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*Full Length Research Paper*

# Angiotensin-converting enzyme gene I/D polymorphism in Pakistani systemic lupus erythematosus patients

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**Angiotensin-converting enzyme (ACE) was first identified as a key component of the rennin-angiotensin system, as its main role is to process angiotensin I to angiotensin II and degrade bradykinin. Human ACE maps to chromosome 17q23 spans 21Kb, includes 26 exons and 25 introns. In humans, ID, DD, and II polymorphism is located in intron 16 of the angiotensin gene. The purpose of this study is to investigate the frequency of ACE gene insertion/deletion (I/D) polymorphism genotype in systemic lupus erythematosus (SLE) patients and to study the correlation between I/D polymorphism of the ACE gene and clinical manifestations of SLE. Sixty one (61) controls and 61 SLE patients were recruited from Punjab-Pakistan. Sixty one SLE patients and 61 control subjects were studied for ACE I/D polymorphism by using Triple primer method with nested polymerase chain reaction (PCR). The frequency of DD, ID and II genotypes was 54, 3 and 4% in SLE patients' and 23, 32 and 6% in healthy controls, respectively. The frequency of DD allele in SLE patients with lupus nephritis is 100%, Sjogren's syndrome 100%, Raynaud's phenomenon 88.88%, and with rheumatoid arthritis it is 78.94%. The frequency of ID allele in SLE patients with Raynaud's phenomenon is 5.55%, and with rheumatoid arthritis it is 10.52%. The frequency of II allele in SLE patients with Raynaud's phenomenon 5.55%, rheumatoid arthritis is 10.52% but the important thing to note is that the frequency of II allele in SLE patients with vasculitis is 100%. This study was undertaken to determine whether DD, ID and II polymorphisms of Intron16 of the ACE gene is associated with SLE and whether the results support such an association. It can be concluded that lupus nephritis, Sjogren's syndrome, Raynaud's phenomenon, rheumatoid arthritis and vasculitis, which are common among Pakistani SLE patients, are related diseases and ACE gene is involved in lupus susceptibility.**

**Key words:** Systemic lupus erythematosus, angiotensin converting enzyme I/D polymorphism, Sjogren's syndrome, Raynaud's phenomenon, rheumatoid arthritis, vasculitis.

## INTRODUCTION

Angiotensin Converting Enzyme (ACE) was first identified as a key component of the rennin-angiotensin system, as its main role of this gene is to process angiotensin I to angiotensin II and degrade bradykinin. In mammals, the key role of ACE is in homeostatic mechanism responsible for the maintenance of normal blood pressure and electrolyte balance (Corvol et al., 1995). Human ACE maps to chromosome 17q23 spans 21Kb, includes 26 exons and 25 introns. In mammals, there are two isoforms of ACE exist; one is expressed in the somatic tissue (sACE) and other one in the germinal cells in the male testes (gACE). These two isoforms exist because of the two different promoter activities, as sACE transcribed from a promoter region upstream of the duplication and gACE from a promoter within Intron 12 (Langford et al., 1991). Human sACE is a type-I membrane bound protein. It consists of a 28-residue carboxy terminal cytosolic domain, a 22 residue hydrophobic transmembrane domain, and a 1227 residue extracellular domain that is heavily

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**Abbreviations: ACE,** Angiotensin-converting enzyme; **SLE,** systemic lupus erythematosus; **I/D,** insertion/deletion.

glycosylated. Both C and N domain of sACE are functional but with different biochemical properties e.g., their inhibitor affinity profiles and the requirement for chloride ions differ (Dive et al., 1991).

