**ACE Gene I/D Polymorphism in Primary Nephrotic Syndrome**

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**Abstract**

Idiopathic Nephrotic syndrome (INS) includes edema, hypoalbuminemia and hyperlipidemia Based on the histopathology Nephrotic syndrome may: Minimal change disease , FSGS (focal segmental glomerulo sclerosis) Depending on the response to standard steroid treatment NS may be steroid sensitive or Non-sensitive. The objective of the current study is to investigate the role of ACE I/D gene polymorphism on the susceptibility, progression and steroid response in INS from South Indian population. The angiotensin converting enzyme (ACE) gene carries insertion (I) and deletion (D) polymorphism within its intron 16. The presence of D-allele in the ACE gene has been reported as a probable genetic risk factor for idiopathic nephrotic syndrome (INS).

A total of 50 individuals comprising 25 patients and 25 controls were enrolled in present study .Sex matched and normal adults without any renal disease were taken as controls. Blood samples were collected in sterile vaccutainers with anticoagulants for DNA isolation. 5 ml of blood was processed for DNA isolation using salting out method.

In conclusion ACE I/D genotypes in the present do not significantly influence the susceptibility, progression and drug response variation in INS of South Indian population. The D allele frequency was significantly higher in INS patients than in controls. Thus D allele can be an independent marker of susceptibility to nephrotic syndrome in south Indian population. D allele frequency was significantly higher in Non-SS (steroid resistant group) than in the SS group. The present study deals with comparison of SSNS and SRNS groups to understand the genotypic distribution or the ACE I/D allele involvement. In order to understand its influence at the physiological level this study should to be studied with large sample size in different ethnic groups. This information may help the clinicians to predict the course of disease and to identify individuals with better prognosis to standard steroid treatment.

**Introduction**

The angiotensin converting enzyme (ACE) gene carries insertion (I) and deletion (D) polymorphism within its intron 16. The presence of D-allele in the ACE gene has been reported as a probable genetic risk factor for idiopathic nephrotic syndrome (INS), especially the subtype of focal segmental glomerulosclerosis (FSGS). The D-allele may be related to poor responsiveness to steroid therapy. To clarify the relationship between the D-allele and INS, we studied the prevalence of the D-allele in the South Indian patients. Additionally, we also analyzed relationship between each genotype and steroid sensitivity among the MCNS patients.

Angiotensin converting enzyme (ACE) is a key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. In some cases, the increase of ACE protein is responsible for the elevation of angiotensin II level. Elevated angiotensin II level makes deleterious effects on renal hemodynamics and induces the expression of other growth factors, leading to glomerulosclerosis. .

The ACE gene carries insertion (I) and deletion (D) polymorphism, and the DD-genotype is reportedly related to an increase in the ACE protein expression . Therefore, it has been thought that the DD genotype may link to the ACE-related pathophysiology of renal diseases. Of the ACE I/D polymorphism impacts on the renal diseases, idiopathic nephrotic syndrome (INS) holds particular attention, especially the focal segmental glomerulosclerosis (FSGS). (Hori2001) . reported that the frequency of DD genotype was higher in FSGS patients than in controls. (Lee1997). reported that FSGS patients with DD genotype showed a lower responsiveness to corticosteroid therapy and a higher incidence of chronic renal failure than those with other genotypes.

Although there are many reports from other populations, there is no studies on the relationships between ACE I/D polymorphism and renal diseases have been reported from the Indonesian population. Here, we determined the distribution of the ACE I/D polymorphism among INS patients and healthy individuals in the south Indian population, and compared our results with the data reported from other populations.

**MATERIALS AND METHODS**

For the present investigation blood samples were collected from a total of 50 subjects, 25 (16 males and 9 females) were clinically proven cases of INS fulfilling criteria of the International Study of Kidney Disease in Children for the diagnosis of INS (International Study of Kidney Disease in Children, 1981) without any secondary reasons for renal problem visiting Nephrology Department, NIMS hospital, Hyderabad, India and 25 were healthy volunteers of South Indian origin without any family history of renal disorders and preferred from the higher age group in order to rule out possibility of developing renal problems. Informed consent was obtained from all the participating subjects prior to blood sample collection. This study was approved by the Ethical committee at Osmania University. The mean age of the patient group was 8.58±5.13 years whereas for the control group it was 32.3±11 years. Blood samples as well as clinical data were collected in a well designed proforma from all the patients and controls for analysis.

