Angiotensin Converting Enzyme Gene Polymorphism and its Association with Hypertension in South Indian Population

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Abstract Hypertension, a well known risk factor for various cardiovascular, peripheral vascular and renal events is an important public health challenge. Renin angiotensin system (RAS) being the most vital pathogenic mechanism of hypertension is mediated by a key component; the angiotensin converting enzyme (ACE). The present study was aimed to know the relationship of ACE gene polymorphism and the possible risk of development of hypertension in south Indian population. The study included 101 clinically diagnosed hypertensive patients without any associated disease condition and 81 age and sex matched apparently healthy controls. Genotyping was performed using a polymerase chain reaction, (PCR) amplification of the intron 16 fragment harboring the 287 bp Alu repeat sequence. Three possible genotypes D/D, I/I homozygous and I/D heterozygous were analyzed where the D/D genotypes corresponds to higher ACE levels (D-Deletion, I-Insertion). The PCR products were separated on 2 % agarose gel. Statistical analysis was performed using SPSS.15 software program. We found a significance in frequency of D/D genotype in the hypertensive patients compared to the control group ($p = 0.0005$,

odd's ratio = 4.157). This suggested that ACE (D/D) genotypes are more prone for the development of hypertension. This is relatively a pilot study; but nevertheless may assist in identifying the pathophysiological cause of hypertension.

Keywords Renin angiotensin system · Angiotensin converting enzyme (ACE) · Polymerase chain reaction (PCR) · Hypertension

Introduction

Hypertension is one of the most common conditions afflicting the population of the world. Because of the associated morbidity, mortality and the cost to society, hypertension is an important public health challenge. From time immemorial it is a well known risk factor for various cardiovascular, peripheral vascular and renal events in our body.

Among a plethora of factors affecting the vascular tone [1], the most important contributor is the renin-angiotensin system (RAS) [2–4]. The RAS regulates long term blood pressure and extracellular volume of the body by the action of Angiotensin II, a potent vasoconstrictor. This system has been implicated in the pathological changes of organ damage through modulation of gene expression, proliferation, fibrosis and inflammatory response. Angiotensin converting enzyme (ACE) is a key component of the RAS. It is responsible for converting the inactive decapeptide, angiotensin I, to the biologic active octapeptide angiotensin II, as well as inactivating the vasodilator nonapeptide, Bradykinin [5, 6]. Angiotensin II contributes to hypertension by its intense vasoconstrictor action, aldosterone stimulation and bradykinin inhibition [7–9].

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The ACE genotypes include the presence (I allele) or absence (D allele) of a 287 bp Alu repeat sequence in intron 16, resulting in 3 genotypes (D/D and I/I homozygote, and I/D heterozygote). An I/D polymorphism of ACE gene at this region correlates with circulating ACE plasma activity [10, 11]. An I/D (region in intron 16) polymorphism of ACE gene correlates with circulating ACE plasma activity. Higher plasma ACE activity is observed in subjects with ACE-D/D genotype [12]. A raised plasma ACE activity may elevate blood pressure through increased production of angiotensin II [13]. Studies have demonstrated that ACE Insertion (I)/Deletion (D) polymorphism are associated with common diseases like hypertension, diabetic nephropathy, coronary heart disease and tuberculosis [14–17].

Our aim of the study was to find an association of ACE gene polymorphism with hypertension in the South Indian population.

Materials and Methods

The study was conducted on 182 individuals who were divided into two groups;

Group-A

The control group included 81 subjects aged between 30 and 70 years (mean age 50). These individuals were attending the master health check up clinic of Sri Ramachandra Hospital, Porur, Chennai, for routine blood investigations. The cut off value for exclusion of hypertension was SBP <140 mmHg and DBP <90 mmHg. There was no associated illness in these subjects.

Group-B

101 cases attending the Hypertension clinic of Sri Ramachandra Hospital in the age group of 30–70 years (mean age 50) were included. Hypertension was defined as SBP >140 mm Hg and DBP >90 mm Hg, according to JNC 7 criteria [18]. Patients with diabetic nephropathy, coronary artery disease, bronchial asthma and tuberculosis were excluded from this study.

