Apolipoprotein E gene polymorphisms and thrombosis and restenosis after coronary artery stenting

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Abstract **Experimental data support a protective function of apolipoprotein E (apoE) against restenosis, the main factor limiting the long-term benefit of percutaneous coronary interventions. We investigated the possibility that the single nucleotide polymorphisms (SNPs) –219G/T, 113G/C, 334T/C, and 472C/T of the gene encoding apoE (APOE) are associated with the incidence of death and myocardial infarction or restenosis after stenting in coronary arteries. In addition, we asked whether the apoE isotype-related ε2/ε3/ε4 polymorphism, defined by specific allele combinations (haplotypes) of the 334T/C and 472C/T polymorphism, and other APOE haplotypes, derived from all four SNPs investigated, are associated with adverse clinical and angiographic outcomes after stenting. Our study included 1,850 consecutive patients with symptomatic coronary artery disease (CAD) who underwent stent implantation. Follow-up angiography was performed in 1,556 patients (84.1%) at 6 months after the intervention. We found that none of the APOE SNPs is associated with death and myocardial infarction or restenosis after stenting. In addition, we observed no relationship between APOE haplotypes and adverse outcomes. In conclusion, the APOE –219G/T, 113G/C, 334T/C, and 472C/T polymorphisms, either alone or in combination, do not represent genetic markers of the risk of thrombotic and restenotic complications in patients with CAD treated with coronary stenting.—** Koch, W., J. Mehilli, A. Pfeufer, A. Schömig, and A. Kastrati. **Apolipoprotein E gene polymorphisms and thrombosis and restenosis after coronary artery stenting. *J. Lipid Res.* 2004.** 45: **2221–2226.**

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Compared with conventional balloon angioplasty, stenting has improved the outcome of patients with coronary artery disease (CAD) (1). However, restenosis remains the principal factor limiting the long-term benefit of stenting (1). Experimental data have pointed to a protective function of apolipoprotein E (apoE) against restenosis (2–4). ApoE was found to inhibit cell signaling events associated with growth factor-induced smooth muscle cell migration and proliferation and to limit neointimal hyperplasia after arterial injury (2, 3). These results correspond to the observation that deficiency of apoE was associated with increased neointima formation after endothelial denudation (3, 4).

The apoE gene (APOE) is polymorphic, and some of its allelic forms are known to differentially affect transcriptional activity or give rise to structurally and functionally distinct protein isoforms (5–8). Evidence exists to suggest that the variability of APOE has differential effects on the atheroprotective potential attributed to apoE (5, 6). The ε2/ε3/ε4 polymorphism of APOE is caused by two single nucleotide polymorphisms (SNPs), 334T/C and 472C/T, which are in close physical proximity and absolute linkage disequilibrium (5, 9). The 334T/C and 472C/T SNPs exclusively determine three haplotypes, known as the ε2 (334T/472T), ε3 (334C/472T), and ε4 (334C/472C) alleles of APOE (5). This heterogeneity causes variation at amino acid positions 112 (cysteine or arginine) and 158 (arginine or cysteine) of apoE, resulting in three different isoforms of apoE (5). The ε2/ε3/ε4 polymorphism is one of the most thoroughly studied polymorphisms, especially for its effects on lipid profiles and CAD risk (5, 6). This polymorphism was found to be relevant for apoE plasma level, receptor binding affinity of apoE, plasma lipid and lipoprotein concentrations, and CAD (5, 6). In particular, the 334C allele and the ε4 allele were observed to impose an increased risk of CAD (5, 10), and, among patients with CAD, the ε4 allele was related to more severe and the ε2 allele was related to less severe disease (11). The presence of the ε4 allele has been associated with increased death rates in patients with CAD (12, 13). The cardiovascular risk attributed to the ε4 allele may be related to, at

Abbreviations: apoE, apolipoprotein E; APOE, gene encoding apolipoprotein E, CAD, coronary artery disease; CI, confidence interval; SNP, single nucleotide polymorphism.

