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# POLYMORPHISM OF ANGIOTENSIN I CONVERTING ENZYME BECOMES AN INNATE PROPERTY IN RENAL FAILURE

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## ABSTRACT

Renal failure or kidney failure is a situation in which the kidneys fail to function adequately. Angiotensin converting enzyme plays a pivotal role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I to angiotensin II. The Angiotensin Converting Enzyme (ACE) gene contains a polymorphism which is believed to be associated with the interpersonal variability of ACE levels in circulating blood. Elevated angiotensin II level makes deleterious effects on renal hemodynamics and induces the expression of other growth factors, leading to glomerulosclerosis. This paper attempts to find the association of ACE polymorphism with renal failure using Random Amplification of Polymorphic DNA technique arriving to the major conclusion that ACE polymorphism is an innate property in End Stage Renal Failure (ESRF) and ACE screening would be a valuable diagnostic tool in screening clinical ESRF.

## KEYWORDS

*Renal failure, Angiotensin I converting enzyme, angiotensin polymorphism,*

## INTRODUCTION

Angiotensin converting enzyme (ACE) is a major component of the Renin angiotensin system and plays an important role in blood pressure regulation by hydrolyzing angiotensin I into angiotensin II, a strong vasopressor. This enzyme is also to degrade Bradykinin, which is a potent vasodilator evidences suggest plasma activity is related to the insertion/deletion (I/D) polymorphism of the ACE gene[1]. Angiotensin converting enzyme also plays a vital role in electrolyte balance by hydrolyzing Angiotensin and initiating synthesis of aldosterone-stimulating peptide. This enzyme is also able to inactivate Bradykinin and angiotensin II vasodialators[2].

The ACE gene contains a polymorphism in the form of either insertion (1) or deletion (D) of a 287 base pair Alu repetitive sequence in intron 16[3]. This polymorphism is shown to be associated with the

interpersonal variability of ACE levels in circulating blood. The deletion allele at this gene site is associated with increased plasma ACE activity. Elevated angiotensin II level makes deleterious effects on renal hemodynamics and induces the expression of other growth factors, leading to glomerulosclerosis[4].

The I/D polymorphism of ACE gene is strongly associated with the level of circulating enzyme. This enzyme plays a key role in the production of angiotensin II and in the catabolism of bradykinin to peptides involved in the modulation of vascular tone and in the proliferation of smooth muscle cells[5]. The ACE I/D polymorphism may be a modifying role in the development of heart failure in hypertensive subjects.

High prevalence of kidney disease without a clear mode of inheritance even in familial cases cannot be explained on the basis of polymorphism/mutation in a single gene but implies a multifactorial origin[6]. Identification of such genetic determinants would be essential for a possible early intervention to prevent End Stage Renal Failure (ESRF). Thus, the genes or genetic loci responsible for excess Ang II production or availability are potential candidates for development of kidney disease. These include all the components of RAAS namely, renin (REN). Angniotnsinogen (AGT), Angiotensin I converting enzyme (ACE), angiotensin type 1 receptor (AGTR1) and aldosterone synthase (CYP11B2)[7]. Hence, it is apposite to analyze the polymorphic nature of ACE alleles in renal failure patients.

## *MATERIALS AND METHODS*

### *Screening and Recruitment of patients*

Cancer patients from Sri Meenakshi mission hospital and research Centre, Madurai (9°48′ N 78°06′ E) and ABC hospital, Trichy (10° 45' 57" N and 78° 42' 53" E) were enrolled in the study after protocol approval by University Human ethical committee. Patient consent Performa and case history were maintained. The patients enrolled were in array of stages of renal failure.

### *Blood collection and Sample processing*

Forty peripheral blood samples were collected intravenously in EDTA coated, sterile polypropylene centrifuge tubes. DNA was extracted according to Welsh and Bunce[8] with slight modification in salting out procedure. Briefly, 5 ml of peripheral venous blood was mixed with 45 ml of RCLB. Incubated with lysis buffer for 5 mins and centrifuged at 2000rpm for 10 minutes. The pelleted clear nucleated cells were treated with 3 ml of NLB and SDS. To this 1ml of 6M NaCL and 2ml of chloroform was added to precipitate protein out. The mixture was then vortexed and centrifuged for 10 minutes at 2000rpm. Two volumes of 95% ethanol were added and the DNA was separated from the supernatant. Drained the Alcohol and resuspended the DNA in 200 µL of TE buffer after drying. The isolated DNA was checked for its purity by taking O.D. at 260 and 280nm in an U.V. Spectrophotometer.

