The effectiveness of hydroxy-methylglutaryl coenzyme A reductase inhibitors (statins) in the elderly is not influenced by apolipoprotein E genotype

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We aimed to assess whether the effectiveness of statins in the prevention of myocardial infarction, stroke and total mortality is influenced by apolipoprotein E (apoE) genotype in an elderly population. We used data from the Rotterdam Study, a prospective population-based cohort study in the Netherlands which started in 1990 and included 7983 subjects aged 55 years and older. Subjects who were treated with cholesterol lowering drugs at baseline or with a serum total cholesterol ≥ 6.5 mmol/l at baseline were included. We compared the incidence of myocardial infarction, stroke and total mortality in subjects who received ≥ 2 years of statin treatment with that in subjects who had been treated for less than 2 years, and in untreated subjects, using a Cox proportional hazard model with cumulative statin use defined as time-dependent covariates. The adjusted relative risk of all-cause mortality was 0.79 [95% confidence interval (CI) 0.51 – 1.22] and of myocardial infarction and stroke 0.50 (95% CI 0.28 – 0.91) for subjects treated with statins for ≥ 2 years compared to untreated subjects. The adjusted relative risks for subjects with the ε4 allele were 0.91 (95% CI 0.45 – 1.84) for all-cause mortality and 0.63 (95% CI 0.23 – 1.78) for myocardial infarction and stroke. In subjects without the ε4 allele, adjusted relative risks were 0.71 (95% CI 0.41 – 1.24) for all-cause mortality and 0.46 (95% CI 0.22 – 0.95) for myocardial infarction and stroke. We found a protective effect of statins on the risk of myocardial infarction and stroke that was independent of apoE genotype. The protective effect of statins on total mortality was not statistically significant, but did not seem to differ between subjects with different apoE genotypes. *Pharmacogenetics* **12:647 – 653** © 2002 Lippincott Williams & Wilkins

Introduction

Statins are inhibitors of hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase, which inhibit cholesterol production in the hepatocyte. This increases the synthesis of low-density lipoprotein (LDL) receptors and thereby lowers serum cholesterol. In recent years, six large trials have established the efficacy of cholesterol lowering therapy in primary and secondary prevention [1–6]. Treatment with statins over a 5-year period was associated with a statistically significant reduction in mortality, myocardial infarction and stroke. However, these reductions are average effects for all patients included in the trials, irrespective of their genetic predisposition. Several polymorphisms, such as the apolipoprotein E (apoE) polymorphism are suspected to have an influence on the effectiveness of statins.

Human apoE is a polymorphic protein, defined by three alleles ε2, ε3 and ε4 at a single gene locus on chromosome 19. These alleles code for three isoforms of apoE (E2, E3 and E4) that differ by one or both of two amino acid substitutions at sites 112 and 158, and thus determine the six phenotypes resulting from the combination of any two of them [7,8]. The polymorphism of the apoE gene influences hepatic cholesterol content because lipoproteins with the E4 isoform are taken up with greater affinity than those with the common E3 isoform, which in turn are cleared more efficiently than those with the E2 isoform. Accelerated lipoprotein clearance by the liver leads to a down-regulation of hepatocyte LDL receptor synthesis and an increase in serum cholesterol [9]. The reports on the influence of the apoE polymorphism on the effectiveness of statins are conflicting. Some studies suggested that HMG-CoA reductase inhibitors (statins) were less effective in reducing cholesterol levels in individuals

with the apoE4 isoform [10–14]. This might be explained by the finding that ε4 carriers already have lower HMG-CoA reductase activities [10].

While other studies could not confirm these findings [15–18], in a substudy of the Scandinavian Simvastatin Survival Study (4S), subjects with the ε4 allele with a history of myocardial infarction or angina pectoris had an almost two-fold higher risk of dying in a follow-up period of approximately 5.5 years compared to patients without an ε4 allele. The increased risk of death was not accompanied by an increased risk of a major coronary event. Simvastatin treatment reduced the risk of mortality by 67% in ε4 carriers and by 34% in non-ε4 carriers [19]. There was no difference in the effect of treatment on the reduction of major coronary events. The excess mortality seen in myocardial infarction survivors with the ε4 allele could be abolished by treatment with simvastatin [19]. These results seem to be in contradiction with the studies that found a stronger lipid lowering effect of statins in subjects without the ε4 allele, and this might be explained by a protective effect of statins which is unrelated to lowering of serum cholesterol.

