Original Article

Predisposition of Angiotensin-converting Enzyme Deletion/Deletion Genotype to Coronary Artery Disease with Type 2 Diabetes Mellitus in South India

Dhivakar Mani, Rathika Chinniah, Padmamalini Ravi, Krishnan Swaminathan[1], R. A. Janarthanan[2], Murali Vijayan[3], Kamaraj Raju, Balakrishnan Karuppiah

Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai, [1]Kovai Medical Center and Hospital, Coimbatore, [2]Arvind Heart Hospital, Madurai, [3]Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

Abstract

Background: Worldwide, South Asians contribute to a high proportion of coronary artery disease (CAD) burden, mainly attributed to a high prevalence of diabetes. Early identification of such high-risk individuals would enable aggressive disease modification and prevention of complications. Definition of susceptible genotypes early in the course of disease may be one such avenue for reduction in morbidity and mortality from CAD. **Aim:** Our study was aimed to investigate the insertion/deletion polymorphism of angiotensin-converting enzyme (ACE I/D) gene and susceptibility to CAD in patients with type 2 diabetes mellitus (T2DM) in a South Indian population. **Subjects and Methods:** ACE (I/D) genotyping was performed by polymerase chain reaction specific primer for 187 CAD patients and 185 age- and sex-matched controls. **Results:** We observed that the ACE"II" genotype was found to be significantly associated with CAD patients (odds ratio [OR] = 1.689; $P = 0.028$). However, multiple logistic regression analysis revealed that ACE "DD" genotype was found to be most predominant risk factor for CAD patients with T2DM (OR = 6.118; $P = 0.001$). **Conclusion:** Our results showed that ACE (I/D) genotypes and alleles presented functional dimorphism in the development of CAD and CAD with T2DM patients in South India. This finding may be extremely useful in identifying subsets of patients where early aggressive treatment of risk factors is warranted.

Keywords: Coronary artery disease, genetic, insertion/deletion, polymorphism, South India, type 2 diabetes mellitus

Introduction

There is often an adage that says that the "genetics loads the gun and the environment pulls the trigger." This is very true of diabetes and coronary artery disease (CAD) in South Asians, where even at birth; Indian infants seem to have a propensity toward insulin resistance compared to their European counterparts.[1] In developing countries such as India, CAD, myocardial infarction (MI), type 2 diabetes mellitus (T2DM), and stroke are the most common causes of mortality and morbidity.[2] CAD has the highest incidental rate of all diseases in India, affecting approximately 35.8 million peoples.[3] The WHO estimates that 60% of global cardiac patients will be in India in the year 2020.

Angiotensin-converting enzyme (ACE) gene is located on the chromosome 17q23 and consists of 26 exons and 25 introns. An insertion (I) and deletion (D) polymorphism (ACE I/D) of 287 base pairs has been identified in intron 16. It was reported that individuals homozygous for the deletion/deletion (DD) allele have higher tissue and plasma ACE concentrations than ID heterozygotes and II homozygotes which determines the level of ACE in plasma and tissues.[4] Previous studies have shown that ACE (I/D) polymorphism, particularly ACE-DD genotype, was associated with increased levels of the plasma ACE and increase vasoconstriction, which would result in increased risk of CAD and T2DM.[5,6] However, this is controversial as some studies showed contradictory results with no association between DD genotype in CAD patients.[7] CAD is a serious complication of T2DM and those with diabetes have 2 to 4-fold higher risk of developing CAD than people without diabetes.[8,9]

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Address for correspondence: Dr. Balakrishnan Karuppiah, Department of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai - 625 021, Tamil Nadu, India.
E-mail: immunobala@mkuniversity.org

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Interaction with other genes and environmental factors was thought to be possible explanations for the contradictory results of associations of the ACE (I/D) polymorphism with disease.[10] Although the etiology of ACE II and DD variants has been extensively studied for CAD, MI, stroke, and diabetes in different populations, the association of these variants with the CAD development in South Indian population is less explored. In our current study, we evaluated the prevalence of ACE II and DD polymorphisms in South Indian population to determine if these variants modulate the risk for CAD.

Subjects and Methods

The study samples composed of 187 patients with CAD with a subset of patients with diabetes and 185 age- and sex-matched healthy controls with no history of cardiac ailments or diabetes. The samples were collected from the selected hospitals in Madurai, during 2011–2013. CAD was confirmed angiographically with clinical and laboratory examinations by experienced physicians. Of 187 CAD patients, 69 (36.89%) patients had T2DM. Information on demographic characteristics, medical history, biochemical profile, and established risk factors was recorded by a standardized questionnaire. Informed written consent was obtained from the study subjects. The study protocol was approved by the Institutional Ethical Committee of Madurai Kamaraj University.