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least in part, a lower antioxidant activity of the apoE4 isoform (112Arg/158Arg) compared with that of the apoE2 isoform (112Cys/158Cys) or the apoE3 isoform (112Cys/158Arg) (14). Another SNP of APOE, –219G/T, located in the promoter of APOE, was reported to be significantly associated with APOE promoter activity, apoE plasma concentration, and CAD (7, 8, 10). In addition, the 113G/C SNP of APOE may be relevant for APOE regulation because of its location in the APOE intron-1 enhancer element that constitutes a binding site for specific nuclear protein factors (15, 16).

Together, a number of findings suggest a significant impact of apoE and genetic variants of APOE on cardiovascular risk. APOE polymorphisms may also be associated with angiographic and clinical outcomes after subcutaneous interventions in coronary arteries. Inconsistent results were obtained regarding the relationship between the $\varepsilon 2/\varepsilon 3/\varepsilon 4$ polymorphism and restenosis after balloon angioplasty in coronary arteries (17–20). It has not been examined whether the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs, or APOE haplotypes based on the $\varepsilon 2/\varepsilon 3/\varepsilon 4$ polymorphism, or combinations of all four SNPs are related to thrombotic and restenotic complications after coronary stenting. We addressed this issue in a study that included a relatively large and consecutive series of patients with CAD.

PATIENTS AND METHODS

Patients

The study included a consecutive series of 1,850 Caucasian patients with symptomatic CAD who underwent stent implantation at Deutsches Herzzentrum München and 1. Medizinische Klinik rechts der Isar der Technischen Universität München. The protocols of stent placement and poststenting therapy were described in detail elsewhere (21, 22). Postprocedural pharmacologic therapy consisted of aspirin (100 mg twice daily, indefinitely) and ticlopidine (250 mg twice daily for 4 weeks). Patients who were considered at a higher risk for ischemic complications received additional therapy with the glycoprotein IIb/IIIa blocker abciximab, which was given as a bolus injection during the stent insertion procedure and as a 12 h continuous infusion thereafter. All patients were scheduled for angiographic follow-up at 6 months. Written informed consent was obtained from the patients for the intervention, follow-up angiography, and genotype determination. The study protocol was approved by the Institutional Ethics Committee, and the reported investigations were in accordance with the principles of the current version of the Declaration of Helsinki.

ApoE genotyping

Genotyping of the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs was performed with TaqMan assays, as previously described (9).

Angiographic evaluation

Lesion morphology was classified according to the modified American College of Cardiology/American Heart Association grading system in type A, B1, B2, and C; lesions of types B2 and C were considered complex lesions. Angiograms were recorded just before and immediately after the intervention and at 6 month follow-up. Matched projections of the target lesions were selected for quantitative computer-assisted off-line analysis of the angiograms with the automated edge-detection system CMS (Medis Medical Imaging Systems, Nuenen, The Netherlands). The angiographic parameters obtained were interpolated reference diameter, lesion length, diameter stenosis before and after stenting and at follow-up, diameter of the maximally inflated balloon during stent placement, and length of the stented segment. Quantitative analysis of angiograms was performed by operators not involved in the stenting procedure and unaware of the laboratory or genetic data.

Definitions and study end points

The primary end point of the study was restenosis. Two definitions of restenosis were used: the incidence of a diameter stenosis of $\geqslant$50% at 6 month follow-up angiography (angiographic restenosis) and the need for target vessel revascularization (percutaneous transluminal coronary balloon angioplasty or aortocoronary bypass grafting) as a result of symptoms or signs of ischemia in the presence of angiographic restenosis at the stented site within 1 year after stent placement (clinical restenosis). A secondary end point was the combined incidence of all-cause death and nonfatal myocardial infarction at 1 year after stenting. The diagnosis of acute myocardial infarction was based on the presence of new pathological Q waves on the electrocardiogram or a value of creatine kinase or its MB isoenzyme at least three times the normal upper limit.