We performed a cohort study to assess whether the effectiveness of statins in the prevention of myocardial infarction, stroke, ischaemic heart disease morbidity and mortality and total mortality is influenced by apoE genotype in an elderly population.

Methods
Setting
The Rotterdam Study started in 1990 as a population-based prospective follow-up study. All 10 275 residents of the suburb Ommoord in Rotterdam, aged 55 years or over were invited to participate, of whom 7983 (78%) subjects gave their written informed consent. The baseline measurements took place until 1993. The design of this population-based study has been described elsewhere [20]. The baseline examination included several details, such as an interview on demographics, current health status, medical history, family history of diseases, smoking habits and current use of medication. During a physical examination, blood pressure, weight and height were measured and blood was drawn for DNA extraction and for determination of several laboratory values, such as total cholesterol and high-density lipoprotein (HDL) cholesterol. Furthermore, subjects in the Rotterdam Study are continuously monitored for major cardiovascular and neurological events and vital status. When an event or death has been reported, additional information is obtained by interviewing the general practitioner and by scrutinizing information from hospital discharge records in case of admittance or referral. Information on each possible stroke and myocardial infarction was

reviewed by a specialist who classified the event as definite, probable or possible. This procedure has been described elsewhere [21]. Pharmacy records were available for approximately 99% of the cohort as of 1 January 1991.

Cohort and outcome definition
Every individual who had a baseline serum cholesterol equal to or above 6.5 mmol/l or who used cholesterol lowering drugs at baseline was included in this cohort study. The end of the study period was set at 31 December 1998. We excluded subjects who died before 1 January 1991, because we did not have pharmacy records available before this date. We also excluded patients of whom the apoE genotype had not been assessed.

In the analyses with myocardial infarction and ischaemic stroke, individuals were followed until myocardial infarction, ischaemic stroke, or a censoring event (death, moving out of the study area or end of the study period), whichever came first. In the analyses of mortality, individuals were followed until death or a censoring event, whichever came first. In the ischaemic heart disease analyses, individuals were followed until myocardial infarction, coronary artery bypass grafting (CABG), percutaneous transluminal coronary angioplasty (PTCA), or ischaemic heart disease mortality or a censoring event.

We used definite and probable strokes and myocardial infarctions in our analyses. If computed tomography (CT) or magnetic resonance imaging (MRI), performed within 28 days after stroke, showed a haemorrhage or infarction, the type of stroke was coded accordingly. The stroke was classified as cerebral infarction in case there was no abnormality on CT or MRI. Strokes without neuroimaging were classified as possible haemorrhagic stroke or cerebral infarction in case of typical symptoms. We excluded haemorrhagic strokes from our analyses, because there is probably no association, and possibly even an inverse association, between serum cholesterol, the use of statins and these events [22].

Exposure definitions
Exposure to medication was assessed on the basis of pharmacy records from all seven pharmacies which serve the Ommoord region. These records include the name of the drug, the day of dispensing, the dosage form, the numbers of units dispensed, the prescribed daily dose and the Anatomical Therapeutic Chemical code of the drug [23]. On the basis of these data, we calculated the cumulative exposure to statins in the study population in the period preceding the outcome of interest. Because of the small numbers, we did not distinguish between individual statins.

Potential confounders and effect modifiers

As potential confounders we considered age, sex, diabetes mellitus, systolic blood pressure, body mass index (kg/m^2), total cholesterol, HDL cholesterol, history of atherosclerotic cardiovascular disease (myocardial infarction, stroke, CABG, PTCA, or use of nitrates at baseline and during the first year), myocardial infarction in parents before the age of 65 years and smoking at baseline. When PTCA or CABG occurred during follow-up, or if cohort members started to use nitrates during follow-up, the first episode was considered as primary prevention and the second episode as secondary prevention.

