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RESEARCH ARTICLE

Lack of association of angiotensin-converting enzyme insertion/deletion polymorphism and myocardial infarction at very young ages

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Abstract

We examined whether angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism is associated with the development of myocardial infarction (MI) at ≤ 35 years of age. The study sample consisted of 201 patients with premature MI and 140 age- and sex-matched healthy individuals. No difference was found in the distribution of *ACE* genotypes between the patients and controls. A higher prevalence of the DD genotype among hypertensives was found compared with the non-hypertensive patients (62.5% vs 35.6%, $p = 0.01$). *ACE* polymorphism is not associated with the development of premature MI and this might be due to the low prevalence of hypertension in young coronary patients.

Keywords: ACE *I/D polymorphism; plasminogen activator inhibitor-1; premature myocardial infarction; risk factors*

Introduction

Coronary artery disease (CAD) is multifactorial and involves the interaction of multiple environmental and genetic factors. The genetic component is stronger when myocardial infarction (MI) develops at a very young age. The exact pathogenetic mechanism of premature MI is unknown. It is usually characterized by less atheromatic burden in coronary arteries, lower prevalence of hypertension and diabetes mellitus and it has been proposed that genetic variations related with the vascular tone of coronary arteries or the haemostatic–fibrinolytic balance may have a greater impact in the pathogenesis (Zimmerman et al. 1995, Rallidis et al. 2003, Marenberg et al. 1994).

Angiotensin I-converting enzyme (ACE) is the rate-limiting enzyme of the renin–angiotensin system that generates angiotensin II and inactivates bradykinin. Insertion (I)/deletion (D) polymorphism in the *ACE* gene is due to the insertion or deletion, respectively, of a 287 base pair Alu repeat sequence in intron 16 of this gene (Rigat et al. 1990). The *ACE* DD genotype is associated with increased plasma levels of circulating ACE which results in enhanced production of angiotensin II. It has been proposed that the *ACE* DD genotype might be associated with the development of CAD or MI probably through the atherothrombotic properties of angiotensin II, such as coronary vasoconstriction and endothelial dysfunction. In addition, angiotensin II impairs the balance of the fibrinolytic system by increasing the levels of plasminogen activator inhibitor (PAI)-1 (Vaughan et al. 1995, Brown et al. 1998, Huber et al. 2001) and it has also been reported that the *ACE* DD genotype is associated with higher PAI-1 levels (Cambien et al. 1992, Kim et al. 1997).

Several studies have explored the role of *ACE* gene polymorphism in MI but the results were conflicting (Pfohl et al. 1999, Araújo et al. 2005, Agerholm-Larsen et al. 2000, Acarturk et al. 2005, Seckin et al. 2006, Bautista et al. 2004, Ludwig et al. 1995, Methot et al. 2005, Andrikopoulos et al. 2004, Covolo et al. 2003); even in a recent meta-analysis the heterogeneity between studies

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was evident (Zintzaras et al. 2008) and only a modest positive association between the *ACE* I/D polymorphism and CAD was reported. Controversy also surrounds the impact of *ACE* I/D polymorphism on the pathogenesis of premature MI or CAD (Petrovitc et al. 2001, Sekuri et al. 2005, Berdeli et al. 2005, Viitanen et al. 2001, Wesolowska et al. 1998, Ramasawmy et al. 1996, van Bockxmeer et al. 2000). In our study we examined whether or not *ACE* I/D polymorphism is associated with the very early development of MI, i.e. at ≤ 35 years of age.

Material and methods

Subjects and study design

This case–control study included 201 consecutive patients who had survived their first acute MI occurring at ≤ 35 years of age (mean age ± SD, 32.2 ± 3.4 years). They had been admitted to the Coronary Care Unit of the University General Hospital 'Attikon' and the General Hospital of Nikea between January 1998 and December 2007. The diagnosis of acute MI was based on the presence of more than two of the following three criteria: (1) characteristic chest pain lasting >30 min, (2) ST elevation >0.1 mV on at least two adjacent electrocardiographic leads and (3) increase of creatine kinase to peak levels of at least twofold the upper limit of normal values.

All patients underwent coronary angiography and left ventriculography by the Judkins technique prior to discharge from hospital. Significant coronary artery stenosis was defined as ≥ 50% reduction in lumen diameter of any of the three coronary arteries or their primary branches. Coronary arteries with smooth contours and no focal diameter reduction or with non-haemodynamically significant atherosclerotic lesions (<50% stenosis) were defined as 'normal'.