Statistical analysis

Determination of haplotypes and haplotype frequencies was performed with the expectation-maximization (EM) algorithm and the Markov chain-Monte Carlo algorithm, as previously described (9). Discrete variables are expressed as counts and percentages and were compared with the Chi-square test or the Fisher exact test, as appropriate. Continuous variables are expressed as means $\pm$ SD and were compared by means of the unpaired, two-sided *t*-test or ANOVA for more than two groups. We tested for independent association of the APOE SNP-related genotypes and haplotype-related genotypes in multivariate models (multiple logistic regressions) of restenosis that included age, gender, arterial hypertension, hypercholesterolemia, current tobacco smoking, diabetes mellitus, unstable angina pectoris, acute myocardial infarction, previous myocardial infarction, previous bypass surgery, target coronary vessel, lesion complexity, ostial lesion, chronic occlusion, restenotic lesion, multivessel disease, reference diameter, lesion length, diameter stenosis before stenting, balloon-to-vessel ratio, maximal balloon pressure, length of the stented segment, diameter stenosis after stenting, and abciximab therapy as potentially confounding factors. Adjusted odds ratios and 95% Wald confidence intervals (CIs) were calculated on the basis of the multiple logistic regression models. Analyses were performed using the S-Plus statistical package (Mathsoft, Inc., Seattle, WA). $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

We determined the genotypes of the –219G/T, 113G/C, 334T/C, and 472C/T SNPs of APOE in 1,850 patients with CAD who underwent stenting in coronary arteries. The distributions of the genotypes were 27.1% –219GG, 48.5% –219GT, 24.3% –219TT, 41.0% 113GG, 44.9% 113GC, 14.1% 113CC, 73.7% 334TT, 24.0% 334TC, 2.3% 334CC,

and 86.8% 472CC, 12.4% 472CT, 0.8% 472TT. For comparisons with the carriers of the abundant 334TT and 472CC genotypes, patients with the rare 334CC and 472TT genotypes were combined with the patients who carried the 334TC and 472CT genotypes, respectively. Patients with the 334TC and 334CC genotypes represented the carriers of the APOE ε4 allele, and patients with the 472CT and 472TT genotypes represented the carriers of the APOE ε2 allele. **Table 1** shows baseline clinical characteristics, lesion-related variables before stenting, and procedural parameters of the patients in relation to the genotypes of the APOE SNPs. We observed no statistically significant differences between the groups, with the following exceptions: *i*) patients with the –219GT genotype were younger than the carriers of the –219GG or –219TT genotype ($P = 0.04$); *ii*) reference diameter increased with the number of 113G alleles ($P = 0.006$); *iii*) in patients with the 334TT genotype, hypercholesterolemia ($P = 0.04$), acute myocardial infarction ($P = 0.03$), and periprocedural treatment with abciximab ($P = 0.03$) were

TABLE 1. Baseline clinical characteristics, lesion variables before stenting, and procedural parameters according to the genotypes of the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs (n = 1,850)

