Clinical Research

Effect of I/D Polymorphism of the Angiotensin Converting Enzyme Gene on Postinfarction In-Hospital Mortality

Findings of the GEMIG Study

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Introduction: I/D polymorphism of the angiotensin converting enzyme (ACE) gene has been associated with left ventricular remodelling and prognosis following acute myocardial infarction (AMI). In this study we investigated whether the different genotypes of this polymorphism were related with higher in-hospital mortality after AMI and whether its influence is exerted via a change in left ventricular ejection fraction (LVEF) after AMI.

Methods: We studied 1603 consecutive patients with AMI (mean age 62±13 years) who participated in the GEMIG (Genetic predisposition and Epidemiology of acute Myocardial Infarction in the Greek population) study. This multi-centre study was designed to assess the genetic predisposition to AMI and the postinfarction prognosis in the Greek population.

Results: The total in-hospital mortality was 7.5%. The incidences of the genotypes studied did not differ significantly between patients who died and those who survived. The LVEF did not differ between patients with and without the DD genotype (45±10 versus 45±10%, p: 0.892). A multifactorial analysis, corrected for all probable confounding factors, found that only age (Relative risk: 1.051, 95% CI: 1.011-1.094, p: 0.013) and LVEF ≤ 45% (Relative risk: 4.932, 95% CI: 1.448-16.796, p=0.011) showed an independent and statistically significant correlation with in-hospital mortality. The different I/D polymorphism genotypes were unrelated to in-hospital mortality or to the patients' LVEF following AMI.

Conclusions: In this multi-centre study no correlation was found between I/D polymorphism of the ACE gene and in-hospital mortality following AMI. In contrast, a low LVEF was an independent predictive factor for a poor short-term prognosis. These findings, from a broad, specially designed multi-centre study, cast doubt on the role of I/D polymorphism of the ACE gene in the pathogenesis of AMI and on its relevance to the prognosis of patients after AMI.

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The renin-angiotensin system plays a primary role in the pathophysiology of a variety of cardiovascular diseases.[1] The gene that codes for angiotensin converting enzyme (ACE) is located on chromosome 17 and is characterised by a polymorphism based on the presence (insertion-I) or absence (deletion-D) of a 287 base pair in intron 16.[2]

Individuals with DD genotype exhibit higher levels of ACE in the plasma and in the myocardium,[3] and many studies have sought correlations between this polymorphism and certain cardiovascular diseases. Reports have linked I/D polymorphism with a higher risk of myocardial infarction[4,5] (although there are also conflicting findings[6-8]), with left ventricular hypertrophy,[9,10] the appearance of hypertension in men,[11,12] and with left ventricular dilation after myocardial infarction.[13]

The possibility of a correlation between the different genotypes of I/D ACE gene polymorphism and postinfarction prognosis has been studied intensively, since DD individuals show a greater incidence of left ventricular hypertrophy,[9,10] higher levels of plasma norepinephrine[13] and tissue ACE,[14] and greater left ventricular dilation following myocardial infarction.[13] However, these studies have produced conflicting findings.[6,8,15,16]

In the present study we investigated, in a relatively homogeneous Greek population at low risk for coronary artery disease, whether I/D polymorphism was related with disturbances of left ventricular function or with an increased risk of in-hospital mortality following acute myocardial infarction (AMI).

Material and methods

Patient population

The GEMIG (Genetics and Epidemiology of acute Myocardial Infarction in the Greek population) study is a multi-centre study that was designed to evaluate the genetic predisposition to myocardial infarction and the postinfarction prognosis in the Greek population. The participating centres were 9 cardiology departments in 7 hospitals in 3 Greek cities (Athens, Thessaloniki, Piraeus) and the Department of Biological Sciences at the University of Warwick in Great Britain. The hospitals that participated in the study are listed in the Appendix. A total of 1749 consecutive patients with AMI were included in the GEMIG study. Blood samples for genetic analysis and a check of the genotype of ACE gene I/D polymorphism were taken from 1603 patients, who comprised the study population.

