

## A Study of Angiotensin Converting Enzyme (ACE) Gene Insertion / Deletion (I/D) Polymorphism in Essential Hypertension

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| Received: 11.02.2019 | Accepted: 22.02.2019 | Published: 25.02.2019

DOI: [10.36347/sjams.2019.v07i02.042](https://doi.org/10.36347/sjams.2019.v07i02.042)

### Abstract

### Original Research Article

Angiotensin converting Enzyme (ACE) plays an important role in maintaining blood pressure homeostasis and vascular integrity. The Insertion/Deletion polymorphism (I/D) in exon 7 of Angiotensin converting Enzyme (ACE) gene have been found to be associated with essential hypertension in different human populations. The aim was to study the insertion/ deletion (I/D) polymorphism of ACE gene in patients with essential hypertension and also to assess the genotype distribution and to determine allelic frequency of ACE gene. The target DNA sequence of ACE gene was amplified by Polymerase Chain Reaction (PCR) technique to detect the presence of ACE gene Insertion / Deletion (I/D) polymorphism in 41 patients with essential hypertension. The study results showed that the frequency of I/I, D/D and I/D genotypes was found to be 44%, 27%, and 29% respectively. The frequency of insertion (I) and deletion alleles was found to be 58.54% and 41.46%. The study data indicates, the wild I/I genotype is predominant and the frequency of I allele is increasingly distributed in the studied hypertensive subjects of our study population.

**Keywords:** Essential hypertension, Angiotensin Converting Enzyme, ACE Gene, Polymorphism.

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## INTRODUCTION

Essential or primary hypertension (HTN) is a major public health problem characterised by sustained elevation of blood pressure without any identifiable causes. It is universally accepted that systemic hypertension is a distinct risk factor for various cardiovascular emergencies, particularly left ventricular failure, myocardial infarction, and stroke[1]. Both environmental and genetic factors may predispose individuals to essential hypertension. Since the underlying genetic pathways remain unclear, currently most studies focus on the genes coding for proteins that regulate blood pressure. Clinical and experimental studies suggest that an Insertion/Deletion polymorphism in the gene responsible for Angiotensin-I converting enzyme (ACE) may be a contributing factor in the pathogenesis of essential hypertension.

Angiotensin-I converting enzyme (somatic isoform of ACE-1) is a significant component of the renin-angiotensin system (RAS). Of the various physiological pathways affecting the homeostasis of blood pressure, the renin-angiotensin system (RAS) is known to play a critical role. Renin, a protease secreted by the juxtaglomerular apparatus of the kidney

catalyzes the conversion of a plasma protein called angiotensinogen (released by the liver) into angiotensin I (Ang-1, a decapeptide)[2]. The Angiotensin Converting Enzyme (ACE), synthesized primarily but not exclusively in the pulmonary alveoli, converts angiotensin I to the active octapeptide angiotensin II (AG-II) by releasing the C-terminal histidyl-leucine dipeptide. Angiotensin II is a potent pressor substance causes direct vasoconstriction, increases systemic vascular resistance, enhances sodium and water reabsorption by the proximal tubule. AG-II acts via angiotensin II type 1 (AT<sub>1</sub>) receptors on cell membranes of the adrenal cortex to stimulate the secretion of aldosterone by the adrenal zona glomerulosa. The aldosterone in turn stimulates salt and water reabsorption by the kidneys, and the constriction of small arteries (arterioles), and consequently causes an increase in blood pressure [2]. In clinical practice, ACE inhibitors are the commonly used antihypertensive agents and are known to significantly reduce mortality or the incidence of cardiovascular disease including myocardial infarction in patients who have hypertension or ischemic cardiovascular diseases. Thus, the ACE gene has been recognized as a top candidate gene for the research in essential hypertension.

The ACE-1 gene, encoding angiotensin converting enzyme (ACE-I) spans 21 kilobases, located on the long arm of chromosome 17 (17q23), and consists of 26 exons and 25 introns [3]. It contains a number of polymorphic variants that can be of potential use in genetic analysis of populations. The accumulated evidence points to the existence and the role of insertion/deletion polymorphism of ACE gene and its contribution in the development of essential hypertension. It has been shown that the ACE gene (ACE) contains a polymorphism based on the presence (insertion) or absence (deletion) of a 287 bp Alu repeat sequence (a nonsense DNA fragment) near the 3' end of intron 16[4]. This insertion/deletion polymorphism in the ACE gene produces three genotypes in population: Deletion homozygotes (D/D); Insertion homozygotes (I/I); and Heterozygotes D/I. Since its identification, several studies have shown that the D/D (del/del) genotype in the ACE gene is associated with hypertension.

India, being a culturally and socially diverse nation, the genetic differences would be noted in the region wise prevalence of hypertension. The genetic research regarding the nature of genetic contribution in accelerating the hypertension is inadequate in south Indian population. Therefore, the present study was planned to determine the frequency of D allele of ACE gene in our study population.