	–219G/T			113G/C			334T/C		472C/T	
Variable	GG (n = 502)	GT (n = 898)	TT (n = 450)	GG (n = 759)	GC (n = 831)	CC (n = 260)	TT (n = 1,363)	TC + CC (n = 487)	CC (n = 1,606)	CT + TT (n = 244)
Age (years)	63.6 ± 10.1	62.4 ± 10.0	63.6 ± 9.9	63.4 ± 10.3	62.5 ± 10.0	63.5 ± 9.2	63.0 ± 9.9	63.0 ± 10.4	62.9 ± 10.1	63.5 ± 9.4
Women	112 (22.3)	174 (19.4)	105 (23.3)	158 (20.8)	184 (22.1)	49 (18.8)	290 (21.3)	101 (20.7)	336 (20.9)	55 (22.5)
Arterial hypertension	336 (66.9)	614 (68.4)	309 (68.7)	510 (67.2)	564 (67.9)	185 (71.2)	931 (68.3)	328 (67.4)	1089 (67.8)	170 (69.7)
Hypercholesterolemia	214 (42.6)	386 (43.0)	192 (42.7)	341 (44.9)	354 (42.6)	97 (37.3)	564 (41.4)	228 (46.8)	688 (42.8)	104 (42.6)
Current smoking	146 (29.1)	289 (32.2)	135 (30.0)	234 (30.8)	253 (30.4)	83 (31.9)	412 (30.2)	158 (32.4)	506 (31.5)	64 (26.2)
Diabetes mellitus	102 (20.3)	191 (21.3)	95 (21.1)	149 (19.6)	178 (21.4)	61 (23.5)	302 (22.2)	86 (17.7)	334 (20.8)	54 (22.1)
Unstable angina pectoris	132 (26.3)	245 (27.3)	138 (30.7)	204 (26.9)	234 (28.2)	77 (29.6)	372 (27.3)	143 (29.4)	453 (28.2)	62 (25.4)
Acute myocardial infarction	98 (19.5)	184 (20.5)	93 (20.7)	159 (20.9)	166 (20.0)	50 (19.2)	260 (19.1)	115 (23.6)	329 (20.5)	46 (18.9)
Previous myocardial infarction	144 (28.7)	232 (25.8)	120 (26.7)	213 (28.1)	216 (26.0)	67 (25.8)	362 (26.6)	134 (27.5)	430 (26.8)	66 (27.0)
Previous coronary bypass surgery	57 (11.4)	91 (10.1)	57 (12.7)	88 (11.6)	90 (10.8)	27 (10.4)	149 (10.9)	56 (11.5)	178 (11.1)	27 (11.1)
Target coronary vessel										
Left main coronary artery	8 (1.6)	13 (1.5)	6 (1.3)	10 (1.3)	14 (1.7)	3 (1.2)	22 (1.6)	5 (1.0)	24 (1.5)	3 (1.2)
Left circumflex coronary artery	94 (18.7)	177 (19.7)	98 (21.8)	147 (19.4)	171 (20.6)	51 (19.6)	269 (19.7)	100 (20.5)	322 (20.1)	47 (19.3)
Left anterior descending coronary artery	210 (41.8)	351 (39.1)	173 (38.4)	307 (40.5)	319 (38.4)	108 (41.5)	548 (40.2)	186 (38.2)	640 (39.9)	94 (38.5)
Right coronary artery	159 (31.7)	300 (33.4)	137 (30.4)	240 (31.6)	275 (33.1)	81 (31.2)	437 (32.1)	159 (32.7)	516 (32.1)	80 (32.8)
Venous bypass graft	31 (6.2)	57 (6.4)	36 (8.0)	55 (7.3)	52 (6.3)	17 (6.5)	87 (6.4)	37 (7.6)	104 (6.5)	20 (8.2)
Complex lesion (ACC/AHA type B2 or C)	379 (75.5)	655 (72.9)	347 (77.1)	570 (75.1)	617 (74.2)	194 (74.6)	1017 (74.6)	364 (74.7)	1202 (74.8)	179 (73.4)
Ostial lesion	36 (7.2)	64 (7.1)	27 (6.0)	51 (6.7)	64 (7.7)	12 (4.6)	88 (6.5)	39 (8.0)	108 (6.7)	19 (7.8)
Chronic occlusion	40 (8.0)	49 (5.5)	29 (6.4)	57 (7.5)	47 (5.7)	14 (5.4)	84 (6.2)	34 (7.0)	99 (6.2)	19 (7.8)
Restenotic lesion	133 (26.5)	213 (23.7)	103 (22.9)	200 (26.4)	189 (22.7)	60 (23.1)	341 (25.0)	108 (22.2)	381 (23.7)	68 (27.9)
Multivessel disease	365 (72.7)	645 (71.8)	318 (70.7)	547 (72.1)	602 (72.4)	179 (68.8)	980 (71.9)	348 (71.5)	1143 (71.2)	185 (75.8)
Reference diameter (mm)	3.06 ± 0.53	3.04 ± 0.52	3.00 ± 0.56	3.08 ± 0.53	3.02 ± 0.54	2.97 ± 0.51	3.02 ± 0.52	3.08 ± 0.56	3.03 ± 0.53	3.07 ± 0.51
Lesion length (mm)	12.2 ± 6.9	12.1 ± 6.7	11.9 ± 6.7	12.3 ± 6.8	12.0 ± 6.6	11.9 ± 6.9	12.0 ± 6.8	12.4 ± 6.6	12.0 ± 6.7	12.4 ± 7.2
Diameter stenosis before stenting (%)	79.2 ± 15.7	78.2 ± 15.2	80.0 ± 14.9	79.1 ± 15.5	78.7 ± 15.3	79.4 ± 14.6	78.8 ± 15.2	79.3 ± 15.5	79.0 ± 15.2	78.2 ± 16.0
Balloon-to-vessel ratio	1.07 ± 0.09	1.07 ± 0.10	1.07 ± 0.10	1.07 ± 0.09	1.07 ± 0.10	1.08 ± 0.11	1.07 ± 0.10	1.07 ± 0.09	1.07 ± 0.10	1.06 ± 0.09
Maximal balloon pressure (atmosphere)	13.9 ± 3.2	13.8 ± 3.4	13.9 ± 3.2	13.8 ± 3.3	13.9 ± 3.3	13.8 ± 3.3	13.8 ± 3.3	13.9 ± 3.2	13.9 ± 3.3	13.8 ± 3.3
Stented segment length (mm)	20.2 ± 14.8	20.2 ± 13.8	20.0 ± 13.2	20.6 ± 14.8	20.0 ± 13.7	19.4 ± 12.0	19.9 ± 13.9	20.8 ± 14.0	20.2 ± 14.1	19.9 ± 13.1
Diameter stenosis after stenting (%)	5.2 ± 7.7	5.3 ± 9.1	5.4 ± 7.4	5.5 ± 9.2	5.2 ± 7.8	4.9 ± 7.6	5.1 ± 7.8	5.8 ± 9.8	5.2 ± 8.3	5.8 ± 8.5
Periprocedural abciximab therapy	90 (17.9)	183 (20.4)	91 (20.2)	148 (19.5)	170 (20.5)	46 (17.7)	252 (18.5)	112 (23.0)	322 (20.0)	42 (17.2)