One hundred and forty healthy age- and sex-matched subjects who had undergone a minor orthopaedic intervention in our institution served as the control group. Diabetic and hypertensive subjects were excluded. In addition, all subjects were free of a personal or family history of cardiovascular or thromboembolic disease. Both patients and controls were living in the same areas during the time period of the study and were of Greek origin. Cardiovascular risk factors were recorded in all individuals. The following definitions were used: hypertension, blood pressure ≥ 140/90 mmHg and/or antihypertensive treatment; hypercholesterolaemia, total cholesterol > 200 mg dl^{-1} (5.7 mmol l^{-1}) and/or lipid-lowering agents; diabetes mellitus, fasting plasma glucose > 125 mg dl^{-1} (6.94 mmol l^{-1}) and/or glucose-lowering treatment. Smokers were defined as participants who reported smoking currently and regularly (at least five cigarettes per day).

The study was approved by the ethics committee of both participating hospitals and all subjects gave their informed consent.

Blood sampling and biochemical analysis

Peripheral blood samples were collected from patients and controls after an overnight fast for assessing levels of lipids and PAI-1, and for DNA analysis. In particular, blood from coronary patients was taken twice: within 24 h of admission for lipid measurements and 2 months after discharge for PAI-1 measurements, in order to minimize the acute reaction effect of MI on PAI-1 levels.

For the measurement of PAI-1, blood samples were collected into siliconized Vacutainer tubes containing trisodium citrate. Samples were centrifuged at 3000*g* for 15 min within 15 min of collection. Plasma samples were stored at –80°C until PAI-1 analysis. PAI-1 levels were determined by a quantitative sandwich immunoenzyme technique (ASSERACHROME PAI-1, Stago, Asneires, France) performed on a plastic support coated with mouse monoclonal antihuman PAI-1 antibody that captures the PAI-1 to be measured. The assay detects the total circulating PAI-1, both free and complexed with tissue plasminogen activator, bound and not bound to vitronectin, both in active and inactive forms. The detection limit of the assay is 0.1 ng ml^{-1} of PAI-1 and the interassay reproducibility of the assay for normal and abnormal (high) results has a coefficient of variation of 8.69% and 6.52%, respectively.

Blood sampling and DNA analysis

Genomic DNA was extracted from EDTA anticoagulated blood by using a silica membrane-based DNA purification method (QIAamp DNA blood kit; Qiagen, Hilden, Germany) which yields up to 60 μg of DNA from 2 ml initial blood volume. The *ACE* I/D polymorphism detection was based on a reverse hybridization principle (CVD strip assay; Viennalab) which includes the following successive steps: polymerase chain reaction (PCR), amplification of the target sequence using biotin-labelled primers, denaturation of the PCR product and selective hybridization to a test strip containing fixed allele oligonucleotide probes complementary to the deletion and insertion alleles. Bound biotinylated sequences were visualized using streptavidin–alkaline phosphatase and a specific colour substrate. The PCR reaction mixture consisted of 0.1 μg of DNA, a master mix provided by the manufacturer that contains primers that flank the I/D area, dNTPs and 1 U Taq DNA polymerase. The PCR cycles were optimized as follows: 2 min of initial denaturation, 30 cycles of amplification (15 s denaturation at 94°C, 30 s annealing at 58°C, 30 s extension at 72°C) and a final extension of 3 min at 72°C.

Statistical analysis

Continuous variables were expressed as mean ± SD and were compared using the unpaired Student's *t*-test. Categorical variables were presented as frequencies and were tested by the χ^2 test corrected for continuity. Odds ratios (OR) and their corresponding 95% confidence intervals (CIs) were calculated using univariate or multivariate logistic regression analysis. We tested the controls for Hardy–Weinberg equilibrium using the χ^2 test with one degree of freedom. The genotype distribution of cases vs controls was compared using a 2 × 3 contingency table with a χ^2 test. A *p*-value <0.05 was considered significant. The SPSS 13.0 software was used for all calculations.

