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SHORT COMMUNICATION

Association of angiotensin converting enzyme gene insertion/deletion polymorphism with essential hypertension in south Indian population



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Abstract Genetic, environmental and demographic factors contribute to the development of essential hypertension. Genetic polymorphism of Rennin-angiotensin-aldosterone system (RAAS) has been extensively studied to determine the genetic susceptibility to hypertension. The insertion/deletion (I/D) angiotensin converting enzyme (ACE) polymorphism has been established as a cardiovascular risk factor in some population, but its association with essential hypertension is controversial. This study sought to determine the association of I/D polymorphism of the ACE gene in south Indian essential hypertensive subjects. A total of 208 clinically diagnosed essential hypertensive patients without any associated diseases and 220 healthy control subjects were included in this study. Distribution and allelic frequency of Insertion (I) and Deletion (D) polymorphism at the 287 base pair Alu repeat sequence in the intron 16 of ACE gene were analyzed. The distribution of II, ID, DD genotypes of ACE gene was 28.3%, 32.6% and 38.9% respectively in essential hypertensive patients and to 53.6%, 26.3% and 20% in controls. The allele frequency for D allele is 0.58 in essential hypertension as compared to 0.34 of control subjects. The genotype and allele frequency of ACE gene polymorphism is significantly differed in patients when compared to controls. In conclusion, the I/D polymorphism of ACE gene is associated with Indian essential hypertension.

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Introduction

Hypertension is a major public health problem in India and in other developing countries.¹ A meta analysis of hypertension prevalence rates in India demonstrated a significant increase.² Prevalence of hypertension in India between three and six decades increased about 30 times among urban residents and by about 10 times among rural residents.³ The cause of essential hypertension is multifactorial; genetic factors also a part of it.⁴

Genetic factors are responsible for about 30–60% of the familial aggregation of blood pressure and the transmission of cultural factors being responsible for the remaining (stress, diet, physical activity).⁵ Blood pressure is mainly regulated by Renin-angiotensin-aldosterone system (RAAS) which also acts as a key regulator of electrolyte balance.⁶ Genetic polymorphism of RAAS has been thoroughly studied to elucidate the genetic susceptibility to Hypertension.⁷ The angiotensin converting enzyme (ACE) gene is the key gene in RAAS and attractive candidate gene to study the development of essential hypertension.⁸ Angiotensin converting enzyme a metalloproteinase (dipeptidyl carboxy peptidase) converts angiotensin-I to angiotensin-II, a potent vasoconstrictor.⁹ The protease activity of ACE inactivates bradykinin, a potent vasodilator.¹⁰ ACE consists of 1306 amino acids sequence.¹¹

Angiotensin converting enzyme insertion/deletion (ACE I/D) polymorphism is one of the well described polymorphism of the RAAS. Several recent studies have demonstrated the association of ACE I/D polymorphism with cardiovascular complications.^{12,13} On the other hand, the association between I/D polymorphism and hypertension is still controversial. Some studies have shown the association of ACE I/D polymorphism with essential hypertension^{14–16} and some studies were failed to show the association.^{17,18} ACE I/D polymorphism associated with increased levels of ACE in plasma which increase Angiotensin II levels a key factor enhancing peripheral resistance. The mean plasma ACE level in DD subject is about twice that of II subject with ID subject having intermediate level.^{19,20} Although the I/D polymorphism of the ACE gene is considered to play a small role in the pathogenesis of hypertension, the higher levels of ACE associated with the D allele may lead to greater angiotensin II formation in cardiac and vascular tissue, predisposing a subject to cardiovascular damage. There are very limited studies available in Indian subjects regarding association of ACE I/D polymorphism in essential hypertension and the data was lacking in the south Indian population. The present study aimed to explore the association of I/D polymorphism of ACE gene with south Indian essential hypertensive patients. This study was conducted first time in the southern part of the Andhra Pradesh (Nellore District) India.

Materials and methods

The study was approved by ethical committee of the Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India. A total of 428 subjects were recruited for this study and they were divided into two groups. Group-I consist of 208 essential hypertensive patients attending

various clinics of Narayana Medical College and Hospital, Nellore city. Group II consist of 220 healthy volunteers (no age, sex matched controls) were collected randomly. Data collected from each subjects as clinical variables including age, height, weight, body mass index, and family history. Hypertension was defined as a systolic blood pressure (SBP) >140 mmHg and sustained diastolic blood pressure (DBP) >90 mmHg. Blood pressure was measured in the subjects who had been seated and rested for five minutes and taken twice to calculate mean SBP and DBP. All the patients were not receiving antihypertensive drugs and patients with secondary hypertension, renal, liver and cardiac abnormalities were excluded from the present study.