Data are presented as mean values ± SD or the number (%) of patients. ACC/AHA, American College of Cardiology/American Heart Association grading system; APOE, gene encoding apolipoprotein E (apoE); SNP, single nucleotide polymorphism.

less frequent and diabetes mellitus ($P = 0.04$) was more frequent than among carriers of the 334C allele (334TC genotype or 334CC genotype).

The apoE isotype-related genotypes ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4, and ε4ε4 were present at 0.8, 11.2, 1.2, 61.7, 22.8, and 2.3%, respectively. Genotype-based evaluation revealed that the alleles of the four APOE SNPs were arranged in eight different haplotypes: haplotype 1 = –219G/113G/334T/472T (GGTT; 7.0%), haplotype 2 = GGTC (41.6%), haplotype 3 = TCTC (36.3%), haplotype 4 = TGTC (0.6%), haplotype 5 = GCTC (0.2%), haplotype 6 = TGCC (11.7%), haplotype 7 = GGCC (2.6%), and haplotype 8 = TCCC (<0.001%). Haplotype 1 was the only haplotype that represented the ε2 allele (334T/472T); haplotypes 2–5 included the ε3 allele (334T/472C); and haplotypes 6–8 contained the ε4 allele (334C/472C). It was possible to assign a genotype, defined by a specific combination of two of the eight haplotypes, to each patient. In total, 23 different haplotype-related genotypes were present in the study population.

