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Original Article

Association of Angiotensin-converting Enzyme Gene Insertion/Deletion Polymorphism with Coronary Artery Disease in South Indian Population

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Abstract

Introduction: The angiotensin-converting enzyme (ACE) gene contains a polymorphism in the form of either Insertion (I) or Deletion (D) of a 287 base pair alu repetitive sequences in intron 16. This polymorphism is shown to be associated with the interpersonal variability of ACE levels in circulating blood and DD genotype having approximately twice the level of ACE activity of II genotype and ID having an intermediate activity.

Aim: The aim of this work was to find the association between ACE gene I/D polymorphisms and coronary atherosclerosis in the South Indian population.

Materials and Methods: Genotype analysis was done on 61 patients with angiographically proven coronary atherosclerosis and 62 healthy controls by polymerase chain reaction (PCR) followed by agarose gel electrophoresis of PCR products.

Results: Results revealed that patients had significantly higher frequency of DD genotype than controls (65.7% vs. 39.3%; *P* = 0.001) with odds ratio of 3 (1.4-6.7); *P* = 0.01 between (II + ID) genotypes and DD genotype.

Conclusion: ACE DD genotype is found to be a significant risk factor for coronary atherosclerosis.

Key words: Angiotensin-converting enzyme gene, Coronary atherosclerosis, Insertion/Deletion polymorphism, Increased angiotensin-converting enzyme activity

INTRODUCTION

Atherosclerosis is the biggest killer of the 21st century.[1] Mechanisms contributing to atherogenesis are multiple and complex. Multiple theories including the role of dyslipidemia, hypercoagulability, oxidative stress, inflammation, and endothelial dysfunction have been put forth.[2,3] Coronary artery disease (CAD) is a polygenic disease whose phenotypic manifestation depends on the interaction of a number of environmental factors. The gene-encoding components of the renin-angiotensin system (RAS) present attractive candidates for cardiovascular disease. The RAS is present in circulating and tissue-based forms,[4-6] and it is involved in sodium homeostasis, cardiovascular remodeling, and maintenance of vascular tone. Angiotensin I (Ang I)-converting enzyme (ACE) is a key component within the RAS, where it hydrolyzes Ang I to generate Ang II (vasoconstrictor)[7] and the Kallikrein-Kinin system, where it inactivates bradykinin (vasodilator).[8] The observation that ACE inhibitors reduce atherosclerosis in cholesterol-fed rabbits supports the potential role for ACE or its substrates in the development of atherosclerosis.[9] ACE could affect smooth muscle cell and fibroblast migration and proliferation, low-density lipoprotein (LDL) oxidation, and endothelial cell function; these are all important factors in atherosclerosis. A polymorphic variant of the ACE gene correlates with higher circulating ACE levels and carries an increased risk of myocardial infarction and cardiomyopathies. The ACE gene located on chromosome 17q23[10,11] contains a polymorphism in intron 16[12-14] of an Insertion/Deletion (I/D) of a 287-bp alu repeat sequence. Insertion allele

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produces 490 bp product and deletion allele produces 190bp product, and this results in 3 possible genotypes of ACE gene as II, DD, and ID. It is said that ACE DD genotype is associated with twice the ACE activity of II genotype and ID with an intermediate activity.[15] Our aim is to test the hypothesis that deletion allele is associated with an increased risk of atherosclerosis. In this study, we sought to determine the distribution of ACE genotypes and the frequency of allele D in patients undergoing coronary angiography at our institution.

MATERIALS AND METHODS

Study Population

Cases

The study sample comprised 61 unrelated South Indian CAD patients (57 males, 4 females) with mean age of 55.4 years. Inclusion criteria were more than 50% stenosis of at least one of the major coronary arteries. Patients with <50% obstruction were excluded. All patients with acute myocardial infarction or unstable angina and patients with ischemic or idiopathic cardiomyopathy were also excluded.