A standard questionnaire was prepared and data collected on clinical variables of age, sex, height, weight, family history, smoking and alcohol habits.

Sampling

Five milliliters of venous blood was drawn from peripheral vein using disodium EDTA vacutainers. All samples were stored as aliquots at -70°C until tested.

Methodology

DNA Extraction

DNA was extracted from whole blood containing EDTA by a standard ‘salting out’ procedure [19]. The quality of the DNA was analyzed by 0.8 % agarose gel electrophoresis (Fig. 1) and the quantity assessed by measuring the absorption at 260 and 280 nm in a standard spectrophotometer.

Polymerase Chain Reaction

To determine the ACE genotype of cases and the control groups, the genomic DNA fragments on the intron 16 of ACE gene were amplified by PCR. The conditions required for amplification were;

1. Initial denaturation: 94°C —5 min
2. Denaturation: 94°C —30 s
3. Annealing: 58°C —45 s
4. Extension: 72°C —45 s
5. Cycling condition: 30 cycles
6. Final extension: 72°C —7 min
7. Hold at 4°C .

Primers for ACE Polymorphism used in this study

The flanking primer sequences as reported by Rigat et al. [12] were;

Forward Primer: 5'CTG GAG ACC ACT CCC ATC CTT TCT 3'

Reverse Primer: 5'GAT GTG GCC ATC ACA TTC GTC AGAT 3'.

Fig. 1 0.8 % gel for assessing the quality of DNA

Fig. 2 Bands visualized under UV lamp

Agarose Gel Electrophoresis

Once the amplicons were obtained, they were subjected to 2 % agarose gel electrophoresis with ethidium bromide and the bands visualized under UV light. With the help of DNA ladder, Deletion (D allele) and Insertion (I allele), were identified at 190 and 490 bp fragment respectively (Fig. 2).

Statistical Analysis

Univariate and bivariate analysis were performed using SPSS.15 software program. Comparison of various parameters like age, sex, diet smoking, alcoholism and BMI between cases and controls were done using independent 't' test. In bivariate analysis, Pearson Chi square test was applied to check the association between the selected variables. Allele and genotype frequencies were compared using 2×2 contingency table using Fisher's exact test. The mean concentration of all numerical values was tested by student's *t* test or ANOVA test. *p* value <0.05 was considered statistically significant. Odd's ratio (OR) at 95 % Confidence Intervals (CI) was determined for the disease susceptibility of the patients.

Results

Polymorphism and Hypertension

On genotype analysis the frequency of I/I, I/D and D/D polymorphism in controls were 25.9, 53.1 and 21.0 % and among cases were 16.8, 30.7 and 52.5 % respectively (Table 1).

Pearson's Chi square test was applied to test the association of the various genotypes with hypertension and a significant association was obtained between D/D

Table 1 Distribution of the polymorphisms between the controls and cases

Types of ACE polymorphisms	Controls <i>n</i> = 81 (%)	Cases <i>n</i> = 101 (%)
I/I	21 (25.9)	17 (16.8)
I/D	43 (53.1)	31 (30.7)
D/D	17 (21.0)	53 (52.5)

P value 0.0005

Distribution of polymorphisms among controls and cases

Table 2 Distribution of genotypes between controls and cases

Types of ACE polymorphisms	Controls <i>n</i> = 81 (%)	Cases <i>n</i> = 101 (%)
D/D	17 (21)	53 (52.5)
I/D + I/I	64 (79)	48 (47.5)

Odd's ratio for cases/ controls: 4.157, 95 % confidence interval 2.144–8.060

Relationship of wild (D/D) and variant genotypes(I/D+I/I)

polymorphism and hypertension with the *p* value of 0.0005 (<0.05).

To check the severity and association of D/D genotype with hypertension, a comparison was made between the wild type (D/D) and the variant types (I/D + I/I) as shown in Table 2.

