Apolipoprotein E polymorphisms influence effect of pravastatin on survival after myocardial infarction in a Mediterranean population: the GISSI-Prevenzione study

Benedetta D. Chiodini1,2, Maria Grazia Franzosi2*, Simona Barlera2, Stefano Signorini3, Cathryn M. Lewis1, Andria D'Orazio5, Paolo Mocarelli3,4, Enrico Nicolis2, Roberto Marchioli5, and Gianni Tognoni5 on behalf of GISSI Investigators† and SIBioC-GISSI Prevenzione Group

1Division of Genetics and Molecular Medicine, King’s College London, London, UK; 2Department of Cardiovascular Research, ‘Mario Negri’ Institute for Pharmacological Research, Via Eritrea, 20157 Milano, Italy; 3University Department of Laboratory Medicine, Hospital of Desio, Milano, Italy; 4University of Milano-Bicocca, Medical School DMS, Milano, Italy; and 5Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy

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Introduction

In recent years, results from a series of landmark clinical trials have confirmed that treatment with statins significantly reduces cardiovascular morbidity and mortality.1 This has been demonstrated both in hypercholesterolaemic patients and in subjects with cholesterol levels in normal limits and both in primary (WOSCOPS and HPS studies)2,3 and in secondary prevention (4S, CARE, LIPID studies).4–6 Besides the well-known effect in lowering serum cholesterol and decreasing the coronary heart disease (CHD) risk, statin treatment may have various beneficial effects. The relatively early reduction in mortality seen in patients at high risk for CHD treated with statins can be attributed also to the pleiotropic effects of statins, including improvement of endothelial function, inhibition of inflammatory response, and antioxidant properties.7,8 However, a wide interindividual variation in response to lipid-lowering drugs exists, depending on factors such as age, gender, concomitant disease, and genetic determinants. The knowledge of allelic variants of genes involved in the metabolism of lipid-lowering drugs could, in future, allow these drugs to be administered according to the patient’s genotype. This could increase the safety, efficacy, and cost-effectiveness of lipid-lowering therapies.9

Amongst all the candidate genes suspected to be involved in CHD susceptibility, the gene encoding APOE protein shows the greatest concordance between studies. The relationship between APOE polymorphisms and serum lipid levels is very well known. In contrast, the association between the ε4
variant of the APOE gene and CHD risk is not completely explained, and it has been demonstrated in some populations but not in others.\textsuperscript{10,11}

Several studies have also investigated the relationship between APOE genotypes and response to statins in terms of plasma lipid changes. In these studies, ε4 carriers were less likely to respond favourably to statin therapy in terms of total and LDL cholesterol decrease than ε2 and sometimes ε3 carriers.\textsuperscript{12–15} Although several studies reported this finding, agreement in the literature is not complete.\textsuperscript{16–19}

Only very few studies have evaluated the effect of the APOE gene on the response to statins in terms of clinical outcome.\textsuperscript{19} A substudy of the Scandinavian Simvastatin Survival Study (4S)\textsuperscript{20} has found that in the 5.4 years following a myocardial infarction (MI), the ε4 carriers were at higher risk of dying compared with non-ε4 carrier patients, and that this gap of survival was neutralized after treatment with simvastatin. The findings obtained in the 4S study proposed for the first time that the effects of statins on the mortality of the overall 4S study population are not directly attributable to a pharmacological effect or proportional to the degree of cholesterol lowering, but are expressed only in the sub-population with the genetically controlled worst cardiovascular prognosis. The effect of statins in this group was to neutralize the excess risk. Although this seems to occur in an opposite direction compared with the effect of statins on plasma lipid levels, the beneficial role of statins in the ε4 carrier group may be explained by the statins’ anti-inflammatory and anti-oxidant properties. Data from the Rotterdam Cohort study, another Nord European on an older population, could not replicate this finding.\textsuperscript{20}

We conducted a genetic study in the patients of the GISSI-Prevención (GISSI-P) trial\textsuperscript{21} to assess the effect of the APOE gene on the response to statin treatment in secondary prevention after MI. This trial is in a Mediterranean population, which is known to be at a lower cardiovascular risk, and involves a large number of patients.

