$\epsilon 3\epsilon 4$ genotype as risk factor of myocardial infarction in middle-aged people in Spain

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Abstract. Apolipoprotein E (apoE) plays an important role in lipid metabolism. Its $\epsilon 4$ allele has been consistently associated with lipoprotein disorders but its connection to myocardial infarction (MI) is controversial. Because $\epsilon 4$ frequency decreases with age we thought that the contradictory results in different studies could be due to the wide age range of the subjects included. To test our hypothesis, ApoE genotyping was performed in 474 MI cases and an analysis was performed by percentiles of age. The frequencies of $\epsilon 3\epsilon 4$ genotype and $\epsilon 4$ allele in the MI group as a whole (subjects aged 31 to 92) were not significantly different from those in our area general population. However, significant differences were observed when comparing by group of age. The frequencies decreased as age increased. The $\epsilon 3\epsilon 4$ and $\epsilon 4$ frequencies were significantly higher in MI subjects aged 31 to 56 than in subjects over 74. The $\epsilon 3\epsilon 4$ genotype prevalence in an age and sex matched control group of subjects aged 31 to 56 was significantly lower than in the 31–56 year-old MI group. In conclusion, our data shows different $\epsilon 3\epsilon 4$ and $\epsilon 4$ frequencies depending on the age range of the subjects with MI, being significantly higher in the middle-aged group. This finding may help explain the discrepancies between studies analyzing association between apoE genotype and MI, and emphasizes the idea of considering apoE genotype for prevention at early age.

1. Introduction

Plasma lipid levels are important predictors of myocardial infarction (MI) risk [1]. Apolipoprotein E (apoE) is one of the key regulators of plasma lipid levels by affecting the hepatic binding, uptake, and catabolism of several classes of lipoproteins [2].

The apo E gene is a polymorphic gene with three codominant alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$), which give rise to six possible genotypes. Previous studies [3], including our own [4], have documented the effects of these alleles on the normal variation of lipid levels in our population. The $\epsilon 4$ allele is associated with higher total cholesterol (TC), LDL-C and apoB levels [3,4]. However, although these polymorphisms are traditionally one of the most

highly correlated genetic risk factors for cardiovascular disease [2], and previous studies have suggested that the $\epsilon 3\epsilon 4$ genotype and the $\epsilon 4$ allele may increase the risk of MI [5] or the extent of atherosclerosis [6–8], there are discrepancies in the literature, with some data contradicting these findings [9,10]. A recent meta-analysis has assessed the relation of apoE genotypes to CHD risk and has attributed the conflicting results of previous studies to several factors such as inadequate statistical power or study design or gene-environment interactions [11].

Because the frequencies of the $\epsilon 4$ allele and $\epsilon 3\epsilon 4$ genotype in the general population have been described as decreasing with age, we decided to investigate if the association between apoE genotype and cardiovascular disease risk in our population depends on age. To do this, we examined ApoE genotype and allele distribution in subjects with MI by age group.

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2. Materials and methods

2.1. Study design and subjects

This study examines apoE genotypes in patients (male and female) diagnosed with myocardial infarction in the Coronary Unit of an area hospital over a two-year period. Patients were considered to have had an MI if they had elevated ST segments or Q waves and enzymes elevation (troponin, CK-MB, etc.).

Controls were randomly selected from among subjects who met the matching criteria of age and who had not had an MI. Samples were collected randomly from blood donor units and from staff members in hospitals corresponding to the area in which MI subject were collected.

The study protocol was approved by the Clinical Research Ethics Committee of our hospital. Participants were required to sign a written consent for participation in the study. Venous blood samples for DNA extraction were obtained from every participant upon admittance. Data on cardiovascular risk factors in subjects with MI were collected by means of questionnaires.

2.2. ApoE genotyping

For apoE genotyping, DNA was amplified by PCR using the primers 5'CGGGCACGGCTGTCCAAGGAG3' and 5'CAGCGCGCCCTGTTCCACGAG3' as described [12]. Amplification products were then digested with the restriction enzyme HhaI and apoE genotype was determined by comparison with the combination of fragment sizes described by Hixon [13].