Clinical parameters from fasting blood samples were tested for total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides (TGL) levels using commercial kit (Accurex, India) through an automatic biochemistry analyzer (Stat Fax, USA). Low-density lipoprotein (LDL) cholesterol level was calculated according to the Friedewald formula.

The volume of 2 ml of venous blood was drawn in ethylenediaminetetraacetic acid-coated tubes and the DNA was isolated by standard salting out method.[11] The concentration of the extracted DNA was estimated spectrophotometrically and stored at −80°C.

The ACE genotype was determined by polymerase chain reaction (PCR) as described previously with minor modifications.[4] PCR amplification was carried with an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 2 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min and a final extension at 72°C for 5 min (Agilent, USA). The PCR products were run in 1.5% agarose gel and gel documented. Homozygosity was identified with 190-bp PCR product for D allele (DD), 490-bp PCR product for I allele (II), and 190-bp and 490-bp PCR products for ID. The DD genotype further confirmed by additional amplification with the second set of primers.[12]

Genotype distribution and allele frequencies were compared between groups using Pearson's Chi-square test. Continuous variables were expressed as means ± standard deviation differences in continuous data among the groups were determined by unpaired Student's *t*-test to compare the clinical and laboratory findings among patients with and without CAD. Multiple logistic regression analysis was performed using SPSS version 16.0 software (SPSS, Chicago, Illinois, USA). The Hardy-Weinberg equilibrium was also tested by Chi-square test. $P < 0.05$ was considered statistically significant.

Results

Clinical and demographical characteristics of CAD patients and controls are listed in Table 1. Fasting blood glucose (FBG), LDL, TC, and TGL levels were observed to be significantly higher in CAD patients than controls. HDL levels were significantly higher in controls than CAD patients. The genotype and allele frequencies of ACE (I/D) gene polymorphism were presented in Table 2. The frequencies of DD, ID, and II genotypes were 14.4%, 52.4%, and 33.1%, respectively, in the CAD patients and 15.1%, 62.1%, and 22.8%, respectively, for controls. The homozygous ACE-II genotype was observed to be significantly higher in CAD patients than in controls (odds ratio [OR] = 1.689; $P = 0.028$). No significant association was observed for ID and DD genotypes in CAD patients and controls. Sex-wise analysis revealed that the frequency of II genotype was significantly associated with female CAD patients (OR = 2.321; $P = 0.047$). The frequency of ID genotype was found to be higher in control females (OR = 0.287; $P = 0.002$).

We analyzed the data for CAD patients with other complications and/or diseases, such as the T2DM. Of 187 CAD patients, 36.89% ($n = 69$) had T2DM and the remaining 63.10% ($n = 118$) were free of diabetes. As opposed to pooled CAD data, the frequencies of DD genotype (OR = 3.351; $P = 0.004$) and D allele (OR = 1.676; $P = 0.022$) were significantly higher in CAD with T2DM compared with CAD without T2DM [Table 3].

Multiple logistic regression analysis for independent variables such as age, sex, TC, LDL, very LDL, HDL, TGL, and FBG are shown in Table 4. In CAD with T2DM patients, hypertension (OR = 3.726; $P < 0.002$) and TGL (OR = 2.809; $P < 0.046$) were found to be the significant risk factors. Among

Table 1: Clinical and biochemical parameters of coronary artery disease patients and controls

Variables	CAD (n=187)	Control (n=185)	*P*
Age	55.26±11.15	55.61±10.82	0.759
Male/female	128/59	128/57	
FBG	142.08±63.54	115.23±48.21	<0.001
Diabetes, *n* (%)	69 (36.89)	Nil	NS
HDL	41.05±9.89	43.62±6.64	0.003
LDL	122.68±30.44	108.31±23.37	<0.001
VLDL	33.59±17.70	28.42±9.82	<0.001
TGL	168±106.83	143.04±59.65	0.005
TC	194.83±37.27	176.19±30.73	<0.001

P<0.05 as significant level. CAD: Coronary artery disease, FBG: Fasting blood glucose, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TGL: Triglycerides, TC: Total cholesterol, VLDL: Very low-density lipoprotein, NS: Not significant

the ACE (I/D) genotypes, DD was found to be with increased risk (OR = 6.118; *P* = 0.001) for CAD with T2DM patients.

Discussion

Diabetes is often referred to "coronary risk equivalent" with studies showing that patients with type 2 diabetes without a history of MI have the same risk of someone without diabetes with a previous MI.[13] Unfortunately, not only do South Asians present with more aggressive and diffuse disease than their Caucasian counterparts, mortality due to premature CAD is also much higher in this population.[14] Conventional risk factor management on its own may be insufficient to deal with the huge burden of CAD in patients with diabetes. A smart strategy to implement primary prevention that complements secondary prevention measures in diabetes is the need of the hour. One such strategy is the use of selected biomarkers or gene polymorphisms to identify high-risk patients with type 2 diabetes needing aggressive treatment to prevent cardiovascular complications.

