

Angiotensin-converting enzyme gene variant and its levels: risk factors for myocardial infarction in a South Indian population

Pulla Reddy B, Srikanth Babu B M V, Venkata Karunakar K, Yasovanthi J, Munshi A, Sampath Kumar P, Sharath A, Jyothy A

ABSTRACT

Introduction: Recently, there has been increasing evidence that genetic variation in the angiotensin-converting enzyme (ACE) plays an important role in myocardial infarction. Therefore, the present study was carried out with the aim of investigating the association of the ACE gene insertion/deletion (I/D) polymorphism and its levels in myocardial infarction patients and their first-degree relatives (FDRs).

Methods: 206 patients with myocardial infarction, 168 FDRs and 210 control subjects were enrolled in the study. ACE I/D polymorphism was determined using the polymerase chain reaction method. Serum ACE levels were measured using the photometric method.

Results: The DD genotype and ACE activity were significantly higher in patients (p-value is 0.00006 and 0.0001, respectively) and FDRs (p-value is 0.003 and 0.04, respectively) compared with the controls.

Conclusion: ACE DD genotype and ACE levels are important risk factors for myocardial infarction. This study indicates that the higher frequency of the DD genotype and ACE levels observed in FDRs may increase susceptibility to developing myocardial infarction.

Keywords: ACE activity, angiotensin-converting enzyme, first-degree relatives, I/D gene polymorphism, myocardial infarction, South Indian population

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INTRODUCTION

Myocardial infarction (MI) is a multifactorial disease that is influenced by environmental and genetic factors.⁽¹⁾ Indians are more prone to cardiovascular diseases, including MI, at a younger age compared to other populations.⁽²⁾ Nearly 25% of all MIs are silent and do not display symptoms such as chest pain. The classical risk factors for developing MI are smoking, alcohol consumption, hypertension, dyslipidaemia, male gender and diabetes mellitus.⁽³⁾ In addition, a family history of cardiovascular disease (CVD) has been found to be a major risk factor for MI,^(4,5) thus indicating the involvement of a genetic component. In recent years, there have been several veins of research conducted to establish the functional variants of some candidate genes and the risk of developing MI.⁽⁶⁻⁹⁾

The angiotensin-converting enzyme (ACE) is a major component of the renin-angiotensin system, which is expressed in the lungs, kidneys, cardiomyocytes and other tissues. It bears two catalytic domains, each of which bears a functional zinc dependent active site.⁽¹⁰⁾ ACE (EC 3.4.15.1) is a type I integral membrane protein that plays a key role in blood pressure homeostasis. ACE converts angiotensin I to angiotensin II,⁽¹¹⁾ a potent vasoconstrictor, and also inactivates bradykinin,⁽¹²⁾ a potent vasodilator. Elevated levels of angiotensin II and decreased bradykinin levels may result in a chronic state of increased vascular resistance and high blood pressure. Furthermore, angiotensin II promotes smooth muscle cell proliferation, migration and macrophage activation, as well as adhesion to the vascular wall, leading to the development of atherosclerotic plaques.^(13,14) The ACE gene is located on chromosome 17q23, and bears 26 exons and 25 introns.^(15,16) Even though the human ACE gene contains a large number of polymorphic regions that can be of potential use in the genetic analysis of populations,⁽¹⁷⁾ the insertion/deletion (I/D) polymorphism that is present in intron 16, where 287 Alu repeat is deleted, has been extensively

Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad 500016, Andhra Pradesh, India

Pulla Reddy B, MSc, PhD
Research Scholar

Srikanth Babu BMV, MSc, PhD
Research Scholar

Venkata Karunakar K, MSc, PhD
Research Scholar

Yasovanthi J, MSc, PhD
Research Scholar

Munshi A, PhD
Scientist

Jyothy A, PhD
Director

Department of Cardiology, Osmania General Hospital, Afzul Gunj, Hyderabad 500012, Andhra Pradesh, India

Sampath Kumar P, MD, DM
Head Cardiologist

Sharath A, MD, DM
Cardiologist

Correspondence to:
Prof A Jyothy
Tel: (91) 040 23403681
Fax: (91) 040 23403681
Email: jyothyce@rediffmail.com

Table 1. Demographic and clinical characteristics of the study population.