The cDNA sequences of human gACE and sACE were determined in 1989 and the genome sequence and structure in 1991 (Corvol et al., 1995). ACE is considered to be a mammalian enzyme but sequence homologues with very similar activities are found in wide range of species e.g., *Drosophilia melanogaster* (Coates et al., 2000). All identified forms of ACE and ACE-like gene products tend to have a signal peptide and are glycosylated as they move through the endomembrane system, before transport to the cell membrane. Soluble circulating ACE is produced by a specific proteolytic cleavage above the C-terminal transmembrane anchors by a "secretase", probably itself a zinc metalloprotease (Turner and Hooper, 2002). Immunochemical staining has revealed that sACE is strongly expressed in many endothelial cells such as in arterioles, small muscular arteries and in the normal capillary endothelial cells of the lungs. Strong expression is seen in the epithelial cells of the kidneys, small intestine, epididymis and in the neuronal cells of the brain; while the expression of the gACE was confined to differentiating male germinal cells (Danilov et al., 1994). Complete knockouts of ACE showed a marked reduction in male fertility, spontaneous hypotension and in kidney malformation (Esther et al., 1996; Krege et al., 1995).

ACE gene contains a polymorphism based on the presence (Insertion I) or absence (deletion D) of a nonsense fragment. In humans, I/D polymorphism is located in Intron 16 of the angiotensin gene and it was initially described by Rigat et al. (1990). The purpose of this study is to investigate the frequency of ACE gene insertion/deletion (I/D) polymorphism genotype in SLE patients and to study the correlation between I/D polymorphism of the ACE gene and clinical manifestations of SLE.

## MATERIALS AND METHODS

Sixty one SLE Patients and 61 control subjects were studied for ACE I/D polymorphism. All SLE patients' fulfill ACR criteria. Genomic DNA was isolated by using kit method (Fermentas). Target DNA was amplified using nested polymerase chain reaction (PCR). Triple-primer method with a "nested" PCR primer situated completely within the insertion sequence of the Iallele. The inclusion of a third internal PCR primer is the most reliable PCR strategy for ACE. The annealing temperature was 53°C. The banding patterns of the 3 possible genotypes were as follows: DD, 210-bp fragment; II, 498-and 264-bp fragments; ID, 498-, 264-, and 210-bp fragments I/D genotyping.

## RESULTS

Genomic DNA isolated from subjects as well as from the matched controls was used for the detection of ACE insertion/deletion polymorphism by triple primer method with nested PCR (Figure 1). We have assayed ACE I/D polymorphism in intron 16 and the sequence was obtained from the database as mentioned below:

ATCCTTTCTCCCATTTCTCTAGACCTGCTGCCTATAC
AGTCACTTTTATGTGGTTTCGCCAATTTTATTCCAGCT
CTGAAATTCTCTGAGCTCCCCTTACAAGCAGAGGTG
AGCTAAGGGCTGGAGCTCAAGGCATTCAAACCCCTA
CCAGATCTGACGAATGTGATGGCCACGTCCCGGAAA
TATGAAGACCTGTTATGGGCATGGGAGGGCTGGCGA
GACAAGGCGGGGAGAGCCATCCTCCAGTTTTACCCG
AAATACGTGGAACTCATCAACCAGGCTGCCCGGCTC
AATGGTGAGTCCCTGCTGCCAACATCAC

The frequency of the insertion and deletion alleles was quite different in the subjects and controls. The frequency of DD, ID and II genotypes was 54, 3 and 4% in SLE patients' and 23, 32, 6% in healthy controls. Comparison between cases and control subjects of genotype frequencies was carried out by paired T test and McNemar's test, respectively (Table 1).

Chi-square test was used to analyze the genotype frequency distribution (Table 2) at 5% level of significance ($\alpha = 0.05$). The observed value of Chi Square (36.909) was greater than 5.991, so we easily reject the null hypothesis of independence and concluded that a dependency or relationship exists between ACE I/D polymorphism and lupus.

High creatinine and proteinuria in systemic lupus erythematosus patients are suggestive of having Lupus nephritis. The frequency of DD allele in systemic lupus erythematosus patients with Lupus nephritis is 20(100%), Sjogren's syndrome 9(100%), Raynaud's phenomenon 16(88.88%), and with rheumatoid arthritis it is 15(78.94%). The frequency of ID allele in systemic lupus erythematosus patients with Raynaud's phenomenon is 1(5.55%), and with rheumatoid arthritis it is 2(10.52%). The frequency of II allele in SLE patients with Raynaud's phenomenon 1(5.55%), Rheumatoid arthritis is 2(10.52%) but the important thing to note is that the frequency of II allele in SLE patients with vasculitis is 2 (100%). This data was presented in Figure 2.