Discussion

Cardiovascular diseases are rapidly emerging as a major health concern in most developing countries. It is estimated

that the number of deaths in India, due to cardiovascular diseases would raise up to 4.6 million by the year 2020. Hypertension is a major modifiable risk factor of morbidity and mortality, responsible for 50 % of deaths due to stroke and 24 % due to all other coronary artery disease [20]. South Asians have an increased risk of heart disease, in comparison to Europeans [21].

The RAS has a central role in regulating blood pressure and sodium homeostasis. ACE is a key enzyme in this system which catalyzes conversion of Angiotensin I to Angiotensin II, a potent vasopressor. Genes encoding components of RAS, including Angiotensinogen, ACE, Angiotensinogen II type-1 receptor and Renin (−5,434 and −5,312) have been extensively investigated as genetic determinants of essential hypertension [22, 23]. The Insertion/Deletion polymorphism in the ACE gene is correlated with the circulating ACE levels. Individuals with the D/D genotype have the highest circulating ACE levels as compared to the I/I genotype. Various studies have shown association between this ACE polymorphism and several cardiovascular diseases like myocardial infarction, left ventricular hypertrophy, cardiomyopathy and hypertension [24]. It has been postulated that the association between the ACE I/D polymorphism and hypertension might be related to gender and ethnicity.

In this present study 101 clinically diagnosed hypertensive patients and 81 age and sex matched controls were taken. A number of confounding factors for hypertension like dietary habits, smoking, alcohol and BMI were studied and analyzed. The distribution of all these variables between the hypertensives and controls were studied and their relationship with the three genotypes, I/I, I/D and D/D analyzed. On genotype analysis the frequency of I/I, I/D and D/D polymorphism among nonhypertensives (controls) was 25.9, 53.1 and 21.0 %. The frequency among hypertensives (cases) was 16.8, 30.7 and 52.5 % respectively (Table 1). On statistical comparison of D/D genotypes between the controls and cases, the cases showed a significant increase of frequency (p value = 0.0005). As per (Table 2), an OR of 4.157 was obtained. This showed that in this South Indian population, subjects with D/D genotypes are four times more prone for the development of hypertension. The 95 % CI was found to be 2.144–8.060. Since it did not include 1, the result was confirmed to be statistically significant.

On comparison of the individual frequencies of the three genotypes (Table 3), it was found that D/D genotypes were more prone to develop hypertension with an OR of 3.85 (D/D vs I/I) and 4.32 (D/D vs I/D). This high association between D/D polymorphism and hypertension can be explained by the fact that D/D genotype is associated with high ACE levels. ACE is responsible for the conversion of Angiotensin I to Angiotensin II. Angiotensin II is a potent

Table 3 Comparison between various genotypes (D/D, I/D, I/I)

Genotypes	Odd's ratio	95 % Confidence Interval
D/D vs I/I	3.85	1.66–8.93
D/D vs I/D	4.32	2.11–8.84
I/D vs I/I	1.12	0.51–2.47

vasoconstrictor, and a stimulator of aldosterone synthesis which causes increased blood pressure.

The association between ACE D/D allele with hypertension has given contrasting results world wide. A significant association of ACE D/D allele with hypertension with African Americans, Chinese and Japanese population has already been established [24–28]. However other studies have failed to show a positive association [29–32]. It has been suggested that these inconsistencies may be due to the difference in background of the population characteristics. Some studies showed that I allele is associated with hypertension in the Australian and Pakistani population. The heterogeneity in association of ACE I/D polymorphism with hypertension may be due to varied ethnicity or the various other genetic and environmental factors implicated in the regulation of blood pressure.

In conclusion, we found a significant association between subjects of D/D genotype and hypertension, throwing out light to the fact that D/D genotype people are more prone for the development of hypertension.

However, more knowledge about the genetics of hypertension can be obtained by performing the study over a wider population in various geographical regions of South India. The serum ACE levels can be analyzed along with ACE genotyping and the relationship correlated thereby. The effectiveness of ACE inhibitors on D/D genotype hypertensive's can also be studied simultaneously.

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