Essential demographic data were recorded immediately after admission. Patients were considered to have hypercholesterolaemia if they were taking hypolipidaemic medication or if they had exhibited total cholesterol levels above 200 mg/dl on repeated measurements prior to their admission. Hypertensive patients were those who were taking antihypertensive medication or who reported repeated measurements of systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. Patients with diabetes mellitus were those who were under antidiabetic treatment or who had repeated resting blood sugar levels above 125 mg/dl. The study form was filled in at the end of the hospitalisation period, when all necessary data relating to treatment and outcome were available. The study protocol did not affect in any way the diagnostic and therapeutic interventions applied in individual cases. Each hospital's scientific committee approved the protocol and all patients gave informed consent to their inclusion in the study.

DNA Analysis

I/D polymorphism of the ACE gene

In order to reduce the likelihood of underestimating the D allele because of insufficient multiplication of the I allele, all samples were analysed by the polymerase chain reaction (PCR) method, using the following initial nucleotide sequence (primer): 5′ limb: 5′-CTg-gAg-ACC-ACT-CCC-ATC-CTT-TCT-3′, 3′ limb: 5′-gAT-gTg-gCC-ATC-ACA-TTC-gTC-AgA-T-3′, and 5′ insertion specific primer: 5′-Tgg-gAT-TAC-Agg-CgT-gAT-ACA-g-3′. The following conditions were applied during the PCR: 0.2-0.5 μg DNA multiplied in 25 μL reaction mixture with the following composition: 10mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM dATP/dCTP/dGTP/dTTP, 1.5 mM $MgCl_2$, 25 ng of each primer and 0.5 units of Taq DNA polymerase. The DNA multiplication cycle was carried out using PCR cycler (Techne GENEE Thermal Cycler) and included the following stages: 1.5 minutes denaturation at 94˚C, followed by 35 cycles of 30 seconds at 94˚C, 30 seconds at 55˚C and 1 minute at 72˚C, followed by another 10 minutes at 72˚C. With the aid of PCR, sequences of 158 bases representing the I allele (derivative of 5′ specific for insertion specific primer) and/or sequences of 192 bases representing the D allele were derived, thus allowing the identification of the II, ID and DD genotypes. During the PCR method we frequently observed the reproduction of an additional sequence of 492 bases, which also represent the I allele.

Table 1. Baseline characteristics of patients in relation to genotype of I/D angiotensin converting enzyme gene polymorphism.

	II or ID (n=1039)	DD (n=564)	P value
Age (years)	62±13	62±12	0.834
Male sex	79%	79%	0.779
Diabetes mellitus	29%	29%	0.917
Smoking	63%	65%	0.437
Hypercholesterolaemia	53%	46%	0.016
Hypertension	45%	45%	0.973
Family history of coronary artery disease	26%	25%	0.473

Statistical analysis

Statistical analysis was performed using the SPSS statistical analysis software package. Two-tailed p values <0.05 for between-group comparisons and <0.10 for interaction tests were used as the criterion for significance. The χ^2 test was used for comparing the incidence of the various genotypes in different groups. The t-test was used for the post hoc comparison of 2 genotypes. The Levene statistical test was used to examine the homogeneity of variance. Logistic regression analysis, incorporating age, sex, smoking, diabetes mellitus, hypercholesterolaemia, location of infarct, history of previous AMI and an echocardiographically determined left ventricular ejection fraction (LVEF) ≤ 45%, was applied to assess the independent effect of the genotypes on the risk of in-hospital mortality following AMI. χ^2 analysis was used to check whether the genotypes of the study were in agreement with the Hardy-Weinberg equivalence.

Results

The percentages of patients with II, ID and DD genotype were 18.4, 46.4 and 52.2%, respectively. Figure 1 shows the incidences of the various genotypes of ACE gene I/D polymorphism in relation to in-hospital mortality. The baseline characteristics of the patients participating in the study in relation to the various genotypes are given in table 1. In the single-factor analysis, only hypercholesterolaemia showed a significantly lower incidence in DD patients compared with the II or ID genotype (Table 1). However, this correlation was not confirmed by the multifactorial analysis (Relative risk (RR): 0.636, 95% CI: 0.279-1.449, p=0.281).