#### Aim & Objectives

- The aim was to study the insertion/ deletion (I/D) polymorphism of ACE gene in patients with essential hypertension
- Also, to assess the genotype distribution and to determine allelic frequency of ACE gene.

## MATERIALS AND METHODS

**Study subjects:** It is a cross-sectional study consisting of 41 hypertensive patients in different age groups (26-92 years) including both sexes [Table 1].

**Inclusion criteria:** Hypertensive patients with family history of hypertension were recruited for this study. All the patients recruited were undergoing treatment with one or more antihypertensive agents.

**Exclusion criteria:** Participants with heart disease, renal failure was excluded and the obese persons (body mass index >30), smokers and alcoholics also excluded.

#### Collection of blood samples

About 2 ml of venous blood samples were collected in ethylene-diamine-tetra-acetic acid (EDTA) tube and the whole blood specimens were stored at -20°C (deep freezer) till further analysis.

#### Deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted from the whole blood samples using spin-column chromatography method, according to the protocol given by the manufacturer (Miniprep Kit, Helini Biomolecules, Chennai, Tamil Nadu, India). A silica-based membrane technology used in the form of a convenient spin column by which the cellular components of the blood were lysed and the cellular DNA that bind to silica membrane are recovered after a series of "wash and spin" steps. The purity and quantity of DNA were assessed by absorbance values in UV spectrophotometer and checked by 0.5 % agarose gel electrophoresis. Then the DNA samples were at -20°C till further analysis.

#### Amplification of target DNA sequence by PCR

The DNA samples were amplified by polymerase chain reaction (PCR) technique. The target DNA fragment was amplified using two oligonucleotide primers flanking the ACE gene: the forward primer (sense): 5'-CTGGAGAGCCACTCCCATCCTTTCT-3' and the reverse primer (anti-sense): 5'-GACGTCGCCATCACATTCGTTCAGAT-3'. Each of the DNA samples was amplified in final reaction volume of 15 µL containing 5 µl of master mix (GoTaq Green Master Mix, Promega -USA), 0.3 µl each of the primers (Eurofins Genomics India Pvt Ltd, Bangalore), 8.4 µl of nuclease free water (NFW) and 1µl of extracted DNA. The PCR conditions consisted of 94°C for 10 min (initial denaturation), followed by 37 cycles with the conditions of 94°C for 45s (denaturation), 56°C for 1 min (annealing), 72°C for 1 min 30 s (extension). The final extension was allowed for 10 min at 72°C and the PCR amplicons were stored at 4°C.

#### Analysis of PCR products by Agarose Gel Electrophoresis (AGE)

Agarose gel plate prepared using 2g of agarose in 30 mL of 1X TBE (Tris-Borate-EDTA) buffer and the amplified PCR products were mixed with loading buffer and loaded into the separate sample wells created on the gel matrix. Molecular weight DNA marker (DNA ladder) was also run along with the test samples. Electrophoresis was performed at 80 V for 60 minutes at room temperature. Then the gel was stained in the presence of 50 ml of 1X TBE buffer containing ethidium bromide (0.5 µg /ml) for 30 min. The DNA bands were visualized on ultraviolet light transilluminator and photographed. On visualization, each DNA sample revealed one of following three genotypic patterns:

- Homozygous I/I genotype: Insertion (I) alleles were detected as a single band of 490 bp fragment.
- Homozygous D/D genotype: Deletion (D) alleles were identified as a single band of 190 bp fragment.
- Heterozygous I/D genotype: Both 490 and 190 bp fragments (I and D) were detected as two separate bands.

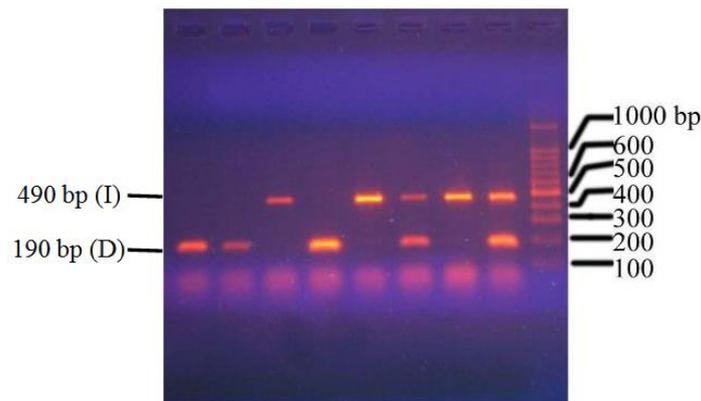
### Statistical analysis

Allele and genotype frequencies were deduced using the gene-counting method and the Hardy-Weinberg (H-W) equilibrium checked by  $\chi^2$  test.  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