### *PCR amplification of ACE*

Amplification with the flanking primer pair, Forward:5’CTGGAGACCACTCCCATCCTTTCT3’; Reverse:5’GATGTGGCCATCACATTCGTCACGAT 3’ resulted in 490bp and 190bp amplification products corresponding to the ‘I’ and ‘D’ alleles respectively. PCR products were detected on a 2% agarose gel containing ethidium bromide. PCR amplification with an insertion specific primer pair
Forward:5’TGGGACCACAGCGCCCGCCACTAC3;
Reverse:5’TCGCCAGCCCTCCATGCCCATAA3’
was performed in all samples, only the I allele produced at 335 bp amplification. The amplified PCR

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products were electrophoresed in 1.5% agarose gel, visualized under UV Transillumninator and documented in a gel documentation unit (Vilbert–Lourmat, France). Alleles were assigned according to the pattern of band formation.

### *Quality control of PCR*

Before SSP typing of DNA samples, the primers were checked for correct amplification with DNA of known allele specificity for each allele. Previously genotyped DNA samples from in-house positive samples were used for the purpose of quality control.

### *Statistical calculations*

The genotype and phenotype frequencies were calculated as below

Genotype frequency = N/2n
Phenotype frequency = N/2n
Where,
N = Total number of each positive allele.
n = Total number of individuals tested.

## RESULTS AND DISCUSSION

The present study was undertaken to identify the ACE I/D polymorphism in kidney failure patients and controls the total number of samples from patients and controls were enrolled in twenty one (n=21). The heterozygote (ID) form was predominantly present in both patient (80.95%) and controls (66.66%; (n = 14)) respectively.

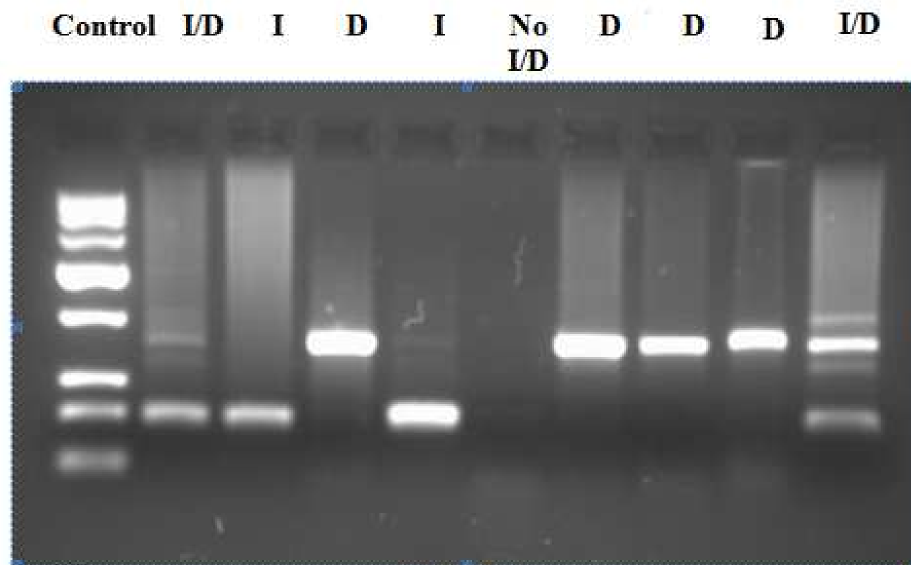


Figure 1

*PCR amplified Genome of Renal Failure patients displaying Insertion / Deletion in ten selected samples (I: Insertion; D: deletion)*

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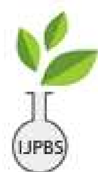
The genotypic frequency for the II allele was 4.76% (n = 1) in patients and 28.57% (n = 6) in controls (P<0.098). The frequency for homozygous DD was 14.28% (n = 3) in patients and 4.76% (n = 1) in controls. The total I allele frequency (Genotype II and ID taken together) was 45.23% (n = 19) in patients and 61.90% (n = 26) in controls. The total D allele frequency (genotype DD and ID taken together) was 54.76% (n = 23) in (02) patients and 38.09% (n = 16) in controls. The odds ratio for D and I allele is 1.94 and 0.52 respectively with a P value < 0.189.

Various genetic markers have been studied to predict susceptibility and course of renal disease. Increased activity of Ang-II alters variety of growth factors and has been shown to have detrimental effect on renal diseases progression. ACE genotype could be one of the risk factors for the development and progression of renal disorders or indicate genetic tendency towards the diseases. Genetic factors may affect the renal disease phenotype in several ways: the susceptibility for acquiring renal disease, the natural course of the disease, and the response to therapy. Distinguishing between these possible mechanisms is necessary to understand the role of genetic factors in renal disease.