Clinical and angiographic outcomes

Follow-up angiography of coronary arteries was performed in 1,556 (84.1%) of the patients 6 months after stenting. The proportions of the patients who underwent 6 month angiography were not substantially different among the SNP-related genotype groups ($P \geq 0.60$), apoE isotype-related genotypes ($P \geq 0.80$), and other haplotype-related genotypes ($P \geq 0.63$). Complete 1 year clinical follow-up data were available for all patients, irrespective of the presence or absence of follow-up angiography.

APOE SNP-related genotypes

The combined incidence of all-cause death and nonfatal myocardial infarction was not significantly different between patients with the –219GG, –219GT, and –219TT genotypes ($P = 0.76$), patients with the 113GG, 113GC, and 113CC genotypes ($P = 0.78$), patients with the 334TT genotype and carriers of the 334C allele (ε4 allele carriers) ($P = 0.21$), or patients with the 472CC genotype and carriers of the 472T allele (ε2 allele carriers) ($P = 0.75$) (**Table 2**).

Similarly, the need for target vessel revascularization because of symptoms or signs of ischemia in the presence of angiographic restenosis at the stented site (clinical restenosis) was not substantially different among the genotype groups of the 219G/T ($P = 0.17$), 113G/C ($P = 0.52$), 334T/C ($P = 0.57$), and 472C/T ($P = 0.22$) SNPs (Table 2). Angiographic restenosis rates were not significantly different between the genotype groups of the 219G/T ($P = 0.56$), 113G/C ($P = 0.59$), 334T/C ($P = 0.10$), and 472C/T ($P = 0.26$) SNPs (Table 2). Continuous measures of angiographic restenosis, diameter stenosis and loss index (the ratio of late lumen loss and acute lumen gain) were not substantially different between the genotype groups.

In a multivariate analysis of angiographic restenosis, we assessed the possible influence of baseline clinical characteristics, lesion-related variables, and procedural parameters on the relationship between the APOE SNPs and angiographic restenosis. After adjustment for these potentially confounding factors, the multivariate analysis did not reveal a significant independent association of the –219G/T ($P = 0.31$), 113G/C ($P = 0.45$), 334T/C ($P = 0.58$), or 472C/T ($P = 0.36$) SNP with angiographic restenosis; the adjusted odds ratios were 0.79 (95% CI = 0.50–1.25), 1.18 (95% CI = 0.77–1.79), 0.91 (95% CI = 0.65–1.27), and 1.17 (95% CI = 0.83–1.64), respectively.

ApoE isotype-related genotypes and other APOE haplotype-related genotypes

We evaluated the association of the six apoE isotype-related genotypes and the seven most frequent APOE haplotype-related genotypes, derived from four SNPs, with death and nonfatal myocardial infarction at 1 year, clinical restenosis, and angiographic restenosis. Patients who carried one of the seven frequent haplotype-related genotypes represented 89.9% of the total study population and 89.5% of the individuals with 6 month follow-up angiography. Data are shown in **Table 3** (apoE isotype-related genotypes) and **Table 4** (other haplotype-related genotypes). The combined incidence of all-cause death and nonfatal myocardial infarction was not significantly different be-

TABLE 2. Death or myocardial infarction, clinical restenosis, and angiographic restenosis according to the genotypes of the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs

Genotype	n	Death or Myocardial Infarction	Clinical Restenosis	n	Angiographic Restenosis
−219 GG	502	34 (6.8)	100 (19.9)	420	147 (35.0)
−219 GT	898	52 (5.8)	162 (18.0)	753	245 (32.5)
−219 TT	450	27 (6.0)	65 (14.4)	383	121 (31.6)
113GG	759	45 (5.9)	142 (18.7)	635	205 (32.3)
113GC	831	54 (6.5)	146 (17.6)	701	240 (34.2)
113CC	260	14 (5.4)	39 (15.0)	220	68 (30.9)
334TT	1,363	89 (6.5)	245 (18.0)	1,145	391 (34.1)
334TC + 334CC	487	24 (4.9)	82 (16.8)	411	122 (29.7)
472CC	1,606	97 (6.0)	277 (17.2)	1,353	439 (32.4)
472CT + 472TT	244	16 (6.6)	50 (20.5)	203	74 (36.5)

Data are presented as number (%) of patients. P = not significant for all comparisons. Patients with the 334TC and 334CC genotypes represent carriers of the apoE isotype-related ε4 allele, and patients with the 472CT and 472TT genotypes represent carriers of the ε2 allele.

