ELSEVIER

Atherosclerosis 145 (1999) 293–300

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

The genotype interactions of methylenetetrahydrofolate reductase and renin-angiotensin system genes are associated with myocardial infarction

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Received 6 July 1998; received in revised form 30 November 1998; accepted 8 February 1999

Abstract

We analyzed the evolution with age of the frequencies of the I/D polymorphism of the angiotensin I-converting enzyme (ACE), a1166c of the angiotensin II AT1 receptor (AT_1R), M235T of the angiotensinogen (AGT) and A225V of their methylenetetrahydrofolate reductase (*MTHFR*) gene in a healthy (H) population and the subsequent comparison to age- and sex-matched groups of myocardial infarction (MI) subjects. A total of 472 H subjects were divided into three groups <30, 30–55 and >55 years old and 277 individuals with MI into two groups 30–55 and >55 years old. The evolution with age showed that the AGT M allele ($P<0.001$) and the MTHFR V allele ($P<0.05$) frequency decreased with age in H men. The comparison between healthy and MI groups showed that the MM genotype frequency increased in MI men >55 years (OR = 4.16; 95% CI; 1.72–10.1) The cc genotype showed a similar behaviour (OR = 3.96; 95% CI; 1.21–12.9). In men, all the combinations with MM genotype presented a high risk, with OR values between 1.10 and 7.22. In women, the cc genotype increased in the MI >55 group (OR = 6.66; 95% CI; 2.02–21.9). All the combinations with the cc genotype showed OR values between 1.71 and 13.3. The MM genotype in men and cc genotype in men and women, are independent risk factors for MI. We propose that the study of the allele frequency evolution in an H population at different ages is essential to determine risk factors for MI in case-control studies, since data from isolated age-matched groups can be misinterpreted. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Myocardial infarction; Angiotensin I-converting enzyme; Angiotensin II AT1 receptor; Angiotensinogen; Methylenetetrahydrofolate reductase; Genotype; Allele; Frequency

1. Introduction

In humans, cardiovascular diseases (CVD) represent the major cause of death in adults. Due to the high incidence of CVD in individuals older than 30 years, the variation with age of the frequency of any CVD-related marker should be detectable in a healthy population (H). Few studies about the evolution with age of genetic polymorphisms have been reported and the majority of them are focused on longevity [1,2]. The genetic polymorphisms I/D of angiotensin I-converting enzyme (ACE), a1166c of angiotensin II (AII) AT1 receptor (AT_1R), M235T of angiotensinogen (AGT) and A225V of methylenetetrahydrofolate reductase (MTHFR) selected in this study have been classified as risk factors for myocardial infarction (MI) and hypertension in previous reports, although contrary results have been also obtained.

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PII: S0021-9150(99)00080-5

The DD genotype from ACE has been found to be a risk factor for MI [3], although it has been also reported as a non-risk [4] or even as a longevity factor [1,2]. The a1166c polymorphism from the AT_1R has been reported as a risk [5,6] or non-risk factor for MI in other papers [7]. The M235T polymorphism from AGT has been related to hypertension [8] and MI [9,10]. Finally, the A225V polymorphism of the MTHFR has been also associated to CVD because of the elevation of plasma homocysteine concentration [11] which is supposed to produce lesions on the vascular wall although its significance as a risk for CVD is also controversial [12].

In this report, we have studied the evolution with age of the genotype and allele frequencies, as well as their interactions, of four CVD-related genes in H subjects and their comparison to age-matched groups of MI individuals in order to determine their risk for MI and the influence of age and sex.