Controls

Totally, 62 controls were studied, and they were recruited from outpatient department during their visit for non-cardiac cases. Age, sex, and other confounding factors such as diabetes mellitus (DM) and hypertension (HT) were matched. For all diabetic controls, treadmill test was done and only those with negative treadmill test were included in the study.

Biochemical Markers

Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TGL) concentration were determined enzymatically using kits and XL-300 auto analyzer at the Centralized Biochemistry Laboratory at G.G.H, Chennai-3. LDL-cholesterol (LDL-c) was calculated using Friedewald's formula.

ACE Gene Polymorphism Screening

DNA was extracted from Buffy coat by high salt method, and using ACE gene, forward 5'-CTGGAGACCACTCCCATCCTTTCT–3' and reverse 5'-GATGTGGCCATCAATTCGTCAGAT–3' primers genomic DNA (1 μg) was amplified in 25μl reaction mixture containing 0.3 μmol/L of each primer and red dye master mix (Bangalore Genei) containing 100 μmol/L of each dNTP, 2.5 μL of ×10 reaction buffer and 0.6 unit of Taq DNA polymerase. After the DNA was denatured for 5 min at 94°C, the reaction mixture was subjected to 30 cycles of denaturation for 1 min at 94°C, 1 min of annealing at 58°C, and 1 min of extension at 72°C. Final extension was carried over at 72°C for 10 min. Amplification products were separated by electrophoresis on a 2% agarose gel and visualized under ultraviolet light after ethidium bromide staining. The polymerase chain reaction (PCR) product is a 190 bp fragment in the presence of a deletion (D) allele and a 490 bp fragment in the absence of a deletion (I) allele. Thus, each DNA sample revealed one of the three possible patterns after electrophoresis: A 490 bp band (II genotype), a 190 bp band (DD genotype), or both 490 and 190 bp bands (I/D genotype).[14,16] Analysis was done using a low molecular weight DNA ladder for Bangalore Genei (Figures 1 and 2).

Statistical Analysis

1. Allele frequencies were calculated by allele counting.
2. Age, body mass index (BMI), and serum lipid levels were compared between controls and patients by Students' *t*-test.

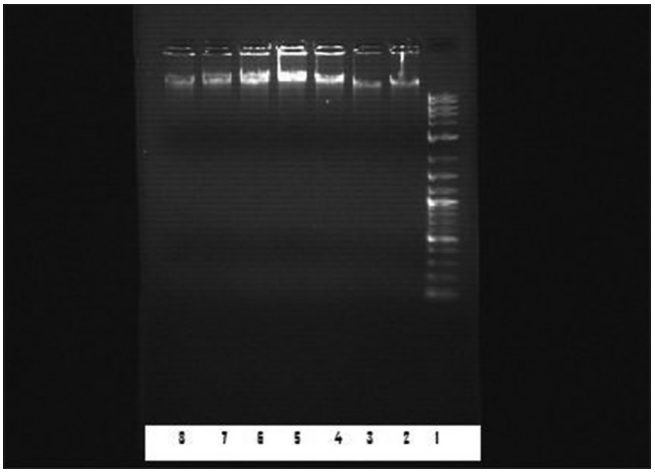

Figure 1: Extracted DNA (lane 2-8) was tested on 1% agarose gel using 1 kb ladder (lane 1) Ladder shows 10000, 8000, 7000, 6000, 5000, 4000, 3000, 2000, 1000 bp fragments