**Methods**

**Setting**

The GISSI-P trial started in October 1993, and 11 324 Italian patients surviving recent (<3 months) MI were randomly assigned supplements of n-3 PUFA, vitamin E, both, or none for 3.5 years.\textsuperscript{22} A second trial assessing the efficacy of a low-dose cholesterol lowering regimen on the cumulative primary endpoint of total mortality, non-fatal MI, and stroke concerned patients who after a period of 3–6 months showed plasma cholesterol levels ≥200 mg/dL despite adequate dietary recommendations. Details on the study design, inclusion and exclusion criteria, and results are reported elsewhere.\textsuperscript{21} Among the 11 324 MI survivors randomized in the first trial, 4271 patients were eligible for the second randomization and were allocated to pravastatin 20 mg daily or no-treatment. An increase in dosage to 40 mg daily was allowed for patients who did not show a decrease in LDL cholesterol of at least 15%. Follow-up visits including clinical examination and lipid determination were scheduled at 6, 12, 18, 30, and 42 months. All the events included in the primary endpoint were adjudicated in a blinded fashion by a committee. The mean follow-up time was 23.0 months (SD = 6.7 months, median 24.3 months) since the GISSI-P second randomization.\textsuperscript{21}

GISSI-P trial had collected and stored blood samples from patients at entry to the GISSI-P trial. The present analysis has been conducted on all the samples of patients who entered the second randomization that were retrievable and found in a good enough condition for the genotyping (nearly 80% of all randomized patients).

The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the hospitals participating in the GISSI-P trial. Informed consent to participate in the study including blood sampling was obtained for each subject before randomization.

**APOE genotyping**

DNA has been extracted from frozen EDTA whole blood using a salting out procedure\textsuperscript{23}, and APOE genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism of a 295 bp region of exon 4.\textsuperscript{24} We defined ε4 carriers as patients with APOE genotypes ε2ε4, ε3ε4, or ε4ε4.

**Statistical methods**

Characteristics of patients for ε4 carriers vs. non-ε4 carriers were compared using χ²-test for the categorical variables or t-test for continuous variables, as the assumption of normality of the distribution was satisfied. The distribution of ε4 genotypes was tested for Hardy–Weinberg equilibrium using a χ²-test with one degree of freedom, and similarly for ε2 and ε3 alleles. All the relevant baseline characteristics (age, hypertension, diabetes, sex, body mass index (BMI), total cholesterol, HDL cholesterol, and triglycerides) were balanced across the randomized treatment. Cox proportional hazards model was initially used to assess if APOE genotype and the randomized treatment were associated with patient outcome. The therapeutic efficacy of pravastatin treatment on APOE genotype was formally tested by the interaction term included in the Cox model.

To estimate the effect of treatment within APOE genotype, the population was stratified into those found to be ε4 carriers and non-ε4 carriers. Cox proportional hazards model was used in each group and the treatment effect on mortality was expressed as hazard ratio (HR) and 95% confidence interval (CI). Mortality curves by treatment were constructed by the Kaplan–Meier method considering the follow-up period from the second randomization of each patient to 15 December 1996, when the study was stopped. Differences between the groups were tested using the log-rank test.

For all analyses, $P < 0.05$ were considered statistically significant. Analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

**Results**

**Baseline characteristics in the 3304 myocardial infarction patients**

Blood samples were available for APOE genotyping for 3304 subjects (2835 males and 469 females). They are representative of the overall GISSI-P study population, since the baseline characteristics are completely overlapping,\textsuperscript{21} and no significant differences were found between genotyped and non-genotyped patients (see Table 1). Mortality rate was slightly higher in the original GISSI-P population (3.75%) compared with the population genotyped that entered this analysis (3.30%). The rate of patients who shifted from 20 to 40 mg/day of pravastatin was 4.07% (87/2138) in the GISSI-P study and 4.53% (75/1657) in our genotyped population.

In the genotyped population, there were 109 deaths during follow-up. The mortality rate was significantly lower in the group treated with statin when compared with the group non-treated with statin (2.66% vs. 3.95%; HR 0.67, 95% CI 0.45–0.97, $P = 0.038$), demonstrating a beneficial effect of pravastatin in terms of survival. Compliance
to the pravastatin was higher in the genotyped population with respect to non-genotyped population, as during the follow-up, the 12.9% of the genotyped patients vs. 17% of non-genotyped permanently discontinued the treatment.