Characteristic	No. (%)			p-value
	Controls (n = 210)	MI Patients (n = 206)	FDRs (n = 168)	
Mean age \pm SD	53.3 \pm 2.8	55.7 \pm 3.1	45.2 \pm 6.5	0.01
Gender				
Male	112 (53.3)	110 (53.4)	85 (50.6)	NA
Female	98 (46.7)	96 (46.6)	83 (49.4)	NA
Mean BMI \pm SD (kg/m ²)	22.0 \pm 1.8	26 \pm 2.3	24 \pm 2.1	0.001
Vegetarian	90 (42.8)	85 (41.3)	70 (41.7)	0.02
Non-Vegetarian	120 (57.1)	121 (58.7)	96 (57.1)	0.001
Smoker	35 (16.7)	52 (25.2)	17 (10.1)	0.0001
Alcoholic	40 (19.0)	56 (27.2)	23 (13.7)	0.0001
Exercise	85 (40.5)	24 (11.7)	30 (17.9)	0.001
Hypertension	26 (12.4)	63 (30.6)	34 (20.2)	0.001
Diabetes mellitus	19 (9.0)	25 (12.1)	16 (9.5)	0.001
Family history of CAD	27 (12.9)	94 (45.6)	168 (100)	0.0001
Family history of hypertension	30 (14.3)	70 (34.0)	62 (36.9)	0.0001

MI: myocardial infarction; FDR: first-degree relatives; SD: standard deviation; BMI: body mass index; CAD: coronary artery disease; NA: not applicable

investigated.⁽¹⁸⁾ ACE genotypes have been reported to affect the levels of ACE, which has been shown to be a high risk factor for the development of MI.⁽¹⁹⁾ Some studies have shown an association with MI but others have failed to do so.⁽²⁰⁻²³⁾ Previous studies from India on this polymorphism have focused on hypertension and coronary artery disease.^(24,25) However, there are no reports on the association of this polymorphism with MI.

The association between the ACE I/D polymorphism and stroke in the South Indian Andhra population has already been previously established.⁽²⁶⁾ In the present study, we investigated the I/D polymorphism and ACE activity in MI patients and their first-degree relatives (FDRs) in comparison with healthy controls in a South Indian population from Andhra Pradesh, India.

METHODS

The study was carried out on 206 MI patients (110 male, 96 female) admitted to the intensive cardiac care unit of Osmania General Hospital, Hyderabad, India. The patients were 45–60 years of age. All patients who were admitted with acute coronary syndrome underwent a coronary angiogram. On the basis of typical electrocardiography changes, elevated cardiac markers and clinical history, they were confirmed as suffering from MI by qualified cardiologists. All the patients had no previous history of coronary artery disease. The study was approved by the Institutional Ethics Committee

and written informed consent was obtained from all patients. 168 (85 male, 83 female) FDRs (siblings of the MI patients in the study) belonging to 95 MI patient families, aged 35–50 years, were included in the study. 210 healthy individuals (112 male, 98 female) aged 40–60 years, who were blood donors from the same hospital, formed the control group.

Information such as the patients' height, weight, body mass index (BMI), cigarette smoking status, alcohol consumption, hypertension, existence of diabetes mellitus, family history of CVDs, was obtained through the use of a structured questionnaire. Exercise was defined as brisk walking or working out the gym daily for one hour. Hypertension was defined, according to the Joint National Committee VII guidelines, as systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg based on the average of the two blood pressure measurements, or a patient's self-reported history of hypertension. Diabetes mellitus was defined as a fasting plasma glucose > 110 mg/100 ml or if the patient was on antidiabetic medications. Smokers were defined as those who reported smoking daily. Ex-smokers and occasional smokers were classified as non-smokers. Since it was found that patients consumed alcohol in different forms and that many were reluctant to admit the exact amount of alcohol consumption, alcohol consumption was defined as at least three alcoholic drinks in a week. Patients who were on ACE inhibitors and patients with nephropathy and other

Table II. Distribution of the ACE genotypes and allelic frequencies of the study population.

Study group	ACE genotypes			Total no.	Allelic frequencies		Total no.
	II	ID	DD		I	D	
Controls	81 (38.5)	91 (43.4)	38 (18.1)	210	253 (0.61)	167 (0.39)	420
Patients	47 (22.8)	94 (45.7)	65 (31.5)	206	188 (0.45)	224 (0.55)	412
FDRs	44 (26.2)	76 (45.3)	48 (28.5)	168	164 (0.49)	172 (0.51)	336

NB. Values for ACE genotypes and allelic frequencies are no. (%).
ACE: angiotensin-converting enzyme; FDR: first-degree relative

kidney diseases were excluded from the study, as ACE inhibitors and kidney diseases alter the levels of ACE.