In the present study, the DD and D allele frequency were higher in patients than in controls (Figure 1). The mechanism accounting for the elevated ACE levels in DD homozygote is unknown. Because the polymorphism is located on an intron, it may simply be in linkage disequilibrium with a functional variant of the ACE gene or suppress transcription. The increase in cellular ACE suggests effects at the transcriptional or translational level rather than increased cleavage from membrane- bound enzyme. Segregation-linkage analysis has identified additional ACE gene polymorphisms, suggesting several quantitative trait loci. These may include I/D polymorphism of ACE gene. DD allele is known to be associated with high (60% higher) levels of ACE than those with II allele[1]. ACE converts angiotensin I to angiotensin II in the rennin-angiotensin aldosterone system[9]. This increased activity is due to increased ACE levels in certain population. It has been suggested that increased formation of angiotensin (Ang) II is involved. As Ang II plays an important role in renal pathophysiology, these findings elicited.

ACE polymorphism affects the susceptibility to acquiring specific renal disorders, overrepresentation of a particular genotype under these conditions would be expected an upsurge of studies on the association of the ACE I/D genotype (and other polymorphisms of the RAAS) and renal disease, giving rise to sometimes conflicting results.

A higher concentration of Angiotensin II in DD genotype is thought to cause progressive renal disease. Acting through various growth factors angiotensin II has been postulated to cause structural changes in renal system and alterations of renal homodynamic[10]. The present study revealed that the II genotype was increased in controls than in patients. The lower enzyme levels led to reduction in glomerular pressure, proteinuria, and tubular damage and scarring, resulting in retardation of disease progression to ESRF. Another mechanism to achieve better disease management and less ESRF outcome in II genotype patients may be in the control of TGF-β level.





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RAS is known for its regulation and maintenance of salt balance systemic and glomerular blood pressure. The hyperactivation of this system leads to an increase in systemic and glomerular blood pressure followed by fibrosis and progressive loss of renal function. Various factors are involved in deregulation of RAS. Polymorphisms in the RAS genes are the most common factor that is responsible for increased RAS activity resulting in hypertension. The predisposing factors for the renal failure such as modifier genes and also candidate genes with polymorphism that function abnormally during adverse condition can cause irreversible damage to the kidney.

Many reports in Indian patients have been published in relation to ACE I/D polymorphism being associated with various diseases[11,12]. These studies have concentrated only on single or multiple gene polymorphisms in candidate or modifier gene alone with respect to a single disease. Here we made an attempt to explore the gene polymorphism frequency in both candidate and modifier genes in South Indian CKD patients. No such observations were reported earlier in Indian population.

## CONCLUSION

The gene polymorphism in candidate ACE gene and their frequency of incident clearly state that ACE polymorphism is an innate property of renal failure. Identification of this genetic determinant made a possible early intervention to prevent End Stage Renal Failure (ESRF). Thus, the genes or genetic loci responsible for excess Angiotensin II production or availability and are potential candidate for development of kidney disease has a knot removed.

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## REFERENCES

1. Rigat B,et al. An insertion/deletion polymorphism in the angiotensin I converting enzyme gene accounts for half the variance of serum enzyme levels. J Clin Invest 86:1343–1346, (1990)
2. Ozaki R, et al. Angiotensin-converting enzyme (ACE) inhibition in type 2, diabetic patients- interaction with ACE insertion/deletion polymorphism. Kidney Int.; 69: 1438-1443. (2006)
3. Muradiye Nacak,et al. Association of Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism with Allergic Contact Dermatitis Basic & Clinical Pharmacology & Toxicology, 101 (2): 101 – 103, (2007)
4. Frank C. New insights into the mechanisms of fibrosis and sclerosis in diabetic nephropathy, Reviews in Endocrine & Metabolic Disorders, 9 (4): 245-254. (2008)
5. François Cambien et al, Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction, Nature, 359, 641 - 644 (1992)

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6. Cambien F, et al. C: Familial ressemblance of plasma angiotensin converting enzyme level: the Nancy study. Am J Hum Genet, 43:774-80. (1988)
7. Jeunemaitre X, et al. JM: Molecular basis of human hypertension: role of angiotensinogen. Cell, 71:169-80. (1992)
8. Welsh K, et al. Molecular typing for the MHC with PCR-SSP. Rev Immunogenet 1:157–176. (1999)
9. Wolf G, et al. Angiotensin II as a renal growth factor. J Am Soc Nephrol 3:1531–1540. (1993)
10. H Kobori et al. The intrarenal renin-angiotensin system: from physiology to the pathobiology of, Pharmacological Reviews, 59 (3): 251-287. (2007)
11. Yuval Heled,et al. Human ACE I/D polymorphism is associated with individual differences in exercise heat tolerance, J Appl Physiol 97: 72-76. (2004)
12. Roltsch MH, No association between ACE I/D polymorphism and cardiovascular hemodynamics during exercise in young women. Int J Sports Med. 26(8): 638-44. (2005)