Table 2 shows the baseline characteristics of the patients included in the study and their distribution by APOE genotype. The frequencies of ε2, ε3 and ε4 alleles were 4.3, 86.9, and 8.8% respectively. APOE genotypes were in Hardy-Weinberg equilibrium. Overall, 554 patients (16.8%) were ε4 carriers and 2750 (83.2%) were non-ε4 carriers. No differences were found at entry in patients with and without the ε4 allele with regards to sex, age, history of hypertension, smoking habits, HDL cholesterol, and triglyceride levels. However, the levels of BMI, total and LDL cholesterol, and the percentages of diabetic subjects were significantly different in patients with and without the ε4 allele. The mean of total cholesterol was 232.6 mg/dL in ε4 carriers and 229 mg/dL in non-ε4 carriers (P = 0.002), and the mean of LDL cholesterol was 154.1 and 150.3 mg/dL, respectively (P = 0.002). The prevalence of diabetes was much lower in the ε4 group compared with the non-ε4 group (8.7% vs. 14.3%, P = 0.0004). No significant difference in terms of mortality was observed between the ε4 and the non-ε4 group (3.61% vs. 3.24%, HR = 1.11, 95% CI 0.68–1.80, P = 0.67; see Table 3). A similar result was observed for non-fatal MI (1.62% in ε4 vs. 2% in non-ε4 carriers, P = 0.56).

Among the 1657 genotyped patients randomized to pravastatin, the rate of patients shifted to 40 mg/day was not significantly different between ε4 (11/270, 4.07%) and non-ε4 carriers (64/1387, 4.61%); P = 0.69.

The efficacy of pravastatin treatment on APOE genotype was formally tested by the interaction term. The results, although not statistically significant (P = 0.14), suggested a different therapy response on patient’s outcome by APOE genotype (Table 3).

Mortality of myocardial infarction patients by ε4-carrier status

We assessed the effect of the treatment with pravastatin on mortality after MI in 2750 non-ε4 carriers participants in GISSI-P trial. In 1363 non-treated and 1387 treated patients, 50 and 39 fatal events occurred, respectively. At the end of the study follow-up, the mortality rates were 3.67% in non-treated patients and 2.81% in treated patients with an HR of 0.77 (95% CI 0.51–1.17, P = 0.21). In non-ε4 carriers, we could not demonstrate a longer survival in patients treated with pravastatin compared with non-treated subjects (log-rank test P = 0.21) (see Figure 1A) or a different distribution of non-fatal MI (1.95% vs. 2.05%, P = 0.84).

We also assessed the effect of the treatment with statin on mortality after MI in 554 ε4 carriers. In 284 non-treated and 270 treated patients, 15 and 5 fatal events occurred, respectively. At the end of the follow-up, the mortality rates were 5.28% in non-treated and 1.85% in treated patients, with an HR of 0.33 (95% CI 0.12–0.90, P = 0.0031) for the treated group, showing a significant effect of statin treatment in the ε4 group. In ε4 carriers, the patients treated with pravastatin survived significantly longer compared with the non-treated subjects (log-rank test P = 0.023) (see Figure 1B) and had also a lower rate of non-fatal MI, even if not significant (0.74% vs. 2.46%, P = 0.11).

Mortality of myocardial infarction patients by treatment

Therefore, we assessed the effect of ε4 allele on mortality after MI in 1647 participants in GISSI-P trial, assigned to no treatment. In 284 ε4 carriers and 1363 non-ε4 carriers, 15 and 50 fatal events occurred, respectively. Although in the two groups of ε4 and non-ε4 carriers non-treated with pravastatin no statistically significant difference in survival could be demonstrated (P = 0.20), the mortality rate of the ε4-carriers was slightly higher when compared with the mortality of the non-ε4-carriers (5.28% vs. 3.67% at the end of the follow-up).

Finally, we assessed the effect of ε4 allele on mortality after MI in 1657 participants in GISSI-P trial, assigned to pravastatin. In 270 ε4 carriers and 1387 non-ε4 carriers, 5 and 39 fatal events occurred, respectively. Although again no statistically significance was achieved, in the treated group the ε4-carriers showed a lower mortality rate when