## DISCUSSION

Angiotensin-converting enzyme is a monomeric, membrane bound, zinc and chloride dependent peptidyl dipeptidase that catalyzes the conversion of the decapeptide angiotensin I to the octapeptide angiotensin II by removing a carboxy terminal dipeptide (Skeggs et al., 1956). In 1990, Rigat et al. identified a biallelic polymorphism in the angiotensin-converting enzyme gene that is characterized by the presence or absence of a 287bpAlu repeat sequence (Rigat et al., 1990). Healthy homozygote for the D allele have high serum angiotensin-

**Table 1.** Distribution of alleles for ACE I/D polymorphism in case and control populations.

| ACE gene I polymorphism | SLE patients (n=61) | Controls (n=61) |
|---|---|---|
| %D | 0.909 | 0.639 |
| %I | 0.0901 | 0.360 |

95% Confidence Interval for mean difference: (-2.881, 3.979). T-Value = 2.03 P-Value = 0.291.

**Table 2.** Genotype frequency distribution.

| ACE gene I polymorphism | SLE patients(n=61) | Controls(n=61) |
|---|---|---|
| ID | 3 | 32 |
| DD | 54 | 23 |
| II | 4 | 6 |

Degree of Freedom = 2, $\chi^2$=36.909.

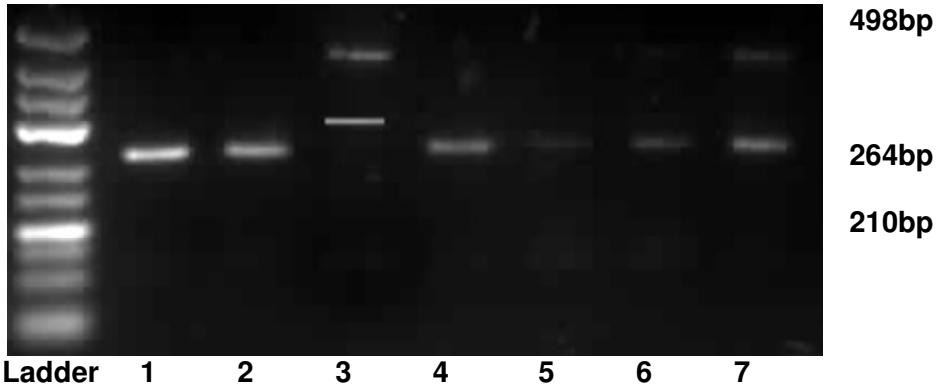


**Figure 1.** Genomic DNA isolated from subjects as well as from the matched controls

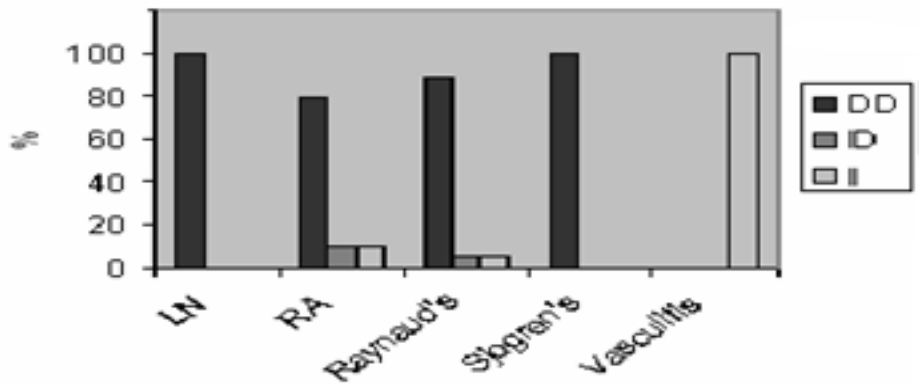


**Figure 2.** Correlation of angiotensin-converting enzyme I/D polymorphism and clinical manifestation of systemic lupus erythematosus. LN: Lupus Nephritis, RA: Rheumatoid Arthritis.