Furthermore, we considered time-dependent current use of coumarin anticoagulants and platelet inhibiting salicylates. In this study, we examined the apoE genotype as an effect modifier in our analyses. DNA was used for apoE genotyping [24]. The apoE gene was amplified by the primer and amplification conditions described by Wenham *et al.* [25]. After amplification, the polymerase chain reaction product was digested with the restriction enzyme *Hha*I and fragments were separated by electrophoresis on a 5% agarose gel. ApoE alleles were visualized by staining with ethidium bromide. We defined ε4 carriers as subjects with ε2/ε4, ε3/ε4 or ε4/ε4 alleles.

Statistical analysis

Chi-square and *t*-tests were used to compare differences at baseline between subjects with and subjects without the ε4 allele. All tests were two-tailed and $P < 0.05$ was considered statistically significant. For every cohort member, we calculated person-time between 1 January 1991 and the occurrence of myocardial infarction, stroke or censoring, whichever came first in the myocardial infarction/stroke analyses. For the ischaemic heart disease morbidity and mortality analyses, we calculated person-time between 1 January 1991 and the occurrence of an ischaemic heart disease event or a censoring event, whichever came first. For the mortality analyses, we calculated person-time between 1 January 1991 and the occurrence of death or censoring event, whichever came first. We calculated relative risks and 95% confidence intervals with a Cox proportional hazards model with the cumulative use of drugs represented by time-dependent covariates. We used PROC PHREG (SAS version 6.12, SAS Institute Inc., Cary; North Carolina, USA) to estimate the incidence of myocardial infarction, stroke and total mortality in relation to the use of statins. We created time-dependent categorical variables by dividing cumulative use of statins during the study period into three mutually exclusive categories: non-use, < 24 months of cumulative use and ⩾ 24 months of cumulative use. These cut-points were chosen to guarantee an adequate number of subjects in each group and are compatible with trials which suggest that a protective effect of statins becomes visible after 18–24 months of therapy [1–5]. The three time-dependent categorical cumulative exposure variables were represented in the models by use of two dummy variables. In this model, a cohort member may contribute person-time to different cumulative exposure categories. Furthermore, we created non-cumulative time-dependent categorical variables (yes/no) for current use of anticoagulants and platelet-inhibiting salicylates.

We calculated the synergy index (SI), which is the ratio of the RR in susceptibles (with the ε4 allele) to the RR of the nonsusceptibles (without the ε4 allele). An SI of one means that the RR in the two subgroups is the same and that there is no interaction on the multiplicative scale. An SI of greater than one means that the joint effect of gene and drug is larger than expected from the product of their individual effects [26].

Results

At baseline, there were 3633 subjects with a total cholesterol ⩾ 6.5 mmol/l who were not treated with statins and there were 180 subjects who were treated with statins. Of these 3813 subjects, seven died before 1 January 1991 and 180 subjects were excluded because of missing apoE genotypes. Therefore, 3626 subjects remained for the analyses. Of these 3626 subjects, 650 subjects were treated with statins during follow-up. Table 1 shows the distribution of patients by apoE genotype and the baseline characteristics of the patients. Subjects with an ε4 allele were slightly younger and had a slightly lower systolic blood pressure at baseline than subjects without an ε4 allele. For the 3626 subjects under study, the median duration of follow-up was 7.2 years, and the total follow-up was 26 244.1 person years. Overall, subjects used statins during 2757.2 years. Of the 23 486.9 person years without statin therapy, 37.7% was contributed by subjects older than 70 years (Table 2). Slightly more than 21% of person-time on statins was contributed by subjects older than 70 years. A relatively large proportion of follow-up time on statins was contributed by subjects with diabetes mellitus, hypertension, a history of atherosclerotic cardiovascular disease, subjects with a total cholesterol level > 7.5 mmol/l or a HDL cholesterol level lower than 1.20 mmol/l, and subjects whose parents had a myocardial infarction before the age of 65 years (Table 2).