| Table 1 Baseline characteristics of the 4271 myocardial infarction patients randomized in the GISSI-Prevenzione trial and comparisons between the genotyped and the non-genotyped patients |
|---------------------------------|------------------|------------------|--------|
| Subjects from the GISSI-Prevenzione original study | Patients genotyped | Patients non-genotyped | P-value |
| No. of patients | 4271 | 3304 | 967 | 0.11 |
| Men | 3684 (86.3) | 2835 (85.8) | 849 (87.8) | 0.64 |
| Age (years) | 60 ± 10.4 | 59.9 ± 10.3 | 59.7 ± 10.8 | 0.38 |
| Diabetes | 581 (13.6) | 442 (13.4) | 140 (14.5) | 0.95 |
| Hypertension | 1559 (36.5) | 1206 (36.5) | 354 (36.6) | 0.64 |
| Smokers | 506 (11.9) | 396 (12.0) | 110 (11.5) | 0.47 |
| BMI (kg/m²) | 26.5 ± 3.4 | 26.5 ± 3.5 | 26.6 ± 3.4 | 0.25 |
| Cholesterol (mg/dL) | 229.3 ± 25.8 | 229.6 ± 25.5 | 228.4 ± 26.9 | 0.21 |
| HDL cholesterol (mg/dL) | 45.7 ± 12.1 | 45.8 ± 12.3 | 45.3 ± 11.8 | 0.14 |
| LDL cholesterol (mg/dL) | 151.5 ± 25.9 | 150.9 ± 26.8 | 149.3 ± 28.9 | 0.16 |
| Triglycerides (mg/dL) | 166 ± 89.0 | 165.1 ± 87.3 | 169.7 ± 91.1 | 0.023 |

Values between brackets are percentages.

*Comparisons have been made for genotyped vs. non-genotyped groups.

**Values are mean ± SD.
Table 2 Baseline characteristics of the 3304 myocardial infarction patients with different APOE genotypes

<table>
<thead>
<tr>
<th>Variables</th>
<th>e4 carriers (n = 29)</th>
<th>Non-e4 carriers (n = 238)</th>
<th>Total (n = 2505)</th>
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<tr>
<td>Age (years)</td>
<td>79 (75.9)</td>
<td>22 (77.1)</td>
<td>52 (75.9)</td>
</tr>
<tr>
<td>Gender</td>
<td>Men (n = 28/51)</td>
<td>Women (n = 462/28)</td>
<td>Total (n = 498)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (10.3)</td>
<td>43 (8.7)</td>
<td>46 (8.7)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (48.3)</td>
<td>209 (34.2)</td>
<td>223 (34.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4 ± 19.8</td>
<td>30.7 ± 10.4</td>
<td>29.0 ± 10.4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>47.1 ± 14.1</td>
<td>47.4 ± 13.8</td>
<td>47.3 ± 13.8</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>151.4 ± 30.7</td>
<td>151.9 ± 29.9</td>
<td>151.7 ± 29.9</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>138.1 ± 9.5</td>
<td>142.1 ± 2.2</td>
<td>140.1 ± 2.2</td>
</tr>
</tbody>
</table>

Table 3 Cox proportional hazards model for mortality

<table>
<thead>
<tr>
<th>Variables</th>
<th>χ²</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>APOE genotype e4 vs. non-e4</td>
<td>0.18</td>
<td>1.11 (0.68–1.80)</td>
<td>0.672</td>
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<tr>
<td>Randomized treatment pravastatin vs. no pravastatin</td>
<td>4.29</td>
<td>0.67 (0.45–0.97)</td>
<td>0.038</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction term APOE genotype * treatment</td>
<td>2.18</td>
<td>0.44 (0.15–1.31)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Model 1: APOE genotype and randomized treatment.
Model 2: variables of Model 1 + the interaction term APOE genotype * treatment.

Discussion

We evaluated the role of the APOE gene on survival of 3304 Mediterranean MI patients treated and non-treated with pravastatin.

In contrast with the original GISSI-P study, where we found just a trend of benefit in patients treated with pravastatin, in this population, we found a significant beneficial effect of statin treatment in terms of survival with a consequent slightly lower overall mortality rate. These different findings with respect to the original study cannot be due to a selection bias. The population genotyped is nearly 80% and is totally representative of the overall GISSI-P study population. All samples effectively retrievable entered in this analysis, with no further selection applied. A better compliance to treatment in our genotyped population compared with the original study population might be the explanation for this discordance in results. The percentage of patients who permanently discontinued the statin treatment during the follow-up was indeed substantially lower in the genotyped compared with non-genotyped population (12.9% vs. 17.0%), and this translates in an increased benefit of statin treatment.

During a follow-up period of approximately 2 years, our study did not observe any significant difference in mortality and in non-fatal MI between e4 and non-e4 carriers, whereas it showed a positive association between e4 allele and total and LDL cholesterol levels. Surprisingly, we found nearly half the prevalence of diabetic patients in the e4 carriers compared with the non-e4 carriers. The most plausible explanation for this result is that in e4 carriers with MI, the occurrence of diabetes largely increases the mortality risk.

Though in the entire population investigated we found a beneficial effect of pravastatin in terms of survival, in effect only the e4 carriers seemed to have gained a significant benefit from this treatment. The same direction of
benefit was found also in the incidence of non-fatal MI. In our population non-treated with statin, ε4 carriers had a slightly higher mortality risk compared with the non-ε4 carriers, whereas in our population treated with statin, ε4 carriers had a slight lower mortality risk compared with the non-ε4 carriers.