Histopathological analysis was done based on biopsy findings for all the patients. The follow up clinical data to evaluate steroid responsiveness was obtainable from all cases. Based on the underlying histopathology MCD could be termed as mild and FSGS as severe. Steroid Sensitivity (SS) was defined as cessation of proteinuria for at least three consecutive days after standard steroid treatment. Steroid Dependence (SD) was defined as two consecutive relapses occurring during the period of steroid tapering or within 14 days of its cessation. No achievement of remission even after four weeks of steroid treatment was classified as steroid resistance (SR). SD and SR were grouped as Non-steroid sensitive (Non-SS).

Genomic DNA was extracted from all the 50 subjects in our laboratory using standard salting out method (Miller *et al*., 1988). ACE *I/D* genotyping was performed as given below and to avoid mistyping of *ID* genotype as *DD*, 5% DMSO was utilized (Shanmugam *et al*.,1993).

**Result and Desiccation**

The total study population comprised of 50 individuals, 25 NS patients and 25 controls. Male to female sex ratio of patients was 1:1 and the mean age was 8.58±5.13 years. The distribution of *II, ID* and *DD* genotypes in patients was 20%, 40% and 40%, whereas in the controls, 44%, 32% and 24% respectively. An odds ratio of 2.25 was obtained for *D vs. I* at 95% confidence interval, indicating that the *D* allele was predominantly higher in the patient group (p=0.007).

Analysis of ACE *I/D* genotypes in different histopathological conditions of NS revealed 28.5%, 42.8%, 28.5%, and 16.6%, 38.8%, 44.4% of *II, ID* and *DD* genotypes in severe and mild forms respectively. Further *I* allele frequency was high in mild form than in severe (0.6 *vs*. 0.36) (Table ).

Distribution of ACE I/D genotypes *II, ID* and *DD* in SSand Non-SS groups was observed to be 42.8%, 28.5%, 28.5% and 11.1%, 40.4%, 40.4% correspondingly. Significantly high frequency of *D* allele (0.66 *vs*. 0.33) was observed in Non-SS group compared to SS group (*P*=0.001) (Table ).

The ACE *DD* genotype is associated with the largest amount of angiotensin converting enzyme and angiotensin II, which has hemodynamic, growth, and prosclerotic effects (International Society of Nephrology, 1996). It is suggested that *DD* genotype acts as a predictor of progressive glomerulosclerosis in diabetic nephropathy (Doria *et al*., 1994; Schmidt *et al*., 1995; Jeffers, 1997), IgA nephropathy (Schmidt *et al*., 1995; Yoshida *et al*., 1995; Syrjanen, 2000), and other chronic renal diseases (Yoshioka *et al*., 1998; Dudley *et al*, 2000; Hohenfeller *et al*., 2001; Konoshita *et al*., 2001). The present study on ACE *I/D* gene polymorphism provides clues for understanding the susceptibility, clinical status and the benefit of steroid therapy in NS patients.

**4.1.ACE I/D gene polymorphisms in the susceptibility to NS:**

The current study dealt with distribution of ACE *I/D* gene polymorphism in NS and normal individuals of South Indian population. Analysis showed that *DD* *vs.* *ID+II* comparison among the two groups were significantly not different. Higher frequency of *D* allele suggests that it can act as an independent marker of susceptibility to nephrotic syndrome in south Indian population. Studies suggest the association of *DD* genotype not only in secondary renal abnormalities (Doria *et al*., 1994; Schmidt *et al*., 1995; Jeffers, 1997; Mesbah *et al*., 2007) but also in primary nephropathy (Lee *et al*., 1997; Serdaroglu *et al*., 2005; Tsai *et al*., 2006). A meta analysis concluded that *II* subjects had a 22% lower risk of diabetic nephropathy than the *D* allele carriers and Asians derived greater protection than Caucasians (Ng *et al*., 2009).

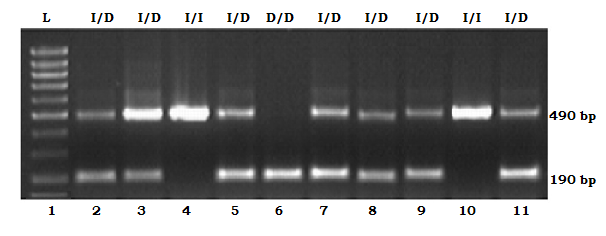
**4.2.ACE I/D gene polymorphisms in relation to severity of INS:**

When patients were categorized on the basis of histopathology and analyzed for the distribution of ACE *I/D* genotypes, no significant difference was observed among the three genotypes in the mild form and severe forms. This is may be due to the small sample size considered in the present study.