2. Material and methods

2.1. Subjects

A total of 472 (197 men and 275 women) H and 277 (220 men and 57 women) individuals with MI were selected for this study, from among residents in Málaga (Southern Spain) whose parents and grandparents were Caucasian and born in this region (Andalucía). H individuals, with no CVD antecedents, were recruited using the Andalusian Health Service identity card. Subjects with confirmed diagnosis of definite MI (typical prolonged chest pain or atypical symptoms, acute congestive heart failure, syncope, and serial cardiac enzymes elevation exceeding twice the upper limit of reference range and dynamic ECG changes typical of MI) were recruited from different cardiology services. After the approval by the University Hospital Ethical Committee, all the subjects were contacted by phone and from those whose consent was obtained, 5 ml of blood were taken. DNA was prepared using standard techniques. The investigation in this paper conforms with the principles outlined in the Declaration of Helsinki.

Subjects were divided by sex and according to their ages. H women were divided into three groups, $H < 30$ (mean age: 21 ± 4), $H = 30–55$ (40 ± 7) and $H > 55$ (70 ± 10), and MI women consisted of only one group $MI > 55$ (mean: 68 ± 8). H men were divided into three groups, $H < 30$ (20 ± 4), $H = 30–55$ (40 ± 6) and $H > 55$ (mean: 68 ± 10), and MI men were divided into two groups, $MI = 30–55$ (45 ± 5) and $MI > 55$ (67 ± 7). The $H < 30$ group has been used as a control to be compared to the other H groups in order to study the evolution with age but is not to be compared to MI groups. All the individuals were genotyped for the different alleles of the *ACE* (II, ID and DD), *AT_1R* (aa, ac and cc), *AGT* (MM, MT and TT) and methylenetetrahydrofolate reductase (AA, AV and VV) gene by polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) or PCR amplification of specific alleles (PASA).

2.2. Identification of ACE genotype

DNA samples were assayed for the I/D polymorphism using DNA amplification by PCR [13]. DD homozygotes were reamplified using specific primers in order to avoid misclassification of heterozygotes as homozygotes [14].

2.3. Identification of AGT genotype

PCR was performed according to the method of Russ et al. [15].

2.4. Analysis of genotype and allele frequencies for AT_1R variants by PASA

AT_1R genotypes were established by PCR using a nesting strategy. In the second round, the upper primer was designed to distinguish between allele a and c. The primers were as follows: upper 5′-gtgaaagaaggagcaagagaacattc-3′, lower 5′-tcgagcagccgtcatctgtctaatgc-3′; PCR was performed with 50 ng DNA. After initial denaturation 94°C 4 min, 35 cycles of PCR consisting of 94°C 1 min, 53°C 1 min and 72°C were carried out. Subsequently for the second round, 1 μl of the first round product was used as template and as internal primers, upper 5′-ctaccaaatcagcc/a-3′ and lower 5′-gctttgctttgtct-3′. In this case the same PCR protocol was used except the annealing temperature was 60°C. To confirm the genotyping, PCR products from ten controls were sequenced.

2.5. Identification of MTHFR genotype

PCR was performed according to the method of Frosst et al. [11].

2.6. Statistical analysis

The chi-square (χ^2) or Fisher's exact test was used to assess genotype and allele frequency differences between $H < 30$ with $H = 30–55$ and $H < 30$ with $H > 55$ and between age-matched H and MI groups, $H = 30–55$ with $MI = 30–55$ and $H > 55$ with $MI > 55$. The odd ratio (OR) and 95% confident interval (CI) were also calculated for age-matched H and MI groups.

As it is not possible to calculate the statistical significance when the frequency value is 0 ($n = 0$), we have considered $n = 0$ to be $n = 1$.

Table 1
Allele frequencies of healthy and myocardial infarction groups in women[a]

Gene	Allele	H<30 ($n=108$)	H=30–55 ($n=80$)	H>55 ($n=87$)	MI>55 ($n=52$)	
					Frequency	OR (CI)
AGT	M	0.58	0.54	0.55	0.56	0.98 (0.64–1.49)
	T	0.42	0.46	0.45	0.44	1.02 (0.67–1.54)
ACE	I	0.38	0.46	0.37	0.45	1.42 (0.87–2.33)
	D	0.62	0.54	0.63	0.55	0.71 (0.44–1.15)
AT_1R	a	0.65	0.79†	0.81¥	0.62‡	0.37 (0.21–0.64)
	c	0.35	0.21†	0.19¥	0.38‡	2.67 (1.55–4.58)
MTHFR	A	0.56	0.61	0.53	0.62	1.43 (0.87–2.35)
	V	0.44	0.39	0.47	0.38	0.70 (0.43–1.15)