There were 329 events of myocardial infarction ($n = 174$) and stroke; in the ischaemic heart disease morbidity and mortality analysis, there were 294 events, and 584 subjects died during follow-up. Compared to untreated subjects, the adjusted relative risk of myocardial infarction and stroke was 1.41 [95% confidence interval (CI) 0.94–2.12] for subjects treated for less

Table 1 Baseline characteristics of subjects according to different apoE genotypes

Characteristic[a]	With ε4: genotype (n)				Without ε4: genotype (n)			
	ε2ε4 (93)	ε3ε4 (942)	ε4ε4 (106)	Total (1141)	ε2ε2 (25)	ε2ε3 (360)	ε3ε3 (2100)	Total (2485)
Women, n (%)	55 (59)	637 (68)	68 (64)	760 (67)	20 (80)	252 (70)	1414 (67)	1686 (68)
Age (years)[b]	66.9	68.4	67.2	68.2*	71.8	68.3	69.0	68.9*
Diabetes mellitus, n (%)	11 (12)	87 (9)	8 (8)	106 (9)	0 (0)	36 (10)	217 (10)	253 (10)
Current smoking, n (%)	23 (25)	195 (21)	22 (21)	240 (21)	3 (12)	76 (21)	441 (21)	520 (21)
Body mass index (kg/m^2)[b]	27.0	26.4	26.5	26.5	28.0	27.0	26.6	26.7
No history of atherosclerotic cardiovascular disease, n (%)	73 (79)	740 (79)	87 (79)	900 (79)	19 (76)	290 (81)	1633 (78)	1942 (78)
Total cholesterol (mmol/l)[b]	7.3	7.5	7.3	7.5	7.9	7.5	7.4	7.4
HDL cholesterol (mmol/l)[b]	1.4	1.4	1.3	1.4	1.3	1.4	1.4	1.4
Systolic blood pressure (mmHg)[b]	137.8	139.0	137.4	138.8*	146.0	140.4	140.6	140.6*

[a]Values are number of patients (%); [b]values are averages. *Significantly different between ε4 and non-ε4 carriers ($P < 0.05$).

than 2 years and 0.50 (95% CI 0.28–0.91) for subjects treated for more than 2 years with statins (Table 3). The adjusted relative risk of mortality from any cause was 1.15 (95% CI 0.78–1.70) for subjects treated for less than 2 years and 0.79 (95% CI 0.51–1.22) for subjects treated for more than 2 years with statins compared to untreated subjects (Table 4). The relative risk of total mortality, stroke or myocardial infarction among subjects using statins for more than 2 years compared to those who were untreated was similar for all apoE genotypes. In Tables 3 and 4, the relative risks are only shown for subjects with and without the ε4 allele. We also performed these analyses for the different genotypes (ε2ε2, ε2ε3, ε3ε3, ε3ε4, ε2ε4, ε4ε4) separately. There were no significant differences between these strata. In Figure 1, the Kaplan–Meier functions of untreated subjects with and without the ε4 allele are shown. These functions were almost equal.

The SI for myocardial infarction or stroke was 1.13 (95% CI 0.33–3.86) and the SI for total mortality was 1.29 (95% CI 0.54–3.04) (Table 5). We also performed analyses with different endpoints, such as ischaemic heart disease morbidity and mortality. The synergy index found in this analysis was 0.82 (95% CI 0.31–2.20). In all analyses, there was no difference in the effectiveness of statins in subjects with or without the ε4 allele.

Discussion

In this observational study of an elderly population, cumulative exposure to statins for more than 2 years was associated with a statistically significantly reduced risk of myocardial infarction or stroke in all apoE genotypes. The risk reduction for myocardial infarction or stroke was stronger than for total mortality. The increased risk of myocardial infarction or stroke which was found in the period up to 2 years of cumulative treatment with statins might be explained by confounding by severity because patients with a high cholesterol or serious comorbidity might more readily be treated with statins.

There were no statistically significant differences in the effectiveness of statins between subjects with and without the ε4 allele in these analyses. The SIs of myocardial infarction or stroke and of total mortality and of ischaemic heart disease morbidity and mortality approached 1 and were not statistically significant. This meant that we were not able to demonstrate an interaction between genotype and treatment with statins. In the untreated patients, the survival curves of subjects with and without the ε4 allele were almost equal. Gerdes *et al.* [19] found that subjects with the ε4 allele had a higher mortality compared to the subjects without the ε4 allele. A first potential explanation for the difference between our findings and those of Gerdes *et*

Table 2 Proportion of follow-up time with and without statin therapy contributed according to the characteristics of 3626 subjects