Results

ACE *I/D polymorphism, premature CAD and extent of CAD*

The prevalence of major cardiovascular risk factors and the distribution of *ACE* I/D genotypes in young survivors of MI and controls are shown in Table 1. The distribution of the *ACE* genotypes in controls was in Hardy–Weinberg equilibrium ($p = 0.289$). Smoking, hypercholesterolaemia and hypertension were more prevalent in patients than in controls at statistically significant levels. There was no difference in the distribution of *ACE* I/D genotypes between patients and controls (all $p > 0.05$). In addition, there was no association between cases, controls and genotype distribution when genotype distribution was also tested using a 2 × 3 contingency table ($p = 0.294$).

We further examined the associations of *ACE* I/D polymorphism and the risk of MI according to the following genetic models (Zintzaras & Lau 2008): (1) the allele contrast (I vs D alleles), (2) the contrast of extremes homozygotes (II vs DD genotypes), (3) the recessive model (ID+II vs DD genotypes) and (4) the dominant model (II vs DD+ID genotypes). There were no statistically significant differences after adjustment for smoking or hypercholesterolaemia (data not shown) in any of the tested models.

Coronary angiogram revealed 'normal' coronary arteries in 35 patients (17.4%), one-vessel disease in 101 (50.2%), two-vessel disease in 38 (18.9%) and three-vessel disease in 27 (13.4%) patients. There was no association between *ACE* I/D genotypes and the number of significantly diseased coronary arteries (Table 2). In patients without significant stenoses the relative frequency of the D allele was 0.55, in patients with one-vessel disease 0.58, with two-vessel disease 0.65 and with three-vessel disease 0.56 ($p = 0.9$).

There was a higher prevalence of the DD genotype in the 24 hypertensive compared with the non-hypertensive patients (62.5% vs 35.6%, $p = 0.01$). In Table 3 we report the genotype distribution of hypertensive and non-hypertensive young MI patients. Patients with hypertension had a three times greater odds of having the DD genotype compared with non-hypertensive patients (95% CI 1.31–7.30, $p = 0.01$). Hypertension remained a significant predictor of having the DD genotype among MI patients after adjustment for diabetes mellitus, hypercholesterolaemia and smoking status (OR 2.57, 95% CI 1.03–6.40, $p = 0.04$). Finally, the prevelance of DD genotype was higher in the hypertensive patients compared to the controls (62.5% vs 34.3%, p = 0.017).

PAI-1 levels

Patients had greater levels of PAI-1 than controls (28.9 ± 22.4 vs 23.4 ± 15.6 ng ml^{-1}, $p = 0.01$). In the whole

Table 1. Prevalence of major cardiovascular risk factors in young survivors of myocardial infarction and control subjects.

	Patients ($n = 201$)	Controls (n = 140)	*p*-Value
Age (years), mean ± SD	32.2 ± 3.4	31.8 ± 3.5	0.27
Males	176 (87.6%)	123 (87.9%)	0.94
Current smokers	188 (93.5%)	71 (50.8%)	<0.001
Hypercholesterolaemia	148 (73.6%)	60 (42.8%)	<0.001
Hypertension	24 (12%)	0 (0%)	<0.001
Diabetes mellitus	6 (3%)	0 (0%)	0.25
ACE I/D polymorphism			
II	35 (17.4%)	19 (13.6%)	0.34
ID	88 (43.8%)	73 (52.1%)	0.13
DD	78 (38.8%)	48 (34.3%)	0.39
Frequency of D allele	0.607	0.604	0.93

ACE, angiotensin-converting enzyme.

Table 2. Relationship between angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) genotypes and extent of coronary artery disease among young survivors of myocardial infarction.

	Number of significantly diseased vessels (>50% stenosis)			
	0	1	2	3
Relative genotype frequency (%)				
DD	26.5	37.6	47.4	39.0
ID	58.8	42.6	36.8	43.5
II	14.7	19.8	15.8	17.5
Odds ratio (95% confidence interval) for having three- vs one-vessel disease	DD vs. ID and II 1.512 (0.804–2.843)			
	DD and ID vs. II 1.358 (0.591–3.123)			

Table 3. Angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) genotype distribution of hypertensive and non-hypertensive survivors of myocardial infarction.