Five milliliters of venous blood samples were collected from each subject. A portion of the blood samples were taken an EDTA tube (Becton Dickson, NJ, USA) to obtain genomic DNA extraction, the rest was left to clot to obtain serum and stored at -20°C for further analysis. Serum samples were analyzed on a HUMASTAR-300 autoanalyser (human, Germany) using kits supplied by human diagnostics (GmbH, Germany) to determine the levels of fasting blood glucose, serum urea, creatinine, total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides (TGL). Low density lipoprotein cholesterol (LDL-C) calculated by Friedwald formula. Third report of the national cholesterol education program guidelines (NCEP report 2001)³⁴ were followed for the classification of lipid profile. Serum sodium, potassium and chloride were estimated on HUMLYTE electrolyte analyzer. (Ion selective electrode method GmbH Germany).

Table 1 Baseline characteristics of study population.

Variable	Hypertension (n = 208)	Controls (n = 220)
Age (years)	43.6 $\pm$ 5.6	42.78 $\pm$ 5.7
Gender (M/F)	121/97	130/90
BMI (kg/m ²)	26.12 $\pm$ 3.2	25.3 $\pm$ 4.1
SBP (mmHg)	151 $\pm$ 9.8*	123.34 $\pm$ 1.8
DBP (mmHg)	92.56 $\pm$ 8.7*	69.89 $\pm$ 5.0
BGL (mg/dl)	93 $\pm$ 6*	89 $\pm$ 4
Serum urea (mg/dl)	22.23 $\pm$ 4.21	21.8 $\pm$ 4.8
Serum creatinine (mg/dl)	0.97 $\pm$ 0.21	0.93 $\pm$ 0.25
Serum sodium (mmol/L)	140.1 $\pm$ 3.2*	139.24 $\pm$ 2.62
Serum potassium (mmol/L)	3.72 $\pm$ 0.21	3.66 $\pm$ 0.32
Serum chloride (mmol/L)	89.32 $\pm$ 4.31	88.63 $\pm$ 3.1
Total cholesterol (mg/dl)	207 $\pm$ 4.6*	157 $\pm$ 6.8
LDL-Cholesterol (mg/dl)	125.19 $\pm$ 6.3*	85 $\pm$ 4.5
HDL-Cholesterol (mg/dl)	38.21 $\pm$ 4.3*	53 $\pm$ 3.2
Triglycerides (mg/dl)	168 $\pm$ 8.3*	133 $\pm$ 6.2

LDL-low density lipoprotein, HDL-high density lipoprotein.

*P = <0.05 highly significant.

Table 2 Distribution of ACE genotypes and allelic frequencies of the study population.

Study group	ACE genotypes			Total	Allelic frequencies		Total
	II	ID	DD		I	D	
Controls n (%)	118 (53.6)	58 (26.3)	44 (20)	220	294 (0.66)	146 (0.34)	440
Patients n (%)	59 (28.3)	68 (32.6)	81 (38.9)	208	186 (0.42)	230 (0.58)	416

Genomic DNA was extracted from peripheral blood using spin column genomic DNA extraction kit (Axygen Biosciences, USA) and ACE intron 16 gene was amplified by Polymerase chain reaction (MG series Thermocycler, USA). For amplification, a flanking primer pair⁸ 5'-CTGGA-GACCACT CCCATCCTTCT-3' and 5'-GATGTGGCCA TCA-CATTC GTCACGAT-3' (synthesized by Bioserve Biotechnology) was used. PCR amplification was performed with a 50 µl reaction mixture contains 40 pmol of each primer, 200 µmol/L each dNTP, 1.5 mmol/L MgCl₂, 1 U of thermo stable DNA polymerase (DYNAZYME II Espoo, Finland) and 20 mMol of Tris-HCL (pH 8.8 at 25 °C) PCR cycling conditions were carried out with an initial denaturation step of 5 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min and extension at 72 °C for 2 min, followed by final extension for 5 min at 72 °C before the storage of sample at 4 °C. PCR products were separated by agarose gel electrophoresis (GENI Bangalore India). DNA fragments were stained with ethidium bromide and visualized under UV light (Gel documentation system Biorad, USA). The PCR fragments consist of three genotypes, a 490 bp band (II), a 190 bp band (DD), and both 490 and 190 bp band (ID). To increase the DD genotyping, PCR amplifications were also performed with insertion specific primer pair²¹ 5'-TGCGACACAGCGCCCGCACTAC-3' and 5'-TCGCCAGCCCTCCATGCCCCATAA-3' for each sample which had the DD genotype to avoid mistyping of ID heterozygote's as D homozygote's. PCR cycling conditions were carried out with an initial denaturation step of 1 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 67 °C for 45 s and extension at 72 °C for 2 min. The PCR product shows a 335 bp band for I allele and no band for DD genotype.