ACE is a key component of renin-angiotensin-aldosterone system that converts inactive angiotensin I to vasoactive angiotensin II, which induces vascular smooth muscle proliferation and hypertrophy.[15] Component gene abnormalities may modulate serum levels of ACE-I and ACE-II accounting for interpersonal variability, which may affect individual susceptibility to CAD in those with type 2 diabetes. However, studies exploring the association between ACE I/D polymorphisms and CAD have shown inconsistent results.[16,17]

In our study, the genotype and allele distribution of ACE (I/D) gene polymorphism of CAD patients with

Table 2: Genotype and allele frequencies of angiotensin-converting enzyme (insertion/deletion) gene polymorphism in coronary artery disease patients and controls

Genotype/allele frequency	CAD Pooled (*n*=187)[a] Male (*n*=128)[b] Female (*n*=59)[c]	Control Pooled (*n*=185)[a] Male (*n*=128)[b] Female (*n*=57)[c]	OR	95% CI	*P*
II	62 (33.1)[a]	42 (22.8)[a]	1.689[a]	1.040-2.745[a]	0.028[a]
	38 (29.7)[b]	29 (22.6)[b]	1.441[b]	0.792-2.626[b]	0.255[b]
	24 (40.7)[c]	13 (22.8)[c]	2.321[c]	0.963-5.649[c]	0.047[c]
ID	98 (52.4)	115 (62.1)	0.670	0.434-1.035	0.060
	73 (57.0)	74 (57.8)	0.969	0.572-1.639	1.000
	25 (42.3)	41 (71.9)	0.287	0.122-0.666	0.002
DD	27 (14.4)	28 (15.1)	0.946	0.514-1.742	0.885
	17 (13.2)	25 (19.5)	0.631	0.305-1.297	0.237
	10 (17.0)	3 (5.2)	3.673	0.859-17.98	0.075
I	222 (59.3)	199 (73.7)	1.255	0.929-1.696	0.139
	149 (58.2)	132 (51.6)	1.308	0.909-1.883	0.155
	73 (61.9)	67 (58.8)	1.138	0.650-1.994	0.688
D	152 (40.6)	171 (46.2)	0.797	0.590-1.077	0.139
	107 (41.8)	124 (48.4)	0.764	0.531-1.100	0.155
	45 (38.1)	47 (41.2)	0.879	0.502-1.539	0.688

OR: Odds ratio, CI: Confidence interval, CAD: Coronary artery disease. a: Pooled, b: Male, c: Female

Table 3: Distribution of angiotensin-converting enzyme genotype and allele frequency in coronary artery disease patients with and without type 2 diabetes compared to controls

Genotype/allele frequency	Controls (*n*=185)	CAD with T2DM (*n*=69)	CAD without T2DM (*n*=118)	OR	95% CI	*P*
II	42 (22.8)	19 (27.5)	43 (36.4)	1.294[a]	0.656-2.540[a]	0.415[a]
				0.663[b]	0.329-1.328[b]	0.260[b]
ID	115 (62.1)	33 (47.8)	65 (55)	0.558	0.307-1.012	0.046
				0.747	0.394-1.416	0.365
DD	28 (15.1)	17 (24.6)	10 (8.5)	1.833	0.879-3.606	0.096
				3.351	1.408-8.986	0.004
I	199 (53.8)	71 (51.4)	151 (64)	0.911	0.604-1.373	0.690
				0.597	0.380-0.935	0.022
D	171 (46.2)	67 (48.6)	85 (36)	1.098	0.729-1.655	0.690
				1.676	1.070-2.628	0.022

[a]CAD with T2DM versus control, [b]CAD with T2DM versus CAD without T2DM. T2DM: Type 2 diabetes mellitus, CAD: Coronary artery disease, OR: Odds ratio, CI: Confidence interval

Table 4: Risk factor analysis of coronary artery disease with type 2 diabetes by multiple logistic regressions

Variables	OR	95% CI	*P*
Age	1.031	0.999-1.064	0.056
Sex	0.707	0.335-1.492	0.363
VLDL	0.996	0.970-1.022	0.743
LDL	0.982	0.893-1.081	0.714
HDL	1.010	0.929-1.098	0.813
TGL	2.809	1.020-7.734	0.046
TC	0.522	0.252-1.078	0.079
FBG	1.002	0.997-1.007	0.402
Hypertension	3.726	1.613-8.607	0.002
DD	6.118	2.099-17.834	0.001

FBG: Fasting blood glucose, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TC: Total cholesterol, VLDL: Very low-density lipoprotein, OR: Odds ratio, CI: Confidence interval, TGL: Triglycerides