[a] ACE, angiotensin I-converting enzyme; AGT, angiotensinogen; AT_1R, angiotensin II AT1 receptor; CI, 95% confident interval; MTHFR, methylenetetrahydrofolate reductase; OR, odd ratio; H<30, healthy individuals younger than 30 years of age; H=30–55, between 30 and 55 of age; H>55, older than 55 years of age; MI=30–55, myocardial infarction individuals between 30 and 55 years of age; MI>55, older than 55.
† $P<0.01$,
¥ $P<0.001$, significance between H<30 with H=30–55 and H>55 groups.
‡ $P<0.001$, significance among age-matched groups of healthy and myocardial infarction subjects.

3. Results

In general, a decrease of genotype frequency of any polymorphic gene with increasing age in a H population can be considered as a risk for mortality or morbidity.

We have used a new approach to case-control studies in which allele frequencies of individuals under 30 years who could suffer from MI, but in whom because of their ages the frequency has not yet been altered, were analyzed. Although these subjects cannot be used as a control group for MI since they are not matched by age, the analysis gives us a perspective about the allele and genotype frequencies before the onset of the disease. Furthermore, it can be used as a control to analyze the variation of frequencies in older H individuals to study the evolution with age.

3.1. Allele and genotype frequencies in women

In relation with the evolution with age in H women, the a allele of the AT_1R a1166c polymorphism showed a significant increase from 0.65 in H < 30 to 0.79 in H = 30–55 ($P < 0.01$) and 0.81 in H > 55 ($P < 0.001$), while in contrast the c allele decreased from 0.35 in H < 30 to 0.21 in H = 30–55 ($P < 0.01$) and 0.19 in H > 55 ($P < 0.001$) (Table 1). These differences are also observed when genotype frequencies are analyzed: the aa genotype increased from 0.40 in H < 30 to 0.61 in H = 30–55 ($P < 0.01$) and 0.66 in H > 55 ($P < 0.001$), and ac decreased from 0.51 in H < 30 to 0.31 in H > 55 ($P < 0.01$) (Table 2).

In women, H > 55 and MI > 55 age-matched groups were only studied because of the low number of individuals in the MI = 30–55 group ($n = 5$). This comparison showed frequencies from 0.81 in H > 55 to 0.62 in MI > 55 ($P < 0.001$) for the a allele, and 0.19 in H > 55 to 0.38 in MI > 55 for the c allele ($P < 0.001$) (Table 1). The most significant results were the increase of the frequency of the cc genotype from 0.03 in H > 55 to 0.19 in MI > 55 ($P < 0.001$, OR = 6.66), and the decrease of the aa genotype from 0.66 in H > 55 to 0.43 in MI > 55 ($P < 0.05$, OR = 0.38) (Table 2). None of the other alleles or genotype frequencies showed significant variations in relation with either the evolution with age or MI groups.

We analyzed the frequencies of paired genotypes, a and c homozygous genotypes with the other studied genes. It was observed that all the cc genotype interactions could represent a risk for women, although only ccMM ($P < 0.01$, OR = 13.3) and ccAA ($P < 0.05$, OR = 9.14), between H > 55 and MI > 55 groups, were statistically significant. In contrast, the majority of the aa genotype interactions showed an OR < 1 (Table 3).