For the genotyping of ACE I/D polymorphism, 2 ml of venous blood was collected in an ethylenediamine tetraacetic acid tube. DNA was isolated using the salting out method.⁽²⁷⁾ Intron 16 of the polymorphic ACE gene was amplified by polymerase chain reaction (PCR) (Thermal cycler, MJ Research Inc, Watertown, MA, USA) using the following primers: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' as the forward primer and 5'-GATGTGGCCATCACATTTCGTCAGAT-3'⁽²⁸⁾ as the reverse primer. Amplified PCR products were separated on 2% agarose gel. The presence of 190 bp fragments represented the D allele and the presence of 490 bp fragments represented the I allele. Mistyping of I/D heterozygotes as DD homozygotes may occur due to the preferential amplification of the D allele and inefficient amplification of the I allele. Hence, to increase the specificity of DD genotyping, PCR amplification was also performed with insertion-specific primers (forward-5' TGGGACCACAGCGCCCGCCACTAC-3' and reverse-5'TCGCCAGCCCTCCCATGCCATAA-3'), with identical PCR conditions except for an annealing temperature of 670°C. The reaction yielded a 335 bp amplified product in the presence of I allele and no product in samples homozygous for the DD genotype.^(29,30)

In terms of the biochemical assay for ACE activity, 3 ml of blood was collected in plain test tubes for serum separation. ACE activity was determined by a semi-auto analyser (ERBA, Chem-7, Transasia Biomedicals Ltd, Ringanwada, Daman, India) using a kit provided by Trinity Biotech (IDA, Business Park, Bray, Ireland). Here, the substrate was N-(3-(2-furylacryloyl)-L-phenyl alanyl glycylglycine (FAPGG), which was hydrolysed to furylacryloylphenyl alanine and glycylglycine. Hydrolysis of the FAPGG resulted in decreased absorbance at 340 nm. The ACE activity in the sample was determined by comparing the sample reaction rate to that obtained with the ACE calibrator. The genotypic and allelic frequencies of the ACE gene

were calculated. The ACE gene polymorphism was in agreement with the Hardy-Weinberg equilibrium. The association between the genotypes and MI was evaluated using the odds ratio (OR) with 95% confidence interval (CI) and chi-square analysis. The association of ACE activity was determined by analysis of variance using the Statistical Package for the Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA), and $p < 0.05$ was considered to be statistically significant.

RESULTS

During the study period, 206 MI patients, 168 FDRs and 210 controls were included in the study. The demographic and clinical characteristics of the participants are summarised in Table I. The patient group had a high prevalence of high BMI, diabetes mellitus, hypertension, a family history of CVD, smoking and alcohol consumption in comparison with the controls ($p = 0.01$). On the other hand, smoking, alcohol consumption and diabetes mellitus were less prevalent among the FDRs, but BMI and hypertension were higher when compared to the controls. The mean age of the MI patients, FDRs and controls was 55.7 ± 3.1 , 45.2 ± 6.5 and 53.3 ± 2.8 , respectively. The mean ACE activity level of the MI patients, FDRs and controls was 55.3 ± 5.4 u/L, 30.1 ± 3.2 u/L and 26.4 ± 2.7 u/L, respectively.

The ACE genotypes and allelic frequencies are represented in Table II. The frequency of the DD genotype in patients and FDRs was significantly higher in comparison with controls (0.55, 0.51 and 0.39, respectively). The genotypic ACE frequencies of patients and controls are presented in Table III. The DD vs. II genotype showed statistical significance ($\chi^2 = 15.82$, OR 2.91, 95% CI 1.72–5.02, $p = 0.00006$). The D vs. I allele also showed significant values ($\chi^2 = 17.79$, OR 1.80, 95% CI 1.37–2.37, $p = 0.00002$). The genotypic distribution of FDRs vs. controls is shown in Table IV. The DD vs. II genotype was statistically significant ($\chi^2 = 8.76$, OR 2.32, 95% CI

Table III. Distribution of ACE genotypes between MI patients and controls.

Patients vs. controls	Chi-square (χ^2)	Odds ratio	95% CI	p-value
DD vs. II	15.82	2.91	1.72, 5.02	0.00006
DD vs. ID	4.01	1.65	1.01, 2.71	0.044
DD vs. II + ID	10.09	2.08	1.32, 3.29	0.001
D vs. I	17.79	1.80	1.37, 2.37	0.00002

ACE: angiotensin-converting enzyme; MI: myocardial infarction; CI: confidence interval

Table IV. Distribution of ACE genotypes between FDRs and controls.

FDRs vs. controls	Chi-square (χ^2)	Odds ratio	95% CI	p-value
DD vs. II	8.76	2.32	1.32, 4.07	0.003
DD vs. ID	2.40	1.50	0.89, 2.55	0.12
DD vs. II + ID	4.64	1.69	1.04, 2.75	0.03
D vs. I	9.84	1.58	1.18, 2.12	0.001

ACE: angiotensin-converting enzyme; FDR: first-degree relative; CI: confidence interval

1.32–4.07, $p = 0.003$). The levels of ACE activity for MI patients, controls and FDRs were significantly different ($F = 462$, $p = 0.0001$). The ACE levels were significantly higher in the patients compared to the controls ($p = 0.001$). Similar results were observed in FDRs compared to controls ($p = 0.04$). The comparison of ACE genotypes and ACE activity among the patients, controls and FDRs is shown in Fig. 1. Patients with the DD genotype had higher ACE concentration levels compared to the ID and II genotypes.