T2DM (CAD + T2DM) compared without T2DM revealed that DD genotype with 3.4-fold increased risk of developing T2DM in CAD patients (OR = 3.351; 95% confidence interval = 1.408–8.986; $P = 0.004$). However, our study shows that ACE (I/D) genotypes and alleles present functional dimorphism in the development of CAD and CAD with T2DM patients in South India. However, a recently published North Indian study of 662 subjects comparing CAD subjects with healthy controls showed a higher frequency of DD genotype in CAD patients compared to healthy controls (40% vs. 28.3%). The presence of DD genotype was associated with a 1.8-fold increase in the risk of CAD in this North Indian population.[18]

Taken together the results of both North and South Indian studies, it seems likely that ACE gene polymorphisms, especially DD genotypes, have a significant influence on CAD and severity of occlusive disease in patients with type 2 diabetes. Identification of such susceptible individuals early in the course of the disease would be extremely helpful in treatment strategies that will lead to better clinical outcomes. From the clinical viewpoint, we envisage a future where ACE genotype testing is offered at affordable rates to patients with diabetes as a part of routine screening similar to a glycosylated hemoglobin test. While plenty of studies have been done on ACE gene polymorphisms worldwide, linking such evidence to clinical parameters that lead to meaningful decision-making is the need of the hour. We therefore need larger studies using predictive analytical models with dedicated software incorporating clinical, biochemical, and genetic parameters. Such modeling may help guide appropriate therapy, especially in high-risk patients, to reduce risk of major cardiovascular complications.

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Conflicts of interest

There are no conflicts of interest.

References

1. Yajnik CS, Lubree HG, Rege SS, Naik SS, Deshpande JA, Deshpande SS, *et al.* Adiposity and hyperinsulinemia in Indians are present at birth. J Clin Endocrinol Metab 2002;87:5575-80.
2. Prasad DS, Kabir Z, Dash AK, Das BC. Cardiovascular risk factors in developing countries: Review of clinico-epidemiological evidence. CVD Prev Control 2010;5:115-23.
3. Government of India Ministry of Health and Family Welfare. Burden of Disease in India. New Delhi: National Commission on Macroeconomics and Health; 2005.
4. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343-6.
5. Beohar N, Damaraju S, Prather A, Yu QT, Raizner A, Kleiman NS, *et al.* Angiotensin-I converting enzyme genotype DD is a risk factor for coronary artery disease. J Investig Med 1995;43:275-80.
6. Ruiz J, Blanché H, Cohen N, Velho G, Cambien F, Cohen D, *et al.* Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A 1994;91:3662-5.
7. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, *et al.* A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995;332:706-11.
8. Schramm TK, Gislason GH, Køber L, Rasmussen S, Rasmussen JN, Abildstrøm SZ, *et al.* Diabetes patients requiring glucose-lowering therapy and nondiabetics with a prior myocardial infarction carry the same cardiovascular risk: A population study of 3.3 million people. Circulation 2008;117:1945-54.
9. Ramprasath T, Senthil Murugan P, Prabakaran AD, Gomathi P, Rathinavel A, Selvam GS. Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione-S-transferases) variants and their association to CAD in patients with type-2 diabetes. Biochem Biophys Res Commun 2011;407:49-53.
10. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: Meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol 2000;20:484-92.
11. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
12. Shanmugam V, Sell KW, Saha BK. Mistyping ACE heterozygotes. Genome Res 1993;3:120-1.
13. Haffner SM. Coronary heart disease in patients with diabetes. N Engl J Med 2000;342:1040-2.
14. Ranjith N, Pegoraro RJ, Naidoo DP. Demographic data and outcome of acute coronary syndrome in the South African Asian Indian population: Cardiovascular topic. Cardiovasc J S Afr 2005;16:48-54.
15. Stroth U, Unger T. The renin-angiotensin system and its receptors. J Cardiovasc Pharmacol 1999;33:21-8.
16. Ganesan M, Bhaskar S, Mani R, Idris MM, Khaja N, Gulla S, *et al.* The relationship of ACE and CETP gene polymorphisms with cardiovascular disease in a cohort of Asian Indian patients with and those without type 2 diabetes. J Diabetes Complications 2011;25:303-8.
17. Grammer TB, Renner W, von Karger S, Boehm BO, Winkelmann BR, Maerz W. The angiotensin-I converting enzyme I/D polymorphism is not associated with type 2 diabetes in individuals undergoing coronary angiography. (The Ludwigshafen Risk and Cardiovascular Health Study). Mol Genet Metab 2006;88:378-83.
18. Bhatti GK, Bhatti JS, Vijayvergiya R, Singh B. Implications of ACE (I/D) gene variants to the genetic susceptibility of coronary artery disease in Asian Indians. Indian J Clin Biochem 2017;32:163-70.