3.2. Allele and genotype frequencies in men

The evolution with age in men showed a decrease of the M allele of the *AGT* gene from 0.59 in H < 30 to 0.37 in H > 55 ($P < 0.001$) and an increase of the T allele from 0.41 in H < 30 to 0.63 in H > 55 ($P < 0.001$) (Table 4). The study of genotype frequencies showed that the MM genotype decreased from 0.32 in H < 30 to 0.10 in H > 55 ($P < 0.01$) and the TT increased from 0.14 in H < 30 to 0.35 in H > 55 ($P < 0.01$) (Table 5). The V allele from *MTHFR* gene decreased from 0.47 in H < 30 to 0.33 in H > 55 ($P < 0.05$) and A allele increased from 0.53 in H < 30 to 0.67 in H > 55 ($P <$ 0.05). When the genotype frequency of this gene was analyzed, no significant variations with age were observed. No changes were found in the frequencies of the *ACE* and *AT_1R* gene polymorphisms in age-related H groups.

Table 2
Genotype frequencies of healthy and myocardial infarction groups in women[a]

Gene	Genotype	H<30 ($n=108$)	H=30–55 ($n=80$)	H>55 ($n=87$)	MI>55 ($n=52$)	
					Frequency	OR (CI)
AGT	MM	0.34	0.32	0.32	0.33	1.02 (0.50–2.06)
	MT	0.48	0.44	0.46	0.46	1.00 (1.00–1.00)
	TT	0.18	0.24	0.22	0.21	0.96 (0.41–2.23)
ACE	II	0.11	0.23	0.16	0.21	1.40 (0.58–3.70)
	ID	0.54	0.46	0.41	0.48	1.31 (0.66–2.60)
	DD	0.35	0.31	0.43	0.31	0.60 (0.29–1.24)
AT_1R	aa	0.40	0.61[†]	0.66[¥]	0.43[§]	0.38 (0.18–0.77)
	ac	0.51	0.36	0.31[†]	0.38	1.39 (0.67–2.87)
	cc	0.09	0.03	0.03	0.19[≠]	6.66 (2.02–21.9)
MTHFR	AA	0.34	0.33	0.24	0.38	1.96 (0.94–4.08)
	AV	0.45	0.54	0.57	0.47	0.63 (0.31–1.27)
	VV	0.21	0.13	0.19	0.15	0.80 (0.30–2.11)

[a] See Table 1 legend.
[†] $P<0.01$,
[¥] $P<0.001$, significance between H<30 with H=30–55 and H>55 groups.
[§] $P<0.05$,
[≠] $P<0.01$, significance among age-matched groups of healthy and myocardial infarction subjects.

The comparison between H = 30–55 and MI = 30–55 did not show any significant variation for any of the studied polymorphisms. However, an increase of the M allele from 0.37 in H > 55 to 0.54 in MI > 55 ($P<0.01$, OR = 1.97) and a decrease of the T allele from 0.63 in H > 55 to 0.46 in MI > 55 ($P<0.01$, OR = 0.51) (Table 4) were observed. Genotype analysis showed a variation from 0.10 in H > 55 to 0.30 in MI > 55 ($P<0.01$, OR = 4.16) for the MM genotype whereas the cc genotype increased from 0.04 in H > 55 to 0.17 in MI > 55 ($P<0.05$, OR = 3.96) (Table 5).

The interactions of paired genotypes only showed significance when H > 55 was compared to MI > 55 group, MMID ($P<0.05$, OR = 5.29), MMac ($P<0.05$, OR = 4.23), MMAV ($P<0.05$, OR = 3.46), MMVV ($P<0.05$, OR = 7.22), TTII ($P<0.05$, OR = 0.14), and TTac ($P<0.05$, OR = 0.36). Although the sizes of the groups are too small to draw conclusions when genotype combinations are analyzed, it can be said that MM interactions could represent a risk and TT a protection for MI (Table 6).

4. Discussion

A population in Hardy-Weinberg equilibrium for a particular polymorphism should display genotype frequencies that remain stable throughout life. Therefore any significant variation of allele frequencies throughout life must be mainly interpreted either as changes of individuals from the healthy to the unhealthy population or because of death.