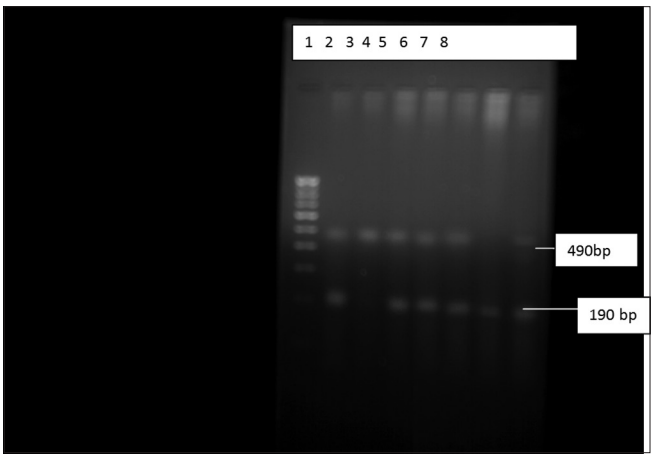

Figure 2: Agarose gel electrophoresis of polymerase chain reaction products. Deleted allele has 190 bp product and inserted allele has 490 bp product. Lane 1 shows ladder (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp), lanes 2, 4, 5, 6, 8 show both the products and indicating ID genotype, lane 7 shows only 190 bp product indicating DD genotype, lane 3 shows only 490 bp product indicating II genotype

3. Genotype frequency distribution between cases and controls was compared with a χ^2 test for 2*2 contingency table.
4. Logistic regression analysis was performed to evaluate the interaction between ACE genotypes II/DD/ID and other variables in relation to the prevalence of CAD Independent variables included in the analysis were age (quantitative), sex (male/female), smoking (Yes/No), alcoholism (Yes/No), HT (Yes/No), DM (Yes/No), serum levels of cholesterol and, TGL (quantitative). The analysis was executed by SAS Statistical program Version 6.10 for Macintosh.

RESULTS

Method of measurement of lipoprotein is not found in part of materials and methods. Table 1 shows age, sex, BMI, HDL levels, and conventional risk factor distribution among patients and controls. Since all the confounding factors were matched, there were no significant differences between the two groups. There was a significant difference in the HDL level - low in cases (37.2 ± 8) and high in controls (51.5 ± 12.5). LDL is high in cases than controls, but it is not significant.

Table 2 shows genotype distribution and allele frequencies of human ACE gene in patients with CAD and controls. ACE genotype distribution was in agreement with the Hardy-Weinberg expectations.

- DD genotype was more frequent among cases (65.7%) when compared to controls (39.3%). In contrast, ID and II were more common among controls (61.7%) when compared to cases (34.3%). There was a significant difference in the distribution of II genotype also between cases and controls (P = 0.001). I + genotype is more common among controls when compared to cases (P = 0.01) (Table 3).

Table 4 shows the age- and sex-adjusted odds ratio between I+ allele (ID+II genotypes) and I− (DD genotype), it was 3. (95% confidence interval, 1.4-6.7; P = 0.01). This shows that I allele protects against atherosclerosis, and homozygous DD genotype favors atherosclerosis.

Table 5 shows the multiple logistic regression analysis and shows that there is no significant difference between cases and controls when comparing with age, sex, DM, HT, smoking, and alcoholism, revealing that they are perfectly matched between cases and controls, and there is a significant difference in HDL levels (P = 0.000) and ace

Table 1: Characteristics of patients with CAD and controls

Variables	Case (61)	Control (62)	*P* value*
Age	55.1±10.7	55.5±12.1	0.94
Sex M: F	56:5	55:7	0.83
DM	13	21	0.10
HT	13	21	0.09
DM+HT	8	13	0.11
Smoking	14	14	0.96
Alcoholism	10	9	0.83
BMI	25.1±4.0	24.8±3.3	0.66
High-density lipoprotein	37.2±8.2	51.5±12.5	0.001
Low-density lipoprotein	91.3±31.5	87.7±41.6	0.59

*Independent Student's *t*-test and Pearson's Chi-square test. CAD: Coronary artery disease, DM: Diabetes mellitus, HT: Hypertension, BMI: Body mass index

Table 2: Genotype distribution of ACE gene between cases and controls

Genotype	Controls *n*=62 (%)	Cases *n*=61 (%)	*P* value
DD	24 (39.3)	40 (65.7)	*P*=0.001
ID	24 (39.2)	19 (31.1)	
II	14 (22.5)	2 (3.2)	