converting enzyme level and high circulating endogenous angiotensin II levels than those with the II genotype (Ueda et al., 1995). Further studies have revealed that the healthy individual with DD genotype may be resistant to angiotensin-converting enzyme inhibitor therapy (Ueda et al., 1996). Sato et al. (1999) showed that the individuals

with II genotype showed a significant increase in systemic lupus erythematosus activity and such patients showed a higher serum level of anti-dsDNA than those with the DD genotype (Sato et al., 1999). Unlike Sato et al. our study group showed that the frequency of D allele was quite high in systemic lupus erythematosus patients than that of the controls and the genotype frequency distribution of this polymorphism was not in Hardy Weinberg equilibrium. In comparison with Brazilian population (Sprovieri and Sens, 2005), Pakistani population also showed that the progression of Lupus nephritis is associated with angiotensin-converting enzyme DD polymorphism. Rabbani et al. (2008) found that in severe systemic lupus erythematosus, D and I alleles were in strong linkage disequilibrium (Rabbani et al., 2008).

In Kuwati population, the presence of DD genotype confers susceptibility to the development of vascular morbidity (Al-Awadhi et al., 2007). Antiphospholipid syndrome is characterized by the development of thrombosis and in systemic lupus erythematosus patients; it can be detected by the presence of antiphospholipid antibodies (Lewis et al., 2001). In the present study, II genotype is 100% associated with antiphospholipid syndrome but we do not consider it significant because of the total systemic lupus erythematosus population, only two were the cases of antiphospholipid syndrome. Parsa et al. (2002) conducted an association of 3 polymorphisms in angiotensin-converting enzyme including I/D polymorphism and 2 polymorphisms were associated with systemic lupus erythematosus and Lupus Nephritis among non-Caucasians that includes Hispanic, Asians, and Pacific population (Parsa et al., 2002). To our knowledge, this study is the first report showing an association of ACE I/D polymorphism with SLE in Pakistani population. One of the reasons of this association is that the DNA sequence variation in ACE gene may influence the risk of developing SLE and Lupus nephritis.

Pullmann et al. (1999) found higher frequency of deletion allele in SLE patients (62.9%) than controls (52%) from the Solvak Republic (Pullmann et al., 1999) but Tassiulas et al. (1998) found the opposite results in African-Americans with the frequency of the D allele lower in Lupus patients (59%) than controls (72.4%) (Tassiulas et al., 1998). Activated angiotensin system may lead to organ damage by enhancement of cellular hypertrophy, proliferation, disruption of the extracellular matrix and induction of cytokine or growth factor secre-tions further exacerbates the injury. Patients with DD genotype have activated angiotensin system and may prone to vascular injury. This may be one of the reasons that the frequency of DD genotype is quite high in SLE patients with Lupus nephritis (100%), Sjogren's syndrome (100%), Raynaud's phenomenon (88.88%) and rheumatoid arthritis (78.94%).

Prakcin et al. (2001) determined angiotensin-converting enzyme genotypes; the frequency of DD, ID, and II genotypes was 50, 28, 22% in systemic lupus erythematosus patients and 25, 50, 25% in healthy controls (Prakcin et al., 2001). The outcome of Prakcin et al. study is somewhat similar to our results because here DD genotype is also common in systemic lupus erythematosus patients than in the control group; the frequency of DD, ID, and II genotypes in SLE patients was 88.52, 4.918, 6.557% and 37.7, 52.45, 9.83% in age and sex-matched controls. Douglas et al. and Lee et al. were unable to find any association of angiotensin-converting enzyme I/D polymorphism with systemic lupus erythematosus and Lupus nephritis (Douglas et al., 2004; Lee et al., 2006; Matsausaka et al., 1996). In contrast, our results suggest that ACE I/D polymorphism might be used as one of the predictive factors for the activity of Lupus.

This study was undertaken to determine whether I/D polymorphism of Intron16 of the ACE gene is associated with systemic lupus erythematosus and the results support such an association. One can conclude that lupus nephritis, Sjogren's syndrome, Raynaud's phenomenon, rheumatoid arthritis and vasculitis, which are common among Pakistani systemic lupus erythematosus patients, are disease related and angiotensin-converting enzyme gene is involved in lupus susceptibility.

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