**4.3. ACE I/D gene polymorphisms and steroid responsiveness of INS patients:**

When steroid sensitivity was correlated with ACE *I/D* polymorphism it was observed that *D* allele carriers were 2 times more resistant to steroids (OR=2.65, *P=*0.001). Though there was difference among the two groups with respect to the ACE *I/D* genotypes, but it did not reach significance. An investigation by Fahmy *et al.,* 2008 in Egyptian children has reported prevalence of *II* individuals in the steroid sensitive group and *DD* individuals in steroid non-sensitive group and no difference with respect to *ID* frequency in cases and controls. However their sample belonged to a different ethnic group. In addition one study from North India (Patil *et al.,* 2005) has reported a significantly higher incidence of *II* genotype than the controls, but their study is restricted to steroid sensitive patients and recommended studies comparing genotype frequency with steroid resistant patients for understanding the influence of ACE genotypes in steroid responsiveness. Understanding the mechanism of action of steroids in reducing the proteinuria may throw light on response to steroids and ACE inhibitors in nephrotic syndrome (Vegter *et al*., 2009).

**Fig(30)**. ACE Gel illustrating homozygous *DD*, homozygous *II* and heterozygous *ID* genotype.



**Table 2:**.**Distribution of genotypes in patient and control groups.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Category** | **ACE Genotypes (%)** | | | **Allele frequency** | | **Comparison of groups** | **OR**  **(95% CI)** | ***P*-value** |
| **II** | **ID** | **DD** | **I** | **D** |
| **Patients**  **N=25** | **5**  **(20)** | **10**  **(40)** | **10**  **(40)** | **0.40** | **0.60** | **D *vs.* I** | **2.25** | **0.007\*** |
| **Controls**  **N=25** | **11**  **(44)** | **8**  **(32)** | **6**  **(24)** | **0.60** | **0.40** | **DD *vs.* Others** | **2.11** | **0.363** |
|  |  |  |  |  |  | **DD *vs.* II** | **1.41** | **0.768** |
|  |  |  |  |  |  | **DD *vs.* ID** | **0.79** | **1** |

**Table 3: Distribution of ACE I/D genotypes among mild and severe forms of histopathology.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Histopathology**  **N=25** | **ACE Genotypes (%)** | | | **Allele frequency** | | **Comparison of groups** | **OR**  **(95% CI)** | ***P*-value** |
| **II** | **ID** | **DD** | **I** | **D** |
| **Severe**  **N=7** | **2**  **(28.5)** | **3**  **(42.85)** | **2**  **(28.5)** | **0.5** | **0.5** | **D *vs.* I** | **1.77** | **0.06** |
| **Mild**  **N=18** | **3**  **(16.66)** | **7**  **(38.88)** | **8**  **(44.4)** | **0.64** | **0.36** | **DD *vs.* Others** | **0.5** | **0.65** |
|  |  |  |  |  |  | **ID *vs.* Others** | **1.17** | **1** |
|  |  |  |  |  |  | **II *vs.* Others** | **2** | **0.65** |

**Table 4. Distribution of ACE I/D genotypes among Steroid Sensitive and Non-Steroid Sensitive patients.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Responsiveness**  **N=25** | **ACE Genotypes (%)** | | | **Allele frequency** | | **Comparison of groups** | **OR**  **(95% CI)** | ***P*-value** |
| **II** | **ID** | **DD** | **I** | **D** |
| **SS**  **N=7** | **3**  **(48.2)** | **2**  **(28.5)** | **2**  **(28.5)** | **0.57** | **0.43** | **D *vs.* I** | **2.65** | **0.001\*** |
| **Non-SS**  **N=18** | **2**  **(11.1)** | **8**  **(40.4)** | **8**  **(40.4)** | **0.33** | **0.66** | **DD *vs.* Others** | **2** | **0.65** |
|  |  |  |  |  |  | **ID *vs.* Others** | **2** | **0.65** |
|  |  |  |  |  |  | **II *vs.* Others** | **0.16** | **0.11** |

**Conclusions**

In conclusion, in the present study ACE *I/D* genotypes do not significantly influence the susceptibility, progression and drug response variation in INS of South Indian population. But a similar study with increased sample size may give useful information which may help the clinicians to predict the course of disease and to identify individuals with better prognosis to standard steroid treatment. Not many studies are cited in literature on the role of ACE *I/D* genotypes and steroid responsiveness, but the ones that are available have limited sample size. However the present study dealt with comparison of SSNS and SRNS groups to understand the genotypic distribution or the ACE I/D allele involvement. In order to delineate the role of different genotypes on the above aspects, its influence at the physiological level has to be studied with large sample size in different ethnic groups.

