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Does Disease Status and Steroid Responsiveness in Idiopathic Nephrotic Syndrome Depend on ACE I/D Gene Polymorphism? A Study from South India

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ABSTRACT

Idiopathic Nephrotic Syndrome (INS) is a malfunction of the kidney glomerular filter that leads to proteinuria, hypoalbuminemia, edema and renal failure. The cause of proteinuria in INS is an injury to the function or structure of glomerular filtration barrier. The present study addresses the role of I/D gene polymorphism of Angiotensin Converting Enzyme (ACE), a pleiotropic molecule on the susceptibility, progression and steroid response variation of INS in South Indian population. For this study, a total of 418 subjects were recruited; 218 clinically proven cases of INS without any secondary reasons for renal problems and 200 healthy controls. Association of ACE I/D gene polymorphism with disease susceptibility, renal histological findings and response to steroid treatment was evaluated. Three significant observations were made (1) Elevated frequency of DD in patients compared to controls (37.6% vs. 17.5%, p = 0.0001) (2) Higher I allele frequency in milder form than in severe form of INS (0.52 vs. 0.29, p = 0.0001) and (3) Patients with ID genotype were 3 times more resistant to steroid treatment compared to other genotypes (z-value = 3.45, p<0.0006). ACE I/D genotypes significantly influences the susceptibility, progression and drug response variation. This information may help the clinicians to predict the course of disease and to identify individuals with better prognosis to standard steroid treatment. In order to delineate the role of different genotypes on the above aspects, its influence at the physiological level is to be studied with large sample in different ethnic groups. This is the first Indian study pertaining to primary nephrotic syndrome dealing with ACE influence on susceptibility, severity and steroid response cumulatively.

Key words: Angiotensin converting enzyme, nephrotic syndrome, steroid response

INTRODUCTION

Kidneys excrete waste products of metabolism and play an important role in maintaining the homeostasis by regulating the body water and solute balance. In addition they also participate in endocrine regulation (Al-Omireeni et al., 2011). Chronic Kidney Disease (CKD) is the major cause of morbidity, mortality and represents an important health problem worldwide. In addition to medical problem, CKD is a huge social and economic problem that needs to be addressed as the cost of dialysis and kidney transplantation is increasing over time (Mortazavi and Rafiee, 2010).

Idiopathic Nephrotic Syndrome (INS) is a malfunction of the kidney glomerular filter and is one of the commonest causes of renal problems in children encountered by nephrologists (Braden *et al.*, 2000). The characteristic features of INS are heavy proteinuria (>40 mg m^{-2} h^{-1}), hypoalbuminemia (<2.5 g dL^{-1}), edema and hyperlipidemia (Saber *et al.*, 2007). The cause of proteinuria is due to injury to the function or structure of glomerular filtration barrier. The estimated annual incidence of INS ranges from 2-7 per 100,000 children and prevalence from 12-16 per 100,000 worldwide. There is epidemiological evidence of a higher incidence of INS in children from South Asia (McKinney *et al.*, 2001). It appears to be a clinically heterogeneous condition characterized by histological variants (Border, 1988; Korbet, 1998; Myllymaki *et al.*, 2003) and different genetic backgrounds (Lenkkeri *et al.*, 1999; Boute *et al.*, 2000; Kaplan *et al.*, 2000) with recessive and dominant inheritances. For these patients the therapeutic option is a standard steroid therapy and on the basis of the patient's response to steroid therapy, it is divided into Steroid-sensitive Nephrotic Syndrome (SSNS) and Steroid-resistant Nephrotic Syndrome (SRNS) which may progress to End-stage Renal Disease (ESRD) (Ruf *et al.*, 2004). It is the final result of various etiologies and prognostic indicators leading to ESRD have been extensively studied, of which genetic factors remain a subject of great concern (Uddin *et al.*, 2007).

In INS the underlying histopathology can be Minimal Change Disease (MCD), Membranous Nephropathy (MGN), Mesangioproliferative Nephropathy (MPGN) or Focal Segmental Glomerulosclerosis (FSGS). MCD is milder with minimum abnormalities of the glomeruli whereas FSGS is the most severe form causing collapse and scarring of some glomeruli while others are intermediate forms (International Study of Kidney Disease in Children, 1981). According to one report rate of apoptosis is significantly higher in patients with FSGS (69%) compared to those with MCD (Kamel *et al.*, 2009). Explanation resides in the fact that apoptosis is highly integrated in the pathogenesis of sclerotic lesions. Schiffer *et al.*, (2001) stated that podocyte depletion leading to podocyte insufficiency and capillary collapse have been invoked as important steps in the development of FSGS. High oxidative stress associated with ACE activity has been suggested to lead to nephropathy as the antioxidant defense systems are overloaded (Ghazi *et al.*, 2009).