Clinical characteristics data of all the subjects were expressed as mean ± SD. All the statistical analysis was carried out using SPSS software version 15.0 for Microsoft windows. Continuous variable was compared between the cases and control group by using two tailed students 't' test. Allelic frequencies were calculated by gene counting method and the genotype distribution with Hardy-Weinberg expectation by a chi-squared test. A level of $P < 0.05$ was considered statistically significant.

Results and discussions

RAAS has always been an attractive model system for the study of the genetics of hypertension. Over the course of lifetime small increase in RAAS activity elevates risk of hypertension. The ACE gene located on chromosome 17q

23, has been suggested by gene targeting and transgenic studies in mice to play key role in blood pressure regulation.²² Studies in genetically hypertensive and their normotensive controls revealed a linkage of chromosomal region containing the ACE gene with blood pressure. This led to the hypothesis that ACE is a possible candidate gene for essential hypertension in humans. The DD genotype of ACE gene was associated with essential hypertension in different ethnic population. Studies conducted in Japanese,²³ Australian,²⁴ Mongolian,²⁵ Pakistanis,²⁶ and Indian populations^{14,16} (Bengalese and Sikh population) have suggested an association between ACE- DD polymorphism with a higher incidence of hypertension. The other studies conducted in Belgian,²⁷ Dutch,²⁸ Italian²⁹ Caucasians³⁰ and Bangladesh population¹⁸ does not shows any association of ACE I/D polymorphism with essential hypertension. Ethnic and geographical variation may influence the ACE I/D polymorphism with hypertension. Similarly, the Indian study consist of 105 patients and 192 controls does not show association of ACE I/D polymorphism with essential hypertension.¹⁶ In the present study the DD genotype of ACE gene is strongly associated with essential hypertension. D-allele associated with early onset of hypertension in Japanese subjects.³¹ Our study represents young hypertensive patients with 38% of prevalence of DD genotype when compared to 20% of control subjects. Allele frequency for D allele is 0.58 in essential hypertension as compared to 0.34 of control subjects and we know that the D allele might increase the susceptibility to hypertension.³² The present study exclusively carried out with essential hypertensive patients. Patients with diabetes mellitus excluded from this study, because the diabetes itself act as risk factor for the development of hypertension.³³ The study group included 208 essential hypertension patients and 220 controls. The base line demographic, clinical and laboratory data of the study population were shown in Table 1. Among patients 121 (58.2%) were men and 87 (41.8%) were women. There was no significant difference between patients and controls with respective to age and BMI ($P > 0.05$). SBH and DBH were higher in patients when compared to controls ($P \leq 0.05$).

Serum total cholesterol, HDL, LDL, VLDL and triglycerides levels are significantly higher in patients when compared to controls ($P \leq 0.05$). Blood glucose, serum sodium levels also shows difference between patients and controls ($P \leq 0.05$). There was no significant difference between patients and controls in serum creatinine, urea, potassium and chloride ($P \geq 0.05$).

In the present study, it was observed that the D-allele of the ACE gene was in statistically significant association with essential hypertension as compared to controls subjects.

Table 3 Distribution of ACE genotypes between HTN and controls.

Patients vs. controls	Chi-square (χ^2)	Odds ratio	CI 95%		P value
			L. limit	U. limit	
DD vs. II	29.07	3.7	2.2	5.9	0.0001*
DD vs. ID	3.04	1.6	0.9	2.6	0.08
DD vs. II + ID	18.5	2.5	1.6	3.9	0.001*
D vs. I	11.2	1.6	1.2	2.1	0.007*

*P-value <0.05 considered highly significant.

The distribution of genotypes in the study subjects showed deviation from hardy Weinberg equilibrium ($P < 0.05$). The ACE genotype distribution and allelic frequency among study subjects are shown in Table 2. In the study population, genotypic frequencies of ACE gene for II, ID and DD are 28.3%, 32.6% and 38.9% respectively in essential hypertensive patients as compared to 53.6%, 26.3% and 20%. The derived allele frequency for D allele is 0.58 in essential hypertension as compared to 0.34 of control subjects, thus the D allele frequency is higher in essential hypertension when compared to controls. It indicates the strong association of D-allele with essential hypertension. The ACE genotype distribution between patients and control are shown in Table 3. The DD vs. II in the patients as compared to control: $\chi^2 = 29.07$, OR = 3.7, 95% CI = 2.2–5.9 ($P = 0.0001$); DD vs. ID: $\chi^2 = 3.04$, OR = 1.6, 95% CI = 0.9–2.6 ($P = 0.08$); DD vs. ID: $\chi^2 = 18.5$, OR = 2.5, 95% CI = 1.6–3.9 ($P = <0.001$); D vs. I: $\chi^2 = 11.2$, OR = 1.6, 95% CI = 1.2–2.1 ($P = 0.007$).

Conclusion

In conclusion our results explore the association of DD genotype with essential hypertension. The present study proves the relation between I/D polymorphism of ACE gene and essential hypertension in south Indian population.

Conflicts of interest

There is no conflict of result.

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