Single-factor analysis of correlation with in-hospital mortality

The overall in-hospital mortality in the patients of the study was 7.5% (121 deaths in a total of 1603 patients). In the single-factor analysis the patients who died during hospitalisation were significantly younger (61±12 versus 71±13 years, p<0.001). Also, female sex (11.7 versus 6.3%, p=0.001), diabetes mellitus (11.2 versus 6.1%, p<0.001), non-smoking status (11.1 versus 5.5%, p<0.001) and hypercholesterolaemia (6.1 versus 9.0%, p=0.024) were significantly related with a poor short-term prognosis

Among the clinical parameters studied, an anterior infarct site, a history of previous myocardial infarction and an LVEF ≤ 45% were significant indexes of in-hospital mortality in the single-factor analysis (Table 2). In contrast, in the single-factor analysis the DD genotype showed no statistically significant correlation with in-hospital mortality (Table 2).

Multifactorial analysis of correlation with in-hospital mortality

In the multifactorial analysis, which included as variables the DD genotype and all the prognostic indexes used in the single-factor analysis (age, sex, diabetes mellitus, smoking, hypercholesterolaemia, infarct location, history of previous AMI and LVEF), only age (RR: 1.051, 95% CI: 1.011-1.094, p=0.013) and LVEF≤45% (RR: 4.932, 95% CI: 1.448-16.796, p=0.011) showed a statistically significant correlation with in-hospital mortality. In contrast, DD genotype (RR: 0.958, 95% CI: 0.403-2.279, p=0.922) had no such correlation. Furthermore, the DD genotype had no significant correlation with the likelihood of treatment with thrombolytic agents or β-blockers and the

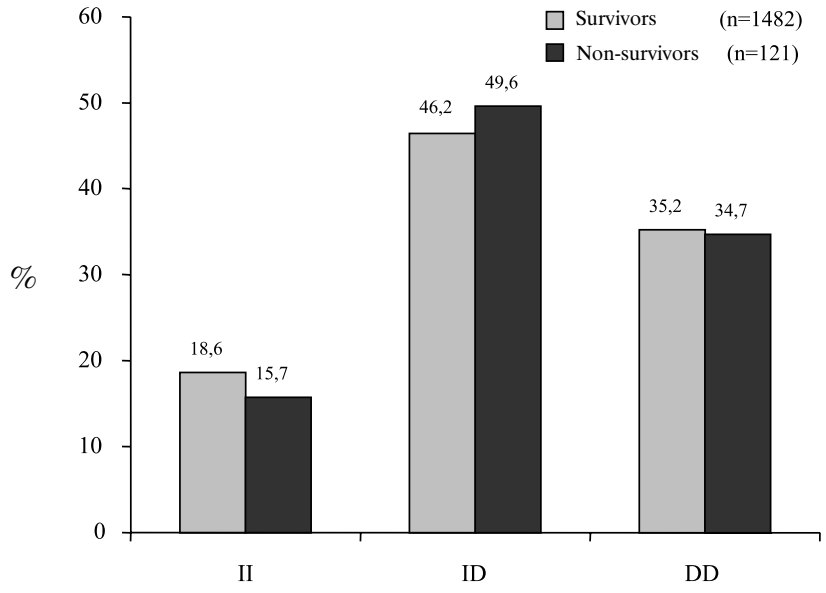

Figure 1. Incidence of genotypes of I/D angiotensin converting enzyme gene polymorphism in relation to in-hospital mortality.

addition of the probability of such treatments to the multifactorial model did not change the above results.

Correlation of genotypes with LVEF

The patients who died during hospitalisation had a significantly lower LVEF compared to those who survived (34±13 versus 46±9%, p<0.001). LVEF did not differ significantly between patients with and without the DD genotype (45±10 versus 45±10%, p=0.892). Taking the mean value of LVEF (45%) as a limit, we investigated whether the various genotypes were independently correlated with LVEF in a multifactorial analysis. The DD genotype was not found to be correlated with LVEF to a statistically significant degree after correction for age, sex, diabetes mellitus and infarct location.

Discussion

This multicentre study, which included a rather large number of consecutive patients with AMI, showed that I/D ACE gene polymorphism is not related either with disturbances of left ventricular systolic function during hospitalisation or with in-hospital mortality following AMI.

Table 2. Single-factor prognostic indexes of in-hospital mortality.