It has been reported that the four studied polymorphisms are related to phenotype features. The I/D is related to variations of the ACE activity [16], the M235T to different angiotensinogen plasma levels [8], the a1166c to a major or minor vasotensional response [17] and the A225V to variable plasma homocysteine levels [11]. Therefore, it can be argued that changes with increasing age of genotype frequency imply that, directly or indirectly, these polymorphisms are linked to a functional status that decreases or increases with age certain genotype frequencies in a healthy population.

MI has been considered as a process of vascular occlusion mediated by vasotensional responses, with or without thrombotic elements, and vascular lesions which lead to inflammation and repairing (etiology of atherogenesis). The VV genotype can be indirectly related to vascular damage in function of higher homocysteine plasma levels [11]. Angiotensinogen levels [18] and AT_1R density [19] are increased during inflammation and repairing process. The cc genotype has been related to a strong vasotensional response in coronary arterial vessels and hypertension [17], and the DD to a faster and more potent vasoconstriction due to the rapid local conversion of AI into AII [20]. Therefore vasoconstriction, vascular wall damage, inflammation and repairing process could be also related to genotype interactions of these genes.

Since MI does not normally appear before 30 years of age, we have established a group of H individuals under this age, who can be considered as H but at risk for cardiovascular events, as a control group to be compared to the other H groups. This control population allowed us to follow up and compare the changes in allele frequency of the H < 30 group to H = 30–55, and to H > 55.

Table 3
Paired genotype frequencies of healthy and myocardial infarction groups in women[a]

Genotype	H < 30 (n = 108)	H = 30–55 (n = 80)	H > 55 (n = 87)	MI > 55 (n = 52)	
				Frequency	OR (CI)
aaII	0.04	0.15*	0.10	0.00	0.16 (0.02–1.07)
aaID	0.22	0.26	0.28	0.33	1.27 (0.60–2.67)
aaDD	0.14	0.20	0.28*	0.10[§]	0.28 (0.10–0.75)
aaMM	0.10	0.23*	0.28[†]	0.12[§]	0.34 (0.13–0.88)
aaMT	0.20	0.21	0.24	0.19	0.74 (0.30–1.78)
aaTT	0.10	0.17	0.14	0.12	0.81 (0.26–2.46)
aaAA	0.11	0.21	0.17	0.10	0.51 (0.17–1.48)
aaAV	0.21	0.36*	0.41[†]	0.21[§]	0.38 (0.17–0.82)
aaVV	0.08	0.04	0.07	0.12	1.76 (0.54–5.70)
ccII	0.00	0.00	0.00	0.06	5.26 (0.67–41.2)
ccID	0.05	0.01	0.00	0.04	3.44 (0.35–33.4)
ccDD	0.05	0.01	0.03	0.09	2.98 (0.86–10.2)
ccMM	0.04	0.01	0.00	0.13[#]	13.3 (2.48–72.2)
ccMT	0.05	0.01	0.03	0.06	1.71 (0.34–8.59)
ccTT	0.01	0.00	0.00	0.00	1.68 (0.10–28.2)
ccAA	0.05	0.00	0.00	0.09[§]	9.14 (1.49–56.1)
ccAV	0.04	0.03	0.03	0.06	1.71 (0.33–8.83)
ccVV	0.01	0.00	0.00	0.04	3.44 (0.35–33.4)

[a] See Table 1 legend.
* $P<0.05$,
[†] $P<0.01$, significance between H < 30 with H = 30–55 and H > 55 groups.
[§] $P<0.05$,
[#] $P<0.01$, significance among age-matched groups of healthy and myocardial infarction subjects.