2.3. Statistical analysis

Statistical analyses were carried out using the SPSS software package, version 9.0. Apo E allele frequencies were calculated by allele counting. Frequencies of the genotypes and the alleles were calculated in each age quartile. *Presence or absence of other cardiovascular risk factors (hypertension, diabetes and smoking) was also compared in MI subjects in each age quartile.* Differences in proportions were tested using the Chi²-test.

Table 1
ApoE genotype and allele distribution (% (n)) in MI subjects

Subjects with MI n = 474	
Genotypes	
$\epsilon 2\epsilon 2$	0.2% (1)
$\epsilon 2\epsilon 4$	1.5% (7)
$\epsilon 3\epsilon 2$	7.4% (35)
$\epsilon 3\epsilon 3$	75.7% (359)
$\epsilon 3\epsilon 4$	14.8% (70)
$\epsilon 4\epsilon 4$	0.4% (2)
Alleles	
$\epsilon 2$	4.6% (44)
$\epsilon 3$	86.8% (823)
$\epsilon 4$	8.6% (81)

3. Results

The study included 474 subjects who had had a myocardial infarction (MI), 365 men (77%) and 109 women (23%). These subjects ranged in age from 31 to 92, with an average age of 64.4 ± 11.7 . The percentile distribution of age for MI subjects shows that 25% of the heart attacks took place before the age of 57, while 50% took place after the age of 66.

ApoE genotype and allele frequency distributions are shown in Table 1. The prevalence of the $\epsilon 3\epsilon 4$ genotype in this IAM population was 14.8% and the prevalence of the $\epsilon 4$ allele was 8.6%.

When analysing the apoE genotype distribution by age quartiles, we observe a progressive decrease in $\epsilon 3\epsilon 4$ frequency as age interval increases (Table 2). The $\epsilon 3\epsilon 4$ genotype and the $\epsilon 4$ allele are significantly ($p < 0.05$) more frequent in subjects between the ages of 31 and 56 (20.5% and 10.7% respectively) than in subjects between the ages of 74 and 92 (9.9% and 5.8%). When analyzing the $\epsilon 3\epsilon 4$ genotype and the $\epsilon 4$ allele prevalences in an age and sex matched 31–56 year-old control group (Table 3), we observed that those frequencies (11.1% and 8.5% respectively) were lower than those in the 31–56 year-old MI group, being significantly lower when comparing the $\epsilon 3\epsilon 4$ genotype frequencies.

In our analysis of other risk factors in MI subjects by age quartile, although the presence of hypertension and diabetes was lower in subjects between 31 and 56 years we found no significant differences between age groups. Nevertheless, we did observe a significantly higher frequency of smokers in the lowest age group of MI subjects versus the higher age groupings. 84.7% of those who have a heart attack while younger than 57 are smokers, while only 19.4% of those who suffer a heart attack after the age of 74 smoke.

Table 2
ApoE genotype and allele distribution (% (n)) in subjects with MI by age group

	31–56 years <i>n</i> = 117	57–66 years <i>n</i> = 118	67–73 years <i>n</i> = 114	74–92 years <i>n</i> = 111	P
Genotypes:					
$\epsilon 2\epsilon 2$	0.9 (1)				—
$\epsilon 2\epsilon 4$	0.9 (1)	3.4 (4)	1.8 (2)		ns
$\epsilon 3\epsilon 2$	6.8 (8)	4.2 (5)	7.9 (9)	11.7 (13)	ns
$\epsilon 3\epsilon 3$	70.9 (83)	76.3 (90)	77.2 (88)	77.5 (86)	ns
$\epsilon 3\epsilon 4$	20.5 (24)	15.3 (18)	13.2 (15)	9.9 (11)	*
$\epsilon 4\epsilon 4$		0.8 (1)		0.9 (1)	ns
Alleles:					
$\epsilon 2$	4.7 (11)	3.8 (9)	4.8 (11)	5.9 (13)	ns
$\epsilon 3$	84.6 (198)	86 (203)	87.7 (200)	88.3 (196)	ns
$\epsilon 4$	10.7 (25)	10.2 (24)	7.5 (17)	5.8 (13)	*

* $p < 0.05$ in the comparison of $\epsilon 3\epsilon 4$ and $\epsilon 4$ frequencies between the 31–56-year old and the 74–92-year-old groups.