TABLE 3. Death or myocardial infarction, clinical restenosis, and angiographic restenosis according to apoE isotype-related genotypes

Genotype	n	Death or Myocardial Infarction	Clinical Restenosis	n	Angiographic Restenosis
ε2ε2	15	1 (6.7)	2 (13.3)	13	5 (38.5)
ε2ε3	207	15 (7.2)	45 (21.7)	170	64 (37.7)
ε2ε4	22	0	3 (13.6)	20	5 (25.0)
ε3ε3	1,141	73 (6.4)	198 (17.4)	962	322 (33.5)
ε3ε4	422	23 (5.5)	74 (17.5)	353	109 (30.9)
ε4ε4	43	1 (2.3)	5 (11.6)	38	8 (21.1)

Data are presented as number (%) of patients. P = not significant for all comparisons. The apoE isotype-related genotypes are combinations of the APOE ε2, ε3, and ε4 alleles. APOE ε alleles were derived from the genotypes of the 334T/C and 472C/T SNPs: ε2 = 334T/472T, ε3 = 334T/472C, ε4 = 334C/472C. To our knowledge, the combination 334C/472T has never been observed. ε2ε2 = 334T/472T and 334T/472T; ε2ε3 = 334T/472T and 334T/472C; ε2ε4 = 334T/472T and 334C/472C; ε3ε3 = 334T/472C and 334T/472C; ε3ε4 = 334T/472C and 334C/472C; ε4ε4 = 334C/472C and 334C/472C.

TABLE 4. Death or myocardial infarction, clinical restenosis, and angiographic restenosis according to APOE haplotype-related genotypes based on four APOE SNPs

Genotype	n	Death or Myocardial Infarction	Clinical Restenosis	n	Angiographic Restenosis
12	114	7 (6.1)	26 (22.8)	90	38 (42.2)
13	91	8 (8.8)	19 (20.9)	78	26 (33.3)
22	323	25 (7.7)	61 (18.9)	271	93 (34.3)
23	535	34 (6.4)	95 (17.8)	450	155 (34.4)
26	196	10 (5.1)	35 (17.9)	160	47 (29.4)
33	257	14 (5.4)	38 (14.8)	218	67 (30.7)
36	148	12 (8.1)	22 (14.9)	125	43 (34.4)

Data are presented as number (%) of patients. P = not significant for all comparisons. Shown are the seven most frequent APOE haplotype-related genotypes; haplotype-related genotypes not shown here represented 2.2% of patients or less. The haplotype-related genotypes are combinations of APOE haplotypes. APOE haplotypes were estimated from the genotypes of the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs with the EM algorithm and the Markov chain-Monte Carlo algorithm (see Ref. 9 for explanations). APOE haplotype-related genotypes are as follows: 12 (haplotype 1 and haplotype 2) = –219G/113G/334T/472T (GGTT) and GGTC; 13 = GGTT and TCTC; 22 = GGTC and GGTC; 23 = GGTC and TCTC; 26 = GGTC and TGCC; 33 = TCTC and TCTC; 36 = TCTC and TGCC. See text for definitions of the eight APOE haplotypes present in the study population.

tween the apoE isotype-related genotype groups ($P = 0.63$) (Table 3) or other haplotype-related genotype groups ($P = 0.95$) (Table 4). Clinical restenosis rates were not substantially different between the apoE isotype-related genotype groups ($P = 0.56$) (Table 3) or other haplotype-related genotype groups ($P = 0.97$) (Table 4). No significant relationship existed between the frequency of the apoE isotype-related genotypes ($P = 0.34$) or other haplotype-related genotypes ($P = 0.85$) and angiographic restenosis (Tables 3, 4, respectively). The apparently lower rate of angiographic restenosis among the carriers of the $\varepsilon4\varepsilon4$ genotype (21.1%) versus patients with the $\varepsilon2\varepsilon2$ genotype (38.5%) (Table 3) was not significant ($P = 0.21$). Finally, multivariate analysis revealed no significant independent association of the apoE isotype-related genotypes ($P = 0.32$) or other haplotype-related genotypes ($P = 0.87$) with angiographic restenosis.