Table 4
Allele frequencies of healthy and myocardial infarction groups in men[a]

Gene	Allele	H < 30 (n = 70)	H = 30–55 (n = 64)	H > 55 (n = 63)	MI = 30–55 (n = 105)		MI > 55 (n = 115)	
					Frequency	OR (CI)	Frequency	OR (CI)
AGT	M	0.59	0.47	0.37[¥]	0.56	1.45 (0.93–2.24)	0.54[#]	1.97 (1.27–3.06)
	T	0.41	0.53	0.63[¥]	0.44	0.68 (0.43–1.07)	0.46[#]	0.51 (0.33–0.79)
ACE	I	0.38	0.45	0.44	0.40	0.80 (0.51–1.26)	0.42	0.90 (0.59–1.37)
	D	0.62	0.55	0.56	0.60	1.24 (0.80–1.92)	0.58	1.12 (0.71–1.76)
AT_1R	a	0.72	0.70	0.71	0.65	0.80 (0.49–1.30)	0.64	0.72 (0.45–1.16)
	c	0.28	0.30	0.29	0.35	1.24 (0.77–1.98)	0.36	1.38 (0.87–2.19)
MTHFR	A	0.53	0.62	0.67*	0.58	0.81 (0.51–1.29)	0.57	0.65 (0.41–1.02)
	V	0.47	0.38	0.33*	0.42	1.22 (0.79–1.89)	0.43	1.54 (0.98–2.42)

[a] See Table 1 legend.
* $P<0.05$,
[¥] $P<0.001$, significance between H < 30 with H > 55 groups.
[#] $P<0.01$, significance among age-matched groups of healthy and myocardial infarction subjects.

We have observed that the comparison of genotype frequencies between H and MI groups, without division by age and sex, did not show statistical significance (data not shown). When subjects were divided by age, only the cc genotype showed significant differences as an individual genotype between H > 55 and MI > 55 groups (OR = 4.83) (data not shown). When subjects were divided by age and sex, the individual genotype significance was extended to MM (OR = 4.16) and cc (OR = 3.96) in men (Table 5), and cc in women (OR = 6.66) (Table 2).

No variations of age-related genotype frequencies between women and men in the H < 30 group were observed. On the contrary, it is remarkable that the MM genotype frequency did not vary in H women (H < 30: 0.34, H = 30–55: 0.32 and H > 55: 0.32) whereas it changed dramatically in men from 0.32 in H < 30 to 0.19 in H = 30–55 and 0.10 in H > 55. This

Table 5
Genotype frequencies of healthy and myocardial infarction groups in men[a]

Gene	Genotype	H<30 (n = 70)	H = 30–55 (n = 64)	H>55 (n = 63)	MI = 30–55 (n = 105)		MI>55 (n = 115)	
					Frequency	OR (CI)	Frequency	OR (CI)
AGT	MM	0.32	0.19	0.10[†]	0.31	1.89 (0.90–3.95)	0.30[≠]	4.16 (1.72–10.1)
	MT	0.54	0.56	0.55	0.51	0.82 (0.43–1.55)	0.47	0.71 (0.38–1.30)
	TT	0.14	0.25	0.35[†]	0.18	0.66 (0.31–1.41)	0.23	0.54 (0.27–1.10)
ACE	II	0.13	0.20	0.21	0.15	0.71 (0.32–1.57)	0.16	0.71 (0.32–1.57)
	ID	0.50	0.50	0.48	0.50	0.98 (0.47–2.04)	0.52	1.20 (0.64–2.24)
	DD	0.37	0.30	0.31	0.35	1.29 (0.66–2.53)	0.32	1.02 (0.50–2.09)
AT_1R	aa	0.53	0.48	0.48	0.42	0.77 (0.41–1.44)	0.45	0.91 (0.49–1.68)
	ac	0.38	0.42	0.48	0.46	1.15 (0.62–2.11)	0.38	0.68 (0.36–1.27)
	cc	0.09	0.10	0.04	0.12	1.36 (0.50–3.71)	0.17[§]	3.96 (1.21–12.9)
MTHFR	AA	0.30	0.44	0.52	0.39	0.82 (0.43–1.57)	0.38	0.56 (0.30–1.04)
	AV	0.46	0.37	0.31	0.37	0.98 (0.41–2.36)	0.38	1.44 (0.74–2.78)
	VV	0.24	0.19	0.17	0.24	1.35 (0.63–2.90)	0.24	1.45 (0.67–3.14)

[a] See Table 1 legend.
[†] $P<0.01$, significance between H<30 with H>55 groups.
[§] $P<0.05$,
[≠] $P<0.01$, significance among age-matched groups of healthy and myocardial infarction subjects.