Genotype	Hypertensive patients (*n*)	Non-hypertensive patients (*n*)	Total (*n*)	*p*-Value
ID	6	82	88	0.04*
DD	15	63	78	
II	3	32	35	
Total	24	177	201	

*indicates association between ACE/ID genotypes and the presence of hypertension in young survivors of myocardial infarction.

population, carriers of the D allele (ID and DD genotypes) had higher PAI-1 levels compared with non-D allele carriers (27.9 ± 21 vs 21.1 ± 11 ng ml^{-1}, $p = 0.002$). Univariate logistic regression analysis revealed that PAI-1 levels were a predictor of the presence of the D allele (OR 1.025, 95% CI 1.001–1.050, $p = 0.04$).

Discussion

Our data indicate that *ACE* I/D polymorphism is not associated with the development of MI at ≤ 35 years of age. The lack of association might be due to the low prevalence of hypertension among young coronary patients as there was higher prevalence of the DD genotype in hypertensive compared with normotensive patients.

The precise pathogenetic mechanisms of premature MI are unknown. However, atherosclerotic plaque rupture or endothelial layer erosion, coronary artery spasm, thrombogenic states or inflammation, per se or in combination, may play an important role in the pathogenesis which is influenced by a complex interaction of genetic and environmental factors (Choudhury & Marsh 1999). The association of the *ACE* DD genotype with increased plasma levels of circulating ACE and subsequently high production of angiotensin II challenged us to investigate whether this genotype is associated with the development of premature MI.

There are a few studies with controversial data regarding the role of *ACE* I/D polymorphism in the development of premature MI. Some (Petrovic et al. 2001, Sekuri et al. 2005, Berdeli et al. 2005) demonstrated that *ACE* I/D polymorphism is associated with premature CAD or MI, while others (Viitanen et al. 2001, Wesolowska et al. 1998, Ramasawmy et al. 1996, van Bockxmeer et al. 2000) failed to establish an association. However, these studies cannot be directly compared with ours as the ethnic make up was different as well the age limit that was applied to define premature MI. In particular, an association was reported in Slovenian (Petrovic et al. 2001) and Turkish (Sekuri et al. 2005, Berdeli et al. 2005) patients, while an absence of association was reported in Finnish patients (Viitanen et al. 2001), French Canadians (Wesolowska et al. 1998), Mauritian Indians (Ramasawmy et al. 1996) and Western Caucasians (van Bockxmeer et al. 2000). In addition, all these studies set higher age limits ranging from 45 to 55 years. Therefore, on the grounds of age limit our study is the first to report the lack of association of *ACE* I/D polymorphism with the development of MI at a very young age, i.e. ≤ 35 years of age. By recruiting very young MI survivors we formed a good model to unmask the impact of a potential genetic risk factor.

In our study we found that, in the whole population, carriers of the D allele (ID and DD genotypes) had higher PAI-1 levels compared with non-D allele carriers. The pathophysiological significance of this finding is unclear as the DD genotype, a genetic variant associated with impaired fibrinolysis, was not associated with premature MI in our study. A possible explanation is that the effect of *ACE* I/D polymorphism might be modified and attenuated by the interaction with other genetic and environmental factors. This also enforces the hypothesis that genotypic combinations are more important than single gene polymorphisms.

We also found a higher prevalence of the DD genotype among young hypertensive survivors of MI compared with normotensive patients. There are contradictory results regarding the association of the DD genotype with essential hypertension and this inconsistency has been mainly attributed to racial/ethnic diversity (O'Donnell et al. 1998, Morshed et al. 2002, Mondry et al. 2005). Whether or not the presence of the DD genotype in young hypertensive subjects predisposes them to develop MI cannot be inferred from our study as our design excluded hypertensive healthy subjects.

A limitation in our study is the fact that we recruited young survivors of an acute MI. Therefore, it cannot be excluded that fatal cases might have a different prevalence of the studied polymorphism. Another limitation is the relatively small size, for a genetic study, of our population. However, in our opinion the selection of very young survivors of MI who represent a rare entity is the strength of our study as there are very limited data in this age group. In addition to this, power analysis calculation revealed that the study had 77% power with $\alpha < 0.05$ to detect a 10% difference in the D allele frequencies between cases and controls for the presence of MI.

In conclusion, our data indicate that *ACE* I/D polymorphism is not associated with the very early development of MI, i.e. at ≤ 35 years of age. However, an association cannot be excluded in certain high-risk young individuals such as the hypertensive, an issue that can be answered by larger studies focused on these high-risk populations.

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