	Relative risk	95% confidence interval	P value
Age	1.065	1.047-1.083	<0.001
Male sex	0.505	0.337-0.757	0.001
Diabetes mellitus	1.963	1.346-2.863	<0.001
Smoking	0.467	0.322-0.678	<0.001
Hypercholesterolaemia	0.650	0.446-0.948	0.025
History of myocardial infarction	2.373	1.561-3.607	<0.001
Anterior infarct location	2.619	1.756-3.907	<0.001
LVEF ≤ 45%	6.511	1.953-21.71	0.002
DD ACE genotype	0.989	0.814-1.201	0.910

LVEF: left ventricular ejection fraction; ACE: angiotensin converting enzyme

Incidence of ACE genotypes

The incidence of the genotypes of I/D ACE gene polymorphism shows significant variation according to the patients' race. A meta-analysis of 145 studies including a total of 49959 individuals found that the incidence of the D allele was 56.2% in Caucasians, higher in blacks (60.3%, $p<0.001$), but lower in Asians (39.1%, $p<0.001$).[17] The present study involved Caucasian subjects and the relative incidences of the DD, ID and II genotypes were 30.5%, 47.0% and 22.5%, respectively, comparable with those reported by the above meta-analysis.

ACE genotypes and LVEF

Left ventricular dysfunction has been clearly established as a factor affecting postinfarction prognosis.[18] According to our findings, the patients who died had a significantly lower LVEF compared to those who survived and LVEF was a significant and independent prognostic factor for short-term outcome.

In this study we also examined whether the DD genotype was related with disturbance of left ventricular systolic function, evaluated echocardiographically based on LVEF, during hospitalisation. Homozygotes of the D allele exhibit elevated levels of ACE in both the plasma and the myocardium.[3,14] Furthermore, the DD genotype is related with elevated levels of plasma norepinephrine.[13] These data are suggestive of increased neurohormonal activation in DD individuals, which would be likely to result in a decline in systolic myocardial function, acceleration of left ventricular remodelling and, in consequence, a reduction in postinfarction survival. According to our findings, LVEF does not differ between patients with or without the DD genotype. Moreover, this polymorphism does not affect the relation between LVEF and in-hospital mortality. These findings agree with those of Zee et al, who showed that there was no correlation between I/D ACE gene polymorphism and the risk of left ventricular remodelling in postinfarction patients who were taking ACE inhibitors.[19] Palmer et al showed that the LVEF immediately after the appearance of myocardial infarction does not differ between patients with different ACE genotypes.[16] However, in the same study DD patients had a significantly greater left ventricular end-diastolic volume, as well as a greater end-systolic volume with marginal significance ($p=0.051$), than did ID and II patients taken together. Also, according to Davis et al, in patients with ischaemic heart disease there is no significant correlation between ACE genotype and left ventricular function, even though the DD genotype is associated with higher levels of ACE in cardiac tissue.[14]

ACE genotype and in-hospital mortality

This study found that ACE gene polymorphism is not related with in-hospital mortality following myocardial infarction. It should be noted that the Greek population is a relatively homogeneous one, with a low incidence of coronary artery disease and low mortality due to ischaemic heart disease,[20] in spite of the relatively high prevalence of major coronary risk factors and the high proportion of smokers. The above characteristics have been confirmed by large epidemiological studies in the general population,[21,22] as well as in patients with proven coronary artery disease.[23,24] Previous studies have also investigated whether I/D polymorphism affects postinfarction prognosis. However, it should be stressed that the present study included a larger number of consecutive, unselected patients with AMI than any other study to date. Keavney et al[6] and Samani et al[8] also maintained that I/D polymorphism does not affect postinfarction survival. Samani et al followed 684 patients over an interval of from 3 to 22 months (mean follow up 15 months) and concluded that the survival of DD patients was similar to that of II and ID patients.[8] Keavney et al, in a multicentre study involving 4629 patients who were followed for 5 years, concluded that survival following AMI was no different in patients with different genotypes of I/D polymorphism.[6] However, it must be stressed that this latter study, though larger and very well designed, included patients with confirmed myocardial infarction who had all received thrombolytic treatment, while our own study material consisted of a consecutive, unselected series of patients with AMI, regardless of any treatment they might have received.