ACE gene has 26 exons and spans 21 kb on chromosome 17q23 (Mattei *et al.*, 1989). The human ACE gene contains a number of variable polymorphic regions that can be of potential use in genetic analysis of populations (Reider *et al.*, 1999). The Insertion/ Deletion (I/D) polymorphism, rs1799752 (Gard, 2010), present in intron 16, in particular has been extensively investigated (Howard *et al.*, 1990). I/D polymorphism consists of the presence (Insertion-I) or absence (Deletion-D) of 287 bp Alu repeat element in intron 16 resulting in 3 genotypes viz, Insertion homozygote (I/I), Insertion/Deletion heterozygote (I/D) and Deletion homozygote (D/D). The ACE I/D polymorphism are associated with overall plasma ACE levels (Rigat *et al.*, 1990). Individuals homozygous for the D allele are characterized by elevated plasma levels of ACE compared with individuals homozygous for I allele. ACE is a key enzyme that converts inactive angiotensin I into a vasoactive and aldosterone-stimulating peptide angiotensin II. High angiotensin II levels make deleterious effects on renal haemodynamics, induces the expression of various growth factors, secretion of extracellular matrix, inflammatory events that play a key role in glomerulosclerosis leading to progression of renal diseases (Egido, 1996). The objective of the current study was to investigate the role of ACE I/D gene polymorphism on the susceptibility, progression and steroid response in INS from South Indian population.

MATERIALS AND METHODS

For the present study, blood samples were collected from a total of 418 subjects, 218 (146 males and 72 females) were clinically proven cases of INS fulfilling criteria of the International Study of Kidney Disease in Children for the diagnosis of INS without any secondary reasons for renal problem visiting Nephrology Department, NIMS hospital, Hyderabad, India and 200 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 15±11 years whereas for the control group it was 32±9 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 218 patients. The follow up clinical data to evaluate steroid responsiveness was obtainable from 180 cases. Based on the underlying histopathology MCD could be termed as mild and MPGN+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 418 subjects in our laboratory using standard salting out method (Miller *et al.*, 1988). ACE I/D genotyping was performed as given elsewhere (Surekha *et al.*, 2011) and to avoid mistyping of ID genotype as DD, 5% DMSO was utilized. (Shanmugam *et al.*,1993). The gel was analyzed on a gel documentation unit (Bio-Rad), PCR product of 490 bp indicate homozygous for Insertion (II), 190 bp indicates homozygous for Deletion (DD) and presence of both 490 and 190 bp indicate heterozygosity (ID) (Fig. 1).

Comparisons of the data among different categories were performed by Chi-square test, Odds ratio and Z test with a 95% confidence interval. The p-value <0.05 was considered significant. Hardy-Weinberg equilibrium was evaluated by χ^2 test for allelic frequency in patient and control group.
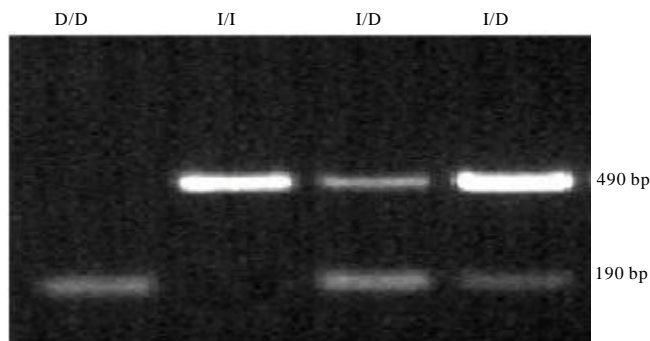

Fig. 1: Agarose gel illustrating homozygous DD, homozygous II and heterozygous ID genotype

RESULTS

The total study population comprised of 418 individuals, 218 INS patients and 200 controls. Male to female sex ratio of patients was 2:1 and the mean age was 15±11 years. The distribution of II, ID and DD genotypes in patients (n = 218) was 20.2, 42.2 and 37.6%, whereas, in the controls (n = 200), 43, 39.5 and 17.5%, respectively (Table 1). DD genotype was predominant in INS group (p = 0.0001). An odds ratio of 2.84 was obtained for DD vs. ID+II at 95% confidence interval.

Analysis of ACE I/D genotypes in different histopathological conditions of INS revealed 13, 33.33, 53.6 and 28.2, 48.3, 23% of II, ID and DD genotypes in severe and mild forms, respectively. An odds ratio of 3.76 was obtained for DD vs. ID+II at 95% confidence interval with p = 0.0001. Further D allele frequency was high in severe form than in mild form (0.7 vs. 0.47, OR = 2.67, p = 0.001) (Table 2). However, it did not deviate from Hardy Weinberg Equilibrium.

Distribution of ACE I/D genotypes II, ID and DD in SS and Non-SS groups was observed to be 18.4, 15.8, 67.5 and 17.3, 40.4, 42.3% correspondingly. With respect to steroid responsiveness DD homozygote behaved differently from that of ID heterozygote (DD vs. others: OR = 2.26, p = 0.002; ID vs. others: OR = 0.27, p = 0.0006). Elevated frequency of D allele (0.74 vs. 0.625) was observed in SS group compared to Non-SS group, however, did not reach to significance (p = 0.085) (Table 3).