DISCUSSION

Cardiovascular diseases are among the major causes of morbidity and mortality in India. Around 25% of deaths can be attributed to CVDs, and it is predicted that India will be host to more than 45% of heart diseases in the world within the next two decades.⁽⁶¹⁾ Since family history is known to play an important role in CVDs, this raises the question of risk for MI in patient's siblings and offspring. Answers to these critical questions rely on the fact that MI has a genetic component. This might help not only in targeting the genes involved in the pathogenesis of the disease but also in developing therapeutic interventions. The present study investigated the ACE gene polymorphism and its levels, along with the demographic and clinical characteristics of MI patients and their FDRs in comparison to the controls.

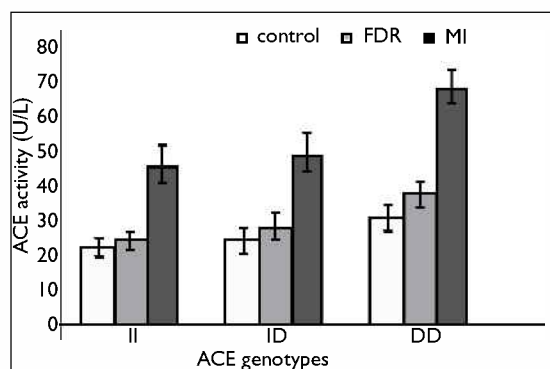


Fig. 1 Comparison between ACE genotypes and activity (mean \pm SD).

FDRs were included in the study as a few reports have indicated an increase in CVDs, by approximately two to three-fold, among FDRs of MI patients. In fact, family history is underrated by many clinicians.^(32,33) In the present study, risk factors such as family history, BMI, smoking, hypertension and alcohol consumption were found to be higher in patients compared to the controls. In FDRs, hypertension was found to be higher when compared to the controls.

The frequencies of the DD and ID genotypes were significantly higher compared to the controls in both patients and FDRs, indicating the association of ACE gene polymorphism with the disease. There was also a significant difference in the allelic frequency among the three groups. An association between the polymorphism in the ACE gene and the risk of MI was first reported by Cambien et al.⁽²¹⁾ Some follow-up studies have also shown a significant association between the ACE DD genotype and an increased risk of MI.^(20,23) A meta-analysis carried out by Samani et al on 3,394 MI cases and 5,047 controls also showed a high frequency of DD genotypes in MI patients.⁽³⁴⁾ Studies from India have revealed that the ACE DD genotype is a risk factor for coronary artery disease and hypertension,^(24,25) which is also a potent risk factor for MI. However, some studies have not been able to establish an association between ACE gene polymorphism and MI.⁽²²⁾ Ethnic differences may best account for these discrepancies. To the best of our knowledge, this is the first study to examine the association of ACE gene polymorphism with MI and their FDRs in the Andhra Pradesh population of South India.

We also observed a positive association between the DD genotype and the levels of ACE. The levels of ACE were significantly higher in the DD genotype followed by the ID and II genotypes. The relationship between the ACE DD genotype and circulating levels was originally shown by Rigat et al, who reported that

the DD genotype of ACE showed twice the levels of ACE activity in comparison to II genotypes.⁽²⁰⁾ However, it is not clear whether the DD genotype results in the over-expression of the ACE gene due to the polymorphism in the intronic region.⁽³⁵⁾ According to Davis et al, this might be on account of a tight linkage to another locus that is involved in the regulation of ACE gene expression.⁽³⁵⁾

There are very few reports on ACE gene polymorphism and ACE levels in FDRs of MI patients. In the present study, we investigated the frequency of the DD genotype and D allele in FDRs of MI. The frequency of the DD genotype was significantly higher in FDRs in comparison with that of the controls. A family history of coronary artery disease has been reported to be a strong risk factor in siblings.⁽³⁶⁾ Similar studies on FDRs have been reported in a Japanese hypertensive population as well.⁽³⁷⁾ This shows that asymptomatic FDRs with a high frequency of D allele might be at a high risk of developing the disease in the future.

In conclusion, our results support the idea that the ACE DD genotype and its levels are important risk factors for MI. However, further studies are required in this area. In addition, a large meta-analysis on families must be carried out in order to assess the level of risk in FDRs. Understanding the mechanism by which the ACE levels affect the MI risk and including the variation in the ACE gene may be the key to new therapeutic strategies.

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