	Follow-up time contributed, %			
Characteristics at baseline	No use 23 486.9 person years	Use < 2 years 1364.3 person years	Use ≥ 2 years 1392.9 person years	Total 26 244.1 person years
Age				
> 70 years	37.7	21.3	21.9	36.0
Sex				
Female	68.6	64.7	64.6	68.2
Current cigarette smoking				
Yes	21.1	23.9	20.4	21.2
Diabetes mellitus				
Yes	8.9	10.2	11.4	9.1
Body mass index (kg/m^2)				
≥ 25	69.2	69.4	69.7	69.2
Hypertension				
Yes	33.8	41.6	47.6	35.0
History of atherosclerotic cardiovascular disease				
Yes	20.8	42.4	47.7	23.4
Total cholesterol (mmol/l)				
> 7.5	36.8	57.1	43.7	38.3
HDL cholesterol (mmol/l)				
> 1.20	74.4	67.0	66.7	73.6
Myocardial infarction in parents < 65 years				
Yes	7.9	12.8	12.9	8.4

Table 3 Association between statin use and myocardial infarction or stroke in subjects treated with cholesterol lowering drugs at baseline or with a baseline total cholesterol ≥ 6.5 mmol/l, stratified by apoE genotype

| Genotype		Person years	Events	IR/1000 person years	RR[a]	RR[b]
All genotypes	Untreated	23 486.9	289	12.3	1	1
	Statins < 2 years	1364.3	28	20.5	1.95 (1.32–2.89)	1.41 (0.94–2.12)
	Statins ≥ 2 years	1392.9	12	8.6	0.70 (0.39–1.26)	0.50 (0.28–0.91)
Without ε4 allele	Untreated	16 161.2	197	12.2	1	1
	Statins < 2 years	904.9	17	18.8	1.81 (1.09–2.99)	1.23 (0.73–2.07)
	Statins ≥ 2 years	974.2	8	8.2	0.69 (0.34–1.41)	0.46 (0.22–0.95)
With ε4 allele	Untreated	7325.7	92	12.6	1	1
	Statins < 2 years	459.4	11	23.9	2.21 (1.17–4.19)	1.73 (0.88–3.39)
	Statins ≥ 2 years	418.8	4	9.6	0.72 (0.26–1.99)	0.63 (0.23–1.78)

[a]Adjusted for age and sex. [b]Adjusted for age, sex, diabetes mellitus, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, smoking, secondary prevention, use of oral anticoagulation, myocardial infarction in parents before the age of 65 years and use of other cholesterol lowering drugs.
IR, Incidence Rate.

Table 4. Association between statin use and total mortality in subjects treated with cholesterol lowering drugs at baseline or with a baseline total cholesterol > 6.5 mmol/l, stratified by apoE genotype

Genotype		Person years	Events	IR/1000 person years	RR[a]	RR[b]
All genotypes	Untreated	23 772.1	532	22.4	1	1
	Statins < 2 years	1501.9	29	19.3	1.33 (0.91–1.94)	1.15 (0.78–1.70)
	Statins ≥ 2 years	1500.2	23	15.3	0.91 (0.59–1.39)	0.79 (0.51–1.22)
Without ε4 allele	Untreated	16 335.1	363	22.3	1	1
	Statins < 2 years	988.8	19	19.2	1.28 (0.80–2.05)	1.16 (0.71–1.87)
	Statins ≥ 2 years	1028.7	14	13.6	0.82 (0.48–1.41)	0.71 (0.41–1.24)
With ε4 allele	Untreated	7437.1	169	22.8	1	1
	Statins < 2 years	513.1	10	19.5	1.45 (0.75–2.79)	1.08 (0.55–2.14)
	Statins ≥ 2 years	471.5	9	19.1	1.09 (0.55–2.17)	0.91 (0.45–1.84)

[a]Adjusted for age and sex. [b]Adjusted for age, sex, diabetes mellitus, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, smoking, secondary prevention, use of oral anticoagulation, myocardial infarction in parents before the age of 65 years and use of other cholesterol lowering drugs.