Table 1: Distribution of genotypes in patients and controls

Category	ACE genotypes (%)			Allele frequency		Comparison of groups	OR (95% CI)	z statistic	p-value
	II	ID	DD	I	D				
Patients									
N = 218	44 (20.2)	92 (42.2)	82 (37.6)	0.41	0.58	D vs. I	2.469	3.06	0.002
Controls									
N = 200	86 (43)	79 (39.5)	35 (17.5)	0.61	0.39	DD vs. others	2.842	4.49	0.0001
						DD vs. II	4.58	5.55	<0.0001
						DD vs. ID	2.27	3.43	0.0006

Table 2: Distribution of ACE I/D genotypes among mild and severe forms of histopathology

Histopathology	ACE genotypes (%)			Allele frequency		Comparison of groups	OR (95% CI)	z statistic	p-value
N = 218	II	ID	DD	I	D				
Severe									
N = 69	9 (13)	23 (33.3)	37 (53.6)	0.29	0.70	D vs. I	2.67	3.28	0.001
Mild									
N = 149	42 (28.2)	72 (48.3)	35 (23.4)	0.52	0.47	DD vs. others	3.76	4.28	0.0001

Table 3: Distribution of ACE I/D genotypes among steroid sensitive and non-steroid sensitive patients

Responsiveness	ACE genotypes (%)			Allele frequency		Comparison of groups	OR (95% CI)	z statistic	p-value
N = 180	II	ID	DD	I	D				
SS									
N = 76	14 (18.4)	12 (15.7)	50 (67.5)	0.29	0.70	D vs. I	1.69	1.71	0.085
Non-SS									
N = 104	18 (17.3)	42 (40.4)	44 (42.3)	0.52	0.47	DD vs. others	2.26	3.08	0.002
						ID vs. others	0.27	3.45	0.0006

DISCUSSION

The ACE DD genotype is associated with the largest amount of angiotensin converting enzyme and angiotensin II which has hemodynamic, growth and prosclerotic effects (Yoshida *et al.*, 1996). It is suggested that, DD genotype acts as a predictor of progressive glomerulosclerosis in diabetic nephropathy (Doria *et al.*, 1994; Schmidt *et al.*, 1995a; Jeffers *et al.*, 1997), IgA nephropathy (Schmidt *et al.*, 1995b; Yoshida *et al.*, 1995; Syrjanen *et al.*, 2000) and other chronic renal diseases (Yoshida *et al.*, 1996; 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 INS patients.

ACE I/D gene polymorphisms in the susceptibility to INS: The current study dealt with distribution of ACE I/D gene polymorphism in INS and normal individuals of South Indian population. Analysis showed that DD vs. ID+II comparison among the two groups were significantly different ($p = 0.0001$) and clearly ascertain that DD genotype is a high risk genotype as the OR value was found to be as high as 2.84 (95% CI $p = 0.0001$). DD genotype with double dose of D allele almost have five fold increased risk of developing INS (DD vs. II, OR = 4.58, $p<0.0001$) compared to II homozygotes which has no D allele. The genotype ID with a single dose of D allele exhibited more than two fold increased risk compared to II (ID vs. II, OR = 2.27 $p = 0.0006$). This shows a graded risk of DD>ID genotypes to INS which may be correlated with the ace levels and suggest that D allele can be 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.*, 1995a; Jeffers *et al.*, 1997; Uddin *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).

ACE I/D gene polymorphisms in relation to severity of INS: When patients were categorized on the basis of histopathology (methodology section) and analyzed for the distribution of ACE I/D genotypes, II homozygotes were significantly predominant in the mild form and DD homozygotes in the severe form. This is in accordance to the earlier reports from Japan and Korea (Lee *et al.*, 1997; Hori *et al.*, 2001). It was stated that, intrarenal concentration of angiotensin II in the individuals with ACE DD genotype was 1,000 times more than that of the concentration in plasma which may increase the intraglomerular pressure, induces transforming growth factor (TGF-β) to exert a prosclerotic activity leading to glomerular sclerosis (Wolf *et al.*, 1993; Egido, 1996; El-Mesallamy *et al.*, 2008), thus a cause for progression of MCD to FSGS in DD homozygotes. Elevated levels of IFN-γ as a consequence of TGF-β signalling have been reported to cause diabetic nephropathy (Nosratabadi *et al.*, 2009).

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 ID individuals are 3 times more resistant to steroids compared to other genotypes ($z = 3.45$, $p = 0.0006$), whereas, DD individuals are almost two times more sensitive to steroids ($z = 2.26$, $p = 0.0021$). II homozygotes were represented equally in both the groups indicating II homozygous condition does not influence steroid responsiveness, thus providing an appropriate example to cite how differently homozygotes

and heterozygotes behave. The involvement of ID genotype with steroid resistance may be due to unexplained allelic interactions among I and D allele. Similar results were obtained by a Turkish study (Celik *et al.*, 2006). 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 contrary to our results; however, their sample size was smaller compared to the present study and also belonged to a different ethnic group. In addition, one study from North India by 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).

In conclusion ACE I/D genotypes do significantly influence the susceptibility, progression and drug response variation in INS of South Indian population. This information 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.

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