The present study investigated genotype distribution and allelic frequency of ACE I/D polymorphism in patients with essential hypertension. The ACE genotypes with gender distribution and the allele frequencies of study subjects are presented in Table-2 and Table-3 respectively. Among the three genotypes, the frequency of homozygous I/I genotype

was higher and the least one in our population was homozygous D/D genotype. Heterozygous genotype showed an intermediate level of frequency. The genotype frequency of the homozygous I/I genotype, homozygous D/D genotype, and the heterozygous I/D genotype was found to be 44 %, (n= 18), 27 % (n=11), and 29 % (n=12) percent respectively. The difference between the observed genotype and expected genotype frequencies in our population was found to be statistically significant ( $p = 0.011$ ). The respective frequencies of D and I allele among the study subjects were 41.46% and 58.54%. The frequency of I allele of ACE gene is found to be high when compared to D allele.



**Fig-1: Genotype pattern of study subjects**

Figure-1 depicts a representative agarose gel electrophoresis of PCR products showing the ACE I/D polymorphism in the studied samples. The length of fragments is compared with molecular weight DNA

marker (DNA ladder). The insertion allele (I) was identified by the presence of 490 bp fragment; the deletion allele (D) was identified by the presence of 190 bp fragment.

**Table-1: Anthropometric parameters of the hypertensive patients**

Hypertensive patients (n=41)	
Age	59.8 ± 12.42
Sex: Male / Female	22/19
BMI (Body Mass Index) Cut-off value (< 29.9)	24.9 ± 2.49
Systolic Blood Pressure (mm/Hg)	134.5 ± 13.44
Diastolic Blood Pressure (mm/Hg)	84.73 ± 6.46

**Table-2: Genotype frequencies and the gender distribution in hypertensive patients**

Genotypes	No. Of patients			Genotype frequency (%)
	Male	Female	n	
I/I	9	9	18	44 %
D/D	4	7	11	27 %
I/D	6	6	12	29 %
Total	19	22	41	

**Table-3: Genotype frequencies and the allele distribution in hypertensive patients**

Genotypes	I/I	I/D	D/D	Chisquare ( $\chi^2$ ) = 6.46 P-Value = 0.011
Observed	18	12	11	
Expected	14.05	19.9	7.05	
H-W Freq.	(34.27%)	(48.54%)	(17.19%)	
Allele Frequencies		I = 48 (58.54%)	D = 34 (41.46%)	

H.W: Hardy Weinberg Frequency

These observations are in line with many of the previous studies conducted in other parts of India including the studies conducted by Randhawa *et al* and Pasha *et al*. [5,6]. These studies have reported that the homozygous I/I genotype is increasingly distributed than the D/D genotype and there was no significant difference noted between the genotype frequencies of hypertensives and control. In our study, the difference between the observed genotype and the expected genotype frequencies in the studied subjects was found to be statistically significant. This shows that the observed genotype frequency is not in accordance with Hardy-Weinberg equilibrium. Our results showed that, the frequency of I allele was higher than the D allele in the studied population. As in our study, many of the Indian studies showed an increased distribution of I allele in hypertensive population. A significant association of I allele with hypertension have been demonstrated in the study conducted by Srivastava *et al*. [7].

In ACE gene polymorphism, the plasma ACE level of D/D genotype is reported to be about double that of I/I genotype; and intermediate level in I/D type [8]. The molecular mechanism underlying this ACE insertion/deletion polymorphism is not clear. It is suggested that the interaction with regulatory elements may be altered due to polymorphism, thus causing increased production of ACE [9]. A significant association of high ACE producing D allele with hypertension has been demonstrated in the African-American, Chinese and Japanese populations [10-13]. A few of the Indian studies also have reported the increased distribution of D allele in hypertensive patients. Interestingly, studies conducted in many other countries were inconsistent with Indian studies including the present study [14,15]. The inconsistent results in different populations may be due to varied ethnicity or the various other genetic and environmental factors implicated in the regulation of blood pressure. In addition to these factors, the result of present study may also be influenced by the study design and the composition of the sample population.

Since hypertension is a complex genetic disorder, it is important to know the genetic information about the other polymorphisms that interact and influence the advent of this disease. Of the various

polymorphisms suggested, the *AT1R* A1166C, *AGT* M268T, *CYP11B2* C-344T, eNOS G894T and eNOS 4a/b polymorphisms have attracted considerable clinical importance [16]. Hence this attempt would help in identifying individuals at an increased risk of developing this disease and to initiate appropriate actions in them to avoid development or delay the onset of essential hypertension.

## CONCLUSION

The present study data indicates, the wild I/I genotype is predominant and the frequency of I allele is increasingly distributed in the studied hypertensive subjects of our population. Our findings may provide a genetic background of our population regarding ACE I/D polymorphism, however a case-control study with larger sample size is needed to establish or refute the role of this polymorphism in the development of essential hypertension.

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