4 preferentially associates with triglyceride-rich lipoproteins and exhibits stronger binding to lipoprotein receptors.[16,17,30] Together, this increases the flux of intestinal cholesterol in hepatocytes. As a consequence, hepatic cholesterol biosynthesis[18,21] and LDL receptors are down-regulated and the concentration of LDL in the blood increases.[16,17] The apoE2 allele exerts opposite effects; it is defective in binding to lipoprotein receptors. This decreases the flux of remnant-derived cholesterol into the liver, up-regulates hepatic sterol biosynthesis and LDL receptors, and subsequently lowers LDL. Consistently, we found the apoE polymorphism associated with the concentrations of both apoB, the major protein constituent of LDL, and with apoE, which is a component of triglyceride-rich lipoproteins and HDL. Compared to apoE3/3 homozygotes, the mean apoB concentration was approximately 9% lower in carriers of at least one apoE2 allele, whereas carriers of apoE4 had 6–7% higher concentrations of apoB than apoE3/3 homozygotes. Moreover, the mean apoE concentration in individuals with at least one apoE2 allele was 33% higher than in apoE3/3 homozygotes, reflecting the accumulation of remnant particles due to impaired receptor-mediated clearance of the binding-defective apoE2.

Presence of at least one apoE2 allele increased CRP by roughly 21%. In contrast, at least one apoE4 allele decreased CRP by 19%. The effect of the apoE polymorphism on CRP hence appears similar in magnitude to the effect on apoB. Furthermore, the difference in CRP due to the polymorphism of apoE is approximately half the difference between smokers and non-smokers and roughly equal to the increments brought about by diabetes mellitus or by the presence of angiographic CAD.

Our study raises the possibility that production, release or catabolism of CRP are related to the activity of the mevalonate pathway in the liver. Support for this possibility comes from the observation that statins, which inhibit the major rate limiting step of the mevalonate pathway, namely the conversion of HMG-CoA into mevalonate, have consistently been shown to lower CRP.[7–9,31] Many researchers have attributed this findings to direct anti-inflammatory effects of statins.[32] However, more than three quarters of an oral dose of any statin is delivered to the liver, the main site of the production of CRP, which makes it difficult to understand how these drugs should directly modify inflammatory processes at extrahepatic sites. Consistently, statins appear to have little, if any, effects on other systemic markers of inflammation such as interleukin 6, the soluble interleukin 6 receptor or circulating adhesion molecules.[8,11–13] For these reasons, we raised the hypothesis that statins modified the expression of CRP in hepatocytes.[14] through their ability to inhibit the mevalonate pathway. The current data are in line with this concept. In carriers of apoE4, the mevalonate pathway is down-regulated and CRP is low. On the contrary, both the mevalonate pathway and CRP are high in carriers of apoE2. Finally, depletion of hepatic sterols by ezetimibe, a compound which inhibits the intestinal absorption of cholesterol, also appears to lower CRP, at least when co-administered with statins.[33,34]

An alternative explanation for our findings would, however, be that the changes in the metabolism of plasma lipoproteins, brought about by the apoE polymorphism, affected CRP indirectly. It is, for instance, conceivable that remnant lipoproteins accumulating in carriers of apoE2 acted as pro-inflammatory agents at the site of the vessel wall thereby initiating or maintaining low-grade systemic inflammation. Although this possibility cannot be ruled out at present, it is not very likely, because fibrinogen and white cell count, two well established indicators of systemic inflammation,[4,29] were not related to the apoE genotype.

An abundance of evidence is now available that CRP improves the prediction of cardiovascular events.[2,3] Consistently, we found a significant association between CRP and angiographic CAD which was independent of the well-established conventional cardiovascular risk factors. The finding that the variation of CRP is in part explained by the apoE polymorphism raises the question whether or not the value of CRP as a marker of CAD would be modified by the apoE polymorphism. We therefore investigated how the odds ratios for CAD changed if tertiles of CRP were generated within subgroups of individuals with different apoE genotypes. This analysis revealed that, in carriers of apoE2, the odds ratio for CAD may be overestimated once the apoE polymorphism is disregarded. On the contrary, in apoE3/4 heterozygous or apoE4/4 homozygous individuals, the odds ratio may be underestimated.

Our study may have some limitations. The control individuals were recruited at a tertiary referral center, underwent coronary angiography and may hence not be representative for a random population sample. This, however, may also be regarded as a strength of the study. The prevalence of clinically asymptomatic coronary atherosclerosis has been reported to be very high at or above 50 years of age.[35] Hence, angiography-based recruitment of controls rules out that individuals with significant, yet clinically unapparent, CAD are inadvertently allocated to the control group. Further, the major

cardiovascular risk factors occur at a similar frequency in our controls compared to the general population. The prevalence of hypertension is close to that found in a random probability sample from Germany.[36] *Prima vista*, diabetes mellitus appears two to three times more frequent as in the German population.[37] This is, however, most likely due to the fact that we did not rely on self-reports. Rather, we measured fasting glucose and performed an oral glucose challenge in individuals not previously known to have DM. Based on clinical history or fasting glucose measurements, the National health and nutrition examination surveys (NHANES) 1999–2000 reports prevalences of DM of 9.2% and 19.3% in adults 40–59 or more than 60 years, respectively.[38] In the current study, 12.1% of the controls had diabetes mellitus according to this criterion, while another 5.6% were detected by elevated post-challenge glucose only.

In the current analysis, only individuals without CAD or with clinically stable CAD not receiving lipid lowering drugs were included. Thus, we may have selected for individuals with low cholesterol or LDL cholesterol concentrations and a lower prevalence of carriers of the apoE4 allele. Compared to the original LURIC cohort, excluding patients presenting with acute coronary syndromes led to an increase in the proportion of subjects without angiographic CAD from 22.3% to 43.6% and to a lower prevalence of cardiovascular risk factors among the individuals studied. However, it is very unlikely that such selection bias would have substantially influenced our results. First, the distribution of apoE genotypes was very similar to that seen in many random population samples;[28] second, the effects of the apoE genotype on circulating levels of apoE and apoB was as expected;[16,17] third, the relationship between the apoE polymorphism and CRP reported here was observed in subjects taking lipid-lowering drugs as well (data not shown); and fourth, we accounted for other factors potentially affecting CRP concentrations by multivariable adjustment.

In conclusion, we demonstrate that the common polymorphism of apoE is associated with circulating concentrations of CRP in clinically stable subjects with and without angiographic CAD. Because the current work provides the first indication for the existence of such a relationship, it needs to be replicated in the future. In particular, research addressing the impact of the apoE polymorphism on the utility of CRP as a prognostic marker in vascular medicine is warranted. Finally, it would be interesting to see whether and how the apoE genotype would modify the decrease in CRP seen during statin therapy.

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