*Pearson's Chi-square test, ACE: Angiotensin-converting enzyme

Table 3: Allelic distribution of ACE gene between cases and controls

Genotype	Control *n*=62 (%)	Case *n*=61 (%)	*P* value*
I+	38 (61.7)	21 (34.3)	0.01
I−	24 (39.3)	40 (65.7)	

*Pearson's Chi-square test, ACE: Angiotensin-converting enzyme

Table 4: Univariate analysis

Genotype	Controls (*n*)	Cases (*n*)	OR (95% CI)
II/ID	38	21	3 (1.4-6.7)
DD	24	40	

OR: Odds ratio, CI: Confidence interval

Table 5: Multiple logistic regression analysis

Parameters	B	SE	Significant	Exp (B)	95.0% CI	
					Lower	Upper
Age	−0.069	0.024	0.794	0.933	0.890	0.978
Sex	−2.001	0.861	0.650	0.135	0.025	0.730
Smoking	−0.418	1.023	0.683	0.659	0.089	4.890
Alcoholism	0.301	1.151	0.793	1.351	0.142	12.887
DM	0.594	0.681	0.383	1.811	0.477	6.882
HT	0.346	0.688	0.616	1.413	0.367	5.446
BMI	0.030	0.078	0.695	1.031	0.885	1.200
TC	0.011	0.009	0.235	1.011	0.993	1.029
TGL	−0.008	0.005	0.083	0.992	0.983	1.001
HDL	−0.185	0.036	0.000	0.831	0.774	0.892
ACE-geno	0.578	0.540	0.045	1.782	0.618	5.134

DM: Diabetes mellitus, HT: Hypertension, BMI: Body mass index, ACE: Angiotensin-converting enzyme, TC: Total cholesterol, TGL: Triglycerides, HDL: High-density lipoprotein

genotype ($P = 0.045$) between cases and controls, proving our hypothesis.

DISCUSSION

Besides very well-known risk factors, genetic factors play a role in the development of CAD. Genetic factors differ in various populations. Among these, ACE gene polymorphism has most frequently been studied and proposed as a CAD risk factor. ACE gene polymorphism determines the serum and tissue ACE activity, which is high in subjects with DD genotype.[17] ACE by causing high Ang II) and low bradykinin levels may increase the risk of CAD.[18] Ang II increases the macrophage-derived growth factor and platelet-derived growth factor, which has a role in the genesis of atherosclerosis.[19] Furthermore, Ang II leads to LDL-C oxidation and stimulates neutrophil, macrophage, and T-lymphocytes.[19,20] ACE decreases nitric oxide release via the bradykinin-kallikrein system and causes endothelial dysfunction which has also an important role in the genesis of atherosclerosis. Homozygous deletion subset of the ACE I/D polymorphism is associated with deteriorated endothelial function.[21] It has been demonstrated by various studies that the ACE D allele is associated with the risk of CAD in various populations. However, other studies show that ACE gene polymorphism is not associated with CAD and MI. A large case–control study by Guardsman *et al*.[22] on Caucasian samples has shown that the D allele was associated with CAD in patients <61.7 years of age.

Hence, the clinical relevance of ACE gene polymorphism remains unclear. As CAD is a multifactorial disease, the ACE gene alone may not have a direct effect on the severity of CAD and premature death. However, the homozygous DD genotype is found to be significantly increased in our study samples.

In this study, we have not confirmed the DD genotypes using insertion-specific primers, as previous studies showed that there is a possibility of mistyping[23] due to preferential amplification of D allele and all the DD genotype positive cases to be confirmed by using another insertion-specific primer. This is the drawback of our study. Moreover, we have not tested the phenotypic variation associated with ACE genotypes, which is one more limitation of our study.

The present study is important as there is a need for confirmation of the risk gene for CAD, even if the effect is small, so as to contribute to our understanding of the pathology of CAD, and determine potential therapeutic strategies.

CONCLUSION

In the present study, ACE homozygous DD genotype is significantly associated with atherosclerosis. The presence of I allele protects against atherosclerosis.

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