Although the role of APOE in survival after CHD has not been widely studied so far, the role of APOE in CHD risk has been extensively investigated. Two meta-analyses\textsuperscript{25,26} identified a 30–40% increased risk for carriers of the ε4 allele compared with carriers of the ε3/ε3 genotype, but this has not been found in all populations. In Europe, this risk has been shown to decrease moving from the North to the South, according to the very similar gradient of CHD occurrence risk. Interestingly, a gradient exists also in ε4 allelic frequency, which decreases from around 20% in...
North-European\textsuperscript{27} to 8–10\% in Mediterranean countries.\textsuperscript{28} Therefore, it is likely that e4 allele either is not a risk factor or it confers a small increased risk of CHD and of death after CHD in Mediterranean populations, so that it would be necessary to investigate thousands of patients to detect this risk.

Our results from analysis by treatment suggest that there is no statistically significant difference in survival after CHD comparing e4 carriers with non-e4 carriers. This finding is consistent with the results of the Rotterdam Cohort study.\textsuperscript{20} Nevertheless, in contrast with the non-randomized Rotterdam Cohort study,\textsuperscript{20} in our population, only carriers of the generally considered high-risk allele seem to benefit from the treatment with statin. This result confirms the 4S substudy findings, but in a large Mediterranean population.

This is particularly noteworthy considering the substantial differences of the two trials in study design and population investigated.

4S and GISSI-P are both multi-centre, double-blind, randomized trials conducted in >4000 men and women affected by angina and/or MI with an average age around 60 years. In both studies, the primary objective was to investigate whether long-term treatment with statin in patients with hypercholesterolaemia will reduce overall mortality. Nevertheless, the population investigated in 4S study was at much higher risk when compared with the GISSI-P population: the 4S population is North European (Danish and Finnish), whereas the GISSI-P population is Italian with Mediterranean dietary and lifestyle habits; randomization involved patients with total cholesterol levels between 212 and 309 mg/dL in the 4S study and between 200 and 250 mg/dL in the GISSI-P study. The total number of fatal events was much higher in 4S patients compared with GISSI-P patients (8.3\% vs. 3.7\% of patients). Similarly, the e4 allelic frequency is more than double in 4S population compared with GISSI-P population, 20.2\% and 8.8\%, respectively.

This difference in e4 allelic frequency in the two populations can be seen as supporting the existence of the already described North–South gradient in e4 allelic frequency, although the slightly different selection criteria in the 4S and GISSI-P studies particularly in relation with the cholesterol levels observed in the two populations.

Further dissimilarities between the two trials exist. In 4S study, patients whose total cholesterol was \(\geq 200\) mg/dL at 6 or 18 weeks of therapy had the simvastatin dose raised to 40 mg (37\%). The GISSI-P study utilized pravastatin at the low dosage of 20 mg, which was increased to 40 mg in 4\% of patients only. Median follow-up time was 5.4 years in the 4S study but only 2 years in the GISSI-P study, because the part of the study assessing statin efficacy was stopped prematurely in late 1996 after the publication of CARE results.

In order to evaluate the prognosis determined by e4 allele and the effect on prognosis of statin in MI survivors, the 4S substudy investigated 966 patients whereas our study included 3304 individuals. Given the above reported differences between the two trials, the GISSI-P substudy needed such a much bigger sample size to also demonstrate a better response to statin treatment in terms of survival in e4 carriers.

In conclusion, the results of the GISSI-P substudy confirm the presence of the association between e4 allele and total and LDL cholesterol. The observation of a much smaller percentage of diabetic subjects in the e4 group compared with the non-e4 group is intriguing and certainly deserves further investigations. The findings also support the existence of the already described North–South European gradient in e4 allelic frequency and suggest the existence of a similar gradient in mortality risk related to this allele. Furthermore, our study shows a particular benefit from statin treatment in e4 carriers compared with non-e4 carriers. The consistency of our findings with those of the 4S is particularly informative because they have been obtained not only independently, but in a population and context that could be expected to produce negative results.

Our findings are suggestive for considering statins as a chapter of therapeutics where pharmacogenetics could be considered a guidance for personalized therapy. The question of whether the effects of statins is of particular interest in a small fraction of the population is so important that an urgent effort should be made by all those groups who have databanks which could help in this direction.

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Conflict of interest: none declared.
References