Table 6
Paired genotype frequency of healthy and myocardial infarction groups in men[a]

Genotype	H<30 (n = 70)	H = 30–55 (n = 64)	H>55 (n = 63)	MI = 30–55 (n = 105)		MI>55 (n = 115)	
				Frequency	OR (CI)	Frequency	OR (CI)
MMII	0.04	0.03	0.00	0.03	0.91 (0.11–7.09)	0.05	3.41 (0.44–26.1)
MMID	0.14	0.13	0.03*	0.16	1.35 (0.54–3.33)	0.15[§]	5.29 (1.37–20.3)
MMDD	0.13	0.03*	0.07	0.12	4.00 (0.94–16.9)	0.10	1.71 (0.54–5.41)
MMaa	0.16	0.08	0.07	0.14	1.96 (0.68–5.60)	0.14	2.38 (0.78–7.22)
MMac	0.12	0.09	0.03	0.13	1.36 (0.50–3.71)	0.12[§]	4.23 (1.03–17.3)
MMcc	0.04	0.02	0.00	0.04	2.49 (0.29–21.4)	0.04	2.81 (0.35–22.6)
MMAA	0.09	0.08	0.05	0.08	0.97 (0.21–4.31)	0.05	1.10 (0.29–4.17)
MMAV	0.14	0.09	0.05	0.14	1.61 (0.59–4.33)	0.15[§]	3.46 (1.04–11.5)
MMVV	0.09	0.02	0.00	0.09	5.90 (0.91–38.1)	0.10[§]	7.22 (1.19–43.6)
TTII	0.01	0.05	0.11*	0.01	0.19 (0.02–1.54)	0.02[§]	0.14 (0.03–0.57)
TTID	0.09	0.13	0.14	0.13	1.07 (0.41–2.75)	0.17	1.18 (0.51–2.71)
TTDD	0.04	0.07	0.10	0.04	0.46 (0.12–1.79)	0.04	0.43 (0.13–1.44)
TTaa	0.07	0.10	0.14	0.08	0.79 (0.25–2.50)	0.11	0.70 (0.28–1.76)
TTac	0.06	0.15	0.19*	0.08	0.44 (0.37–1.18)	0.08[§]	0.36 (0.15–0.88)
TTcc	0.01	0.00	0.02	0.02	1.85 (0.19–17.9)	0.04	2.81 (0.35–22.6)
TTAA	0.03	0.14*	0.16[†]	0.10	0.71 (0.27–1.85)	0.12	0.67 (0.27–1.67)
TTAV	0.04	0.06	0.11	0.04	0.59 (0.14–2.48)	0.05	0.44 (0.14–1.34)
TTVV	0.07	0.05	0.08	0.04	0.80 (0.15–4.29)	0.06	0.75 (0.22–2.49)

[a] See Table 1 legend.
* $P<0.05$,
[†] $P<0.01$, significance between H<30 with H = 30–55 and H>55 groups.
[§] $P<0.05$, significance among age-matched groups of healthy and myocardial infarction subjects.

fact can be interpreted as suggesting that genotype frequency differences between sex do not appear in the H < 30 group since MI arises in later ages. On the other hand, changes in polymorphism frequency in subjects older than 30 years could be due to the higher incidence of MI in men, that could be related to renin-angiotensin system physiological variations between sex [21–23].

According to the observed variations with age for the MM genotype frequency in men (Table 5), this genotype can be considered as a risk factor for morbimortality. Furthermore, when this increased genotype frequency is observed in MI = 30–55 (0.31) and MI > 55 (0.30) and compared to their age-matched H groups, the MM genotype can be considered as a risk factor for MI.