**References**

1. Doria , A. , J. Warram and A. Krolewski, 1994. Genetic predisposition to diabetic nephropathy. Evidence for a role of the angiotensin I-converting enzyme gene. Diabetes,43:690-695.
2. Dudley, J., E. Afifi, A. Gardner , E. J. Tizard and M.E. McGraw, 2000. Polymorphism of the ACE gene in Henoch-Schonlein purpura nephritis .Pediatric Nephrol.,14:218-220.
3. Fahmy, M.E., Fattouh , R.A. Hegazy and M.L. Essawi , 2008. ACE gene polymorphism in Egyptian children with idiopathic nephrotic syndrome . Brastisl lek listy , 109:298-301.
4. Hohenfeller, K., A. M. Wingren, O. Nauroth, E. Wuhl, O. Mehls and F. Schaefer , 2001. Impact of ACE I/D gene polymorphism on congenital renal malformation. Pediatric Nephrol.,16:356-361
5. Hori, C., M Hiraoka ,N. Yoshikawa, k. Tsuzuki and Y. Yoshida et al., 2001 Significance of ACE genotypes and medical treatments in childhood focal glomerulosclerosis .nephron.88:313-319
6. Jeffers, B. W., R. O. Estacio, M. V. Raynolds and R. W. Schrier, 1997 Angiotensin converting enzyme gene Polymorphism in non-insulin dependent diabetes mellitus and its relationship with Diabetic nephropathy . kidney Int., 52: 473-477.
7. Lee, D. Y. ,W. Kim, S. K. Kang, G. Y. Koh and S. K. park ,1997. Angiotensin – converting enzyme gene polymorphism in patients with minimal – change nephrotic syndrome and focal segmental glomerulosclerosis .Nephron 77:471-473
8. Mesbah K , Joshi RL, Lamothe B, Cordonnier N, , Monthioux E, Jami J, Bucchini D. Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality. EMBO J vol. 15, p. 1542-7, 1996.
9. Miller, S. A., D. D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleate cells. Nucleic Acids Res., 16:1215-1215.
10. NG, D., S.N.B. Ramli, K.S. Chia, D. Koh and B.C. Tai, 2009. Genetic variation at the angiotensin -1 converting enzyme locus and the risk for diabetic nephropathy : A study based on 53studies and 17791 subjects .salud I Ciencia, 16:751-754.
11. Patil, S,J., S. Gulati , F. Khan, M. Tripathi, M. Ahmed and S. Agrawal, 2005. Angiotensin converting enzyme gene Polymorphism in indian children with steroid sensitive nephrotic syndrome .Indian J. Med. Sci., 59:431-435.
12. Schmidt, S., N. Schone and E. Ritz, 1995b. The diabetic nephropathy study group. Association of ACE gene polymorphism and diabetic nephropathy? Kidney Int., 47:1176-1181.
13. Serdaroglu , E., S. Mir, A Berdeli , N Aksu and M. Bak , 2005. ACE gene insertion /deletion polymorphism in childhood idiopathic nephrotic syndrome . Pediatric Nephrol ., 20:1738- 1743
14. Shanmugam, V., K. W. Sell and B. K. Saha, 1993. Mistyping ACE heterozygotes. Genome Res.,3:120-121.
15. Tsia, I. J., Y.H. Yang Y. H. Lin, V.C. Wu, Y.K. Tsau and F.J. Hsieh ,2006. Angiotensin converting enzyme gene Polymorphism in children with idiopathic nephrotic syndrome.Am. J. Nephrol.,26;157-162
16. Vetgter , S. , A. Perna , W. Hiddema ,P. Ruggenenti , G. Remuzzi , G. Navis and M.j. Postma, 2009. Cost-effectiveness of ACE inhibitor therapy to prevent dialysis in nondiabetic nephropathy: Influence of the ACE insertion / deletion polymorphism .Pharmacogenet. Genomic, 19:695-703.
17. Yoshioka K, Hiraoka M, Yoshikawa N, Tsuzuki K, Yoshida Y, Fujisawa K. *et al*. Significance of ACE genotypes and medical treatments in childhood focal glomerulosclerosis. Nephron. 2001;88(4):313–319