Fig. 1
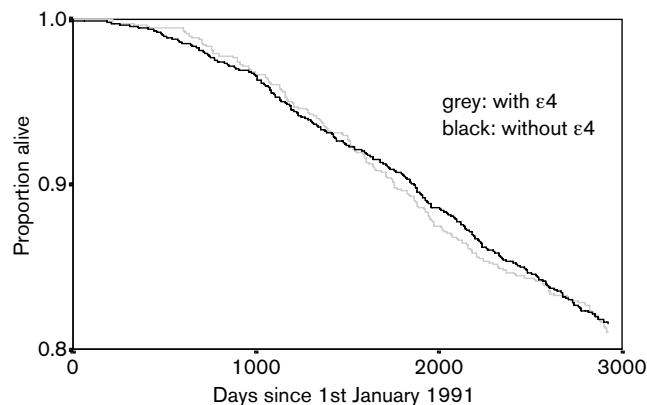
Kaplan–Meier survival curve: all-cause mortality in untreated patients.

Table 5. Synergy indices for subjects with the ε4 allele against subjects without the ε4 allele for myocardial infarction and stroke and for total mortality

	SI[a]	SI[b]
Total mortality	1.25 (0.53–2.95)	1.29 (0.54–3.04)
Myocardial infarction and stroke	1.07 (0.32–3.66)	1.13 (0.33–3.86)

[a]Adjusted for age and sex. [b]Adjusted for age, sex, diabetes, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, smoking, secondary prevention, use of oral anticoagulation, myocardial infarction in parents before the age of 65 years and use of other cholesterol lowering drugs.

al. is that they analysed data from a trial which included only subjects with myocardial infarction or angina pectoris in their history. In our population, both subjects with and without a history of atherosclerotic cardiovascular disease were present. Stratification for this aspect was not feasible because of the small numbers of events. A second potential explanation for the difference may be the age range of the populations. The 4S study population included men and women aged 35–70 years. In our population, only subjects over the age of 55 years were included. Ilveskoski *et al.* [27] found an association between the ε4 allele and coronary atherosclerosis, but only in early middle-aged individuals. In men older than 53 years, the ε4 allele was not a significant risk factor for coronary atherosclerosis. This suggests that, at older age, other risk factors may play a more important role in the atherosclerotic process than apoE polymorphisms [27].

The strengths of this observational study include the completeness of case identification, the validation of case diagnosis and the use of computerized pharmacy records to assess lipid lowering drug use in a similar and unbiased fashion for all subjects. An important limitation in an observational study is that medication

is not randomly assigned. Physicians decided who was treated based on the prognosis of their patients. By multivariate adjustment, we attempted to minimize the influence of confounding by other risk factors for the outcome of interest. However, the risk estimate in the total population after 2 years of cumulative treatment with statins was similar to the effect found in clinical trials, suggesting that our results are probably accurate. In trials, it was shown that the protective effects of statins on cardiovascular events and death become visible after 1.5–2 years [1–5]. Apart from confounding by severity, this may be the reason why we were unable to reproduce a protective effect in the analyses of myocardial infarction or stroke in the first 2 years of treatment.

The absence of an interaction between the apoE genotype and statins in our study was not in agreement with the interaction in a trial which suggested that individuals with the ε4 allele had an elevated risk of mortality and were particularly prone to benefit from simvastatin treatment. Although the differences in hazard ratios due to treatment were not statistically significant between subjects with and without the ε4 allele, we think that this difference in the results may partly be explained by the higher average age of our population. It is also possible that subjects with the ε4 allele are treated differently because some studies reported that their lipid response to statins is less favourable compared to the other genotypes [10–14]. However, other studies did not find such differences [15–18]. A lack of statistical power may also explain why we did not find a difference in effectiveness of statins in individuals with and without the ε4 allele. For example, the protective effect of statins on myocardial infarction and stroke in subjects with the ε4 allele was not statistically significant. Larger studies are needed to confirm our findings.

In the literature, more polymorphisms have been suggested that might influence the effectiveness of statins; for example, a polymorphism coding for the cholesteryl ester transfer protein [28] which plays an important role in the reverse cholesterol transport. Further research is needed to identify those polymorphisms, or combinations of polymorphisms, that are relevant to individualizing cholesterol lowering therapy. Our results suggest that the relevance of these polymorphisms as a determinant of the effectiveness of lipid lowering therapy may vary with age.

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