In contrast to our findings, Palmer et al[16] found a significant correlation between the D allele and mortality after AMI in a study of 978 patients who had survived for at least 24 hours after the infarction. However, because of the specific exclusion criterion in this study there is likely to have a been a systematic error in the patient selection, since the patients at highest risk, those who died during the first 24 hours after admission, were not included in the study. In consequence, it is not possible to generalise the find-

ings to all patients with AMI. Yoshida et al, in a retrospective study that included only 176 patients with a mean follow up period of 5.2 years, concluded that the D allele of the ACE gene was a risk factor for the occurrence of secondary events after AMI, including death of cardiac aetiology.[15] Despite the long follow up period, the small number of patients and the retrospective design of the study are important methodological limitations.

In conclusion, this multicentre, prospective study of a large series of consecutive patients with AMI casts doubt on the value of I/D ACE gene polymorphism in the evaluation of postinfarction prognosis and on the role of this genotype in the pathogenesis of AMI.

Limitations of the study

The main limitation of the study is the lack of data concerning levels of ACE and angiotensin II, which could have been obtained from a random sample of patients. Furthermore, the present study does not include data concerning the pre-hospital mortality of patients with AMI. Even though a significant proportion of patients die before reaching the hospital, it was not possible to obtain sufficient genetic and clinical data from these patients because of methodological and ethical considerations.

Appendix

Participating Centres:

- Athens University Cardiology Department, Hippokration General Hospital, Athens
- State Cardiology Department, Hippokration General Hospital, Athens
- 1st Cardiology Department, Evangelismos Hospital, Athens
- 2nd Cardiology Department, Evangelismos Hospital, Athens
- 1st Cardiology Department, "Agios Panteleimonas" Hospital, Nikaia
- 2nd Cardiology Department, "Agios Panteleimonas" Hospital, Nikaia
- Cardiology Department, Laiko General Hospital, Athens
- Cardiology Department, Tzaneio Hospital, Nikaia, Piraeus
- Cardiology Department, Hippokration Hospital, Thessaloniki
- Department of Biological Sciences, Warwick University, Coventry, Great Britain

Researchers (posts during the time of the study)

- George Andrikopoulos – Hippokration Hospital, Athens.
- Dimitrios Richter – Hippokration Hospital, Athens.
- Edward Needham – Warwick University, Coventry, Great Britain.
- Stylianos Tzeis – Evangelismos Hospital, Athens.
- Mihalis Zairis – Tzaneio Hospital, Piraeus.
- Elias Karabinos – Evangelismos Hospital, Athens.
- Katerina Avgeropoulou – Hippokration Hospital, Athens.
- Natasha Katinioti – Hippokration Hospital, Athens.
- Polychronis Dilaveris – Hippokration Hospital, Athens.
- Elias Gialafos – Hippokration Hospital, Athens.
- Dimitrios Athanasias – Evangelismos Hospital, Athens.
- Dimitrios Manolatos – Evangelismos Hospital, Athens.
- Dimitrios Kostakis – Hippokration Hospital, Thessaloniki.
- Konstantinos Thomaidis – "Agios Panteleimonas" Hospital, Nikaia.
- Konstantinos Parabolidakis - "Agios Panteleimonas" Hospital, Nikaia.
- Paraskevi Vogiatzi – Laiko Hospital, Athens.
- Andreas Synetos– "Alexandra" Hospital, Athens.
- Thomas Apostolou – "Agios Panteleimonas" Hospital, Nikaia.
- Fotios Kardaras - Evangelismos Hospital, Athens.
- Evangelos Papasteriadis – "Agios Panteleimonas" Hospital, Nikaia.
- Athanasios Kontopoulos – Hippokration Hospital, Thessaloniki.
- Stefanos Foussas – Tzaneio Hospital, Piraeus.
- Lambros Anthopoulos, Professor of Cardiology, Athens University Nursing School.
- Mihalis Kyriakidis – Associate Professor of Cardiology, Athens University, Laiko Hospital, Athens.
- Christodoulos Stefanadis – Professor of Cardiology, Athens University, Hippokration Hospital, Athens.
- John Gialafos – Professor of Cardiology, Hippokration Hospital, Athens.
- Raj Mattu – Walsgrave Hospital, Senior Lecturer, Department of Biological Sciences, Warwick University, Coventry, Great Britain.

- Pavlos Toutouzas – Professor of Cardiology, University Cardiology Department, Hippokration Hospital, Athens.

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