The age-related evolution of the AGT allele frequency is a clear example of the importance of selecting exact age-matched populations. According to the variations with age of the MM genotype frequency in men, a non-well adjusted mean age between case and control populations could change the result significancies. For instance, the TT genotype frequency increases with age in H subjects (H < 30: 0.14; H = 30–55: 0.25; H > 55: 0.35). If these changes with age are not studied and age-matched groups with very different mean ages are used, results could be misinterpreted. This could be the case for previous reports that defined the TT genotype as a risk factor for MI [9,10]; the increased frequency in MI groups does not necessarily imply a risk factor for individuals older than 60 years. On the contrary, perhaps there is a higher frequency of TT genotype because these individuals survive.

In relation to ACE individual genotypes, we have not found any significant association with MI. In this sense, no statistically significant associations were found for genotype combinations with the exception of DDaa. However when the DD genotype was associated with the cc genotype in women, it presented an OR = 2.98, whereas with the aa it presented an OR = 0.28 (Table 3). And the association DDMM in men presented an OR = 1.71, whereas with TT it presented an OR = 0.43 (Table 6). These genotype interactions could explain the controversial results obtained in several reports among the approximately 54 published papers up to 1996, in which the authors studied the OR for the ACE genotypes in relation with MI and most of them established the D allele as a risk factor (35 versus 7). But up to November 1997, 12 reports showed inverted proportions (four versus eight) in which the correlation between the D allele and MI was not found.

The individual frequency of the MTHFR VV genotype did not decrease significantly with age in H subjects, so it should not be considered as a risk factor. However when it was associated with the MM in men, this genotype presented an OR = 7.22 whereas with the TT it presented an OR = 0.75 (Table 6). When it was associated with the cc genotype in women, it presented an OR = 3.44 whereas with aa it was OR = 1.76 (Table 3). Like the DD genotype, contradictory results about the VV and its role as a risk factor for MI have been reported [11,12,24,25]. The VV genotype combined with MM or cc enhances the risk, but when it interacts with TT or aa it does not have any effect. The absence of MMVV subjects in the H > 55 group is remarkable, when ten individuals out of 150 should be found, whereas 15 out of 167 are observed in the MI > 55 group (data not shown). The AA genotype frequency decreased, though not significantly, in women H > 55, contrary to a significant increase of the A allele in men. This could be explained by the pleiotropic features of this gene, taking into account its influence on nucleotide synthesis and methyl groups availability. This means that the MTHFR genotype frequency variations could be related to other diseases besides CVD, and different pharmacological or dietary intake of folic acid [26,27].

The AT_1R polymorphisms are considered as intervening factors for MI. The c allele has being referred to as a risk factor in men, mainly when it interacts with the DD genotype [5,6,28]. Other reports have not found the cc genotype and its interactions with the DD genotype a risk factor [7]. We have found the cc genotype to be a strong risk factor in women and men and it should be pointed out that the cc genotype has been recently related to a strong vasoconstrictor response in coronary arterial vessels [29].

In conclusion, to establish a polymorphic allele as a risk factor for high incidence diseases, we consider it essential to study first the evolution with age of its frequency in a healthy population. The renin-angiotensin system and *MTHFR* gene polymorphisms are related, directly or indirectly, to a functional status, different between sexes and associated with MI risk. The cc genotype in women and men, and the MM in men are independent risk factors for MI, whereas the VV genotype could enhance this risk. We think that further studies are needed to assess these risk factors in larger populations, especially if genotype interactions are analyzed.

Acknowledgements

This study was supported by two grants, Ref. SAF95-0804 and SAF98-0163 from the C.I.C.Y.T., Spanish Ministry of Education and Culture; and the PREDICE project supported by SQUIBB Industria Farmacéutica S.A. (Bristol-Meyer Squibb).We thank the Ambulatory Centers of Limonar, Portada Alta and Torcal of the Andalusian Health Service for providing samples.

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