DISCUSSION

ApoE activities confer protection against various forms of vascular disease, including atherosclerosis and injury-induced restenosis (3, 4, 23, 24). Stent deployment elicits local inflammation and neointima formation (25, 26). ApoE is able to inhibit the proliferation of lymphocytes and vascular smooth muscle cells (2, 27) and, therefore, may interfere with the cascade of events that leads to restenosis. We asked whether APOE polymorphisms with potential impact on APOE regulation or apoE function are suitable as predictors of clinical and angiographic outcomes after coronary stenting. The results presented here strongly suggest that the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs, the $\varepsilon2/\varepsilon3/\varepsilon4$ polymorphism, and APOE haplotype-related genotypes based on the four SNPs are not associated with death or myocardial infarction and the incidence of restenosis after stenting in coronary arteries.

The distribution of the apoE isotype-related genotypes in the present study cohort is in good agreement with the distribution of the corresponding apoE phenotypes in a Tyrolean population (28), and the genotype distribution of the –219G/T and 113G/C SNPs is similar to that observed in a study group from Spain (7).

Combined evidence suggested an association of the $\varepsilon4$ allele with a higher cardiovascular risk than the $\varepsilon2$ or $\varepsilon3$ allele (5, 6, 8, 10–13, 17, 18). With regard to the clinical and angiographic outcomes after stenting, we observed no significant difference between patients who carried the $\varepsilon4$ allele and patients who did not carry the $\varepsilon4$ allele. The same was true when we compared patients with the $\varepsilon2$ allele and patients without the $\varepsilon2$ allele.

The association of the $\varepsilon2/\varepsilon3/\varepsilon4$ polymorphism with restenosis after balloon angioplasty of coronary arteries has been observed (17, 18), but not in all studies that addressed this subject (19, 20). In the first of the positive reports (17), the $\varepsilon4$ allele and $\varepsilon4\varepsilon4$ genotype were significantly more prevalent among 59 patients with restenosis than among 91 patients who did not develop restenosis ($P < 0.01$ and $P < 0.04$, respectively). Similarly, in the second study with a positive finding (18), the $\varepsilon4\varepsilon4$ genotype was present more often among 88 patients with restenosis than among 118 patients without restenosis ($P < 0.05$). Enrollment in these studies was restricted to patients who fulfilled several criteria, including the absence of acute myocardial infarction and previous balloon angioplasty or coronary bypass surgery (17, 18). We do not know the reason for the differences between the results we achieved in a much larger series of consecutive patients and the results reported in relatively small samples of selected patients (17, 18). The difference in population size may be an explanation, but differences in study design and baseline characteristics of the patients offer further reasons. In addition, the disparity may result from the fact that balloon angioplasty and stenting in coronary arteries provoke distinct vascular responses: restenosis after balloon angioplasty is characterized mainly by a remodeling process that results in shrinking of the artery (29); restenosis after stenting is caused primarily by neointimal hyperplasia caused by the proliferation of vascular smooth muscle cells and the accumulation of extracellular matrix (30). For this reason, the impact, if any, of the $\varepsilon2/\varepsilon3/\varepsilon4$ polymorphism on the outcomes after balloon angioplasty and stenting may be different.

In conclusion, the present results suggest that the APOE –219G/T, 113G/C, 334T/C, and 472C/T SNPs, either alone or in combination, are not useful as indicators of adverse outcomes in patients who undergo stenting in coronary arteries.

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