Table 3
ApoE genotype and allele distribution (% (n)) in healthy subjects aged 31–56 and sex and age matched with MI subjects aged 31–56

	31–56 years <i>n</i> = 117
Genotypes:	
$\epsilon 2\epsilon 2$	
$\epsilon 2\epsilon 4$	2.6 (3)
$\epsilon 3\epsilon 2$	19.7 (23)
$\epsilon 3\epsilon 3$	65.0 (76)
$\epsilon 3\epsilon 4$	11.1 (13)
$\epsilon 4\epsilon 4$	1.7 (2)
Alleles:	
$\epsilon 2$	11.1 (26)
$\epsilon 3$	80.4 (188)
$\epsilon 4$	8.5 (26)

4. Discussion

Upon analyzing apoE genotype distribution in subjects with MI between the ages of 31 and 92, we observed that, neither the genotype nor the allele distributions, differed significantly from data published by our group [14] and by others [3] in the area where the patients come from. The frequencies of the $\epsilon 3\epsilon 4$ genotype and the $\epsilon 4$ allele in the group of subjects with MI (14.8% and 8.6% respectively) appeared even lower than those described in the general population in Spain (16.8% and 10.1% respectively). This finding of a lower $\epsilon 4$ allele frequency in this MI subject group would seem surprising.

Data in the literature suggested a possible explanation. The $\epsilon 4$ allele and the $\epsilon 3\epsilon 4$ genotype frequencies in the general population have been found to vary with age, becoming lower in older age groups [15]. Furthermore, among MI patients, Gerdes et al. found in the 4S Study (Scandinavian Simvastatin Survival Study), that patients with the $\epsilon 4$ allele that have survived a first MI

have twice the risk of dying of patients with other genotypes after a 5.4-year follow up period [16]. Based on this evidence, we decided to analyze the apoE genotype distribution in MI subjects by age quartile.

We found significantly higher prevalences (almost twice as high) of the $\epsilon 4$ allele and the $\epsilon 3\epsilon 4$ genotype in younger MI patients (31–56 years) as compared to people who suffered heart attacks after the age of 74. Thus, in the older subjects, the $\epsilon 4$ allele and the $\epsilon 3\epsilon 4$ genotype frequencies are lower than in the general population, but these frequencies are higher in young MI subjects than in the general population of the region. The $\epsilon 3\epsilon 4$ genotype frequency in young MI subjects result to be also significantly higher than that in an age and sex matched 31–56 year-old control group.

The high $\epsilon 4$ allele prevalence in young MI subjects seems to indicate a genetic predisposition for an elevated MI incidence at early ages and increased fatalities among those MI victims. Other studies have pointed out the relevance of the $\epsilon 4$ allele as a cardiovascular risk factor in young people [17] and have indicated that its influence on coronary heart disease is weak in older individuals [18], but the important differences in apoE frequencies between MI subject age groups, as far as we know, have not been demonstrated before. The different $\epsilon 3\epsilon 4$ and $\epsilon 4$ frequencies in different age intervals for patients with MI could be another important reason for the discrepancies between studies regarding the association between apoE genotype and MI. Wide age ranges in these studies may cause their differing results.

When analyzing the presence of other cardiovascular risk factors in MI subject classified by age range, we found, in addition to an increased frequency of $\epsilon 3\epsilon 4$ carriers, a significantly higher frequency of smokers among the younger group of subjects with MI (younger

than 57) than among the older group. In our population, these two risk factors (apoE genotype and smoking) coincide in those who suffer heart attacks early in life. Smokers tend to occupy the lower age ranges in MI studies as do $\epsilon 4$ allele carriers. Humphries et al. demonstrated that male smoker carriers of the $\epsilon 4$ allele showed significantly increased risk of CHD in middle-age [19], pointing to a potential gene-environment interaction in determining CHD risk.

Our data confirms the need to evaluate the apoE genotype to make preventive recommendations, especially at early ages, when other risk factors, such as smoking, may confer an additive risk for MI.

5. Conclusion

$\epsilon 3\epsilon 4$ and $\epsilon 4$ frequencies depend on the age range of the subjects with MI. The high $\epsilon 4$ allele prevalence in young MI subjects seems to indicate a genetic predisposition for an elevated MI incidence at early ages. This finding may help explain the discrepancies between studies analyzing association between apoE genotype and MI, and emphasizes the idea of considering apoE genotype for prevention at early age.

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