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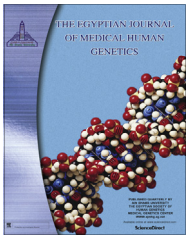

ORIGINAL ARTICLE

ACE DD genotype associated with the female Chronic Kidney Disease patients of Tamilnadu population

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Abstract *Background:* The Renin-Angiotensin System (RAS) is an important regulator for blood pressure and kidney disease. The level of vaso active peptide Angiotensin-II is mainly determined by the RAS enzyme angiotensin converting enzyme-1 (ACE-1).

Aim: To investigate the association of ACE I/D polymorphism and Chronic Kidney Disease (CKD) in south India.

Methods: In the present study, we have collected CKD patients (n = 147) and control subjects (n = 211) from Tamilnadu. Genotyping was carried out by polymerase chain reaction (PCR) on the basis of allele specific primers.

Results: The DD genotype is associated with the female population (OR-CI = 2.40 (1.05–5.51), p = 0.04) as compared to the male population (OR-CI = 0.75 (0.37–1.51), p = 0.42). Further, we found the over representation of "I" – allele (homozygous II and heterozygous ID) in unaffected males [OR (CI) – 0.58 (0.32–1.04), p = 0.07] which suggests its protective role in male population.

Conclusion: The DD genotype of ACE is associated with CKD in south India.

1. Introduction

Chronic Kidney Disease (CKD) is an International public health problem which is encompassing large phenotypes. Each phenotype is the combination of an underlying kidney disease and superimposing environmental and genetic factors. The CKD is a polyphonic and complex disorder which results from

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gene–gene and gene–environmental interactions. The disease pathogenesis of renal failure is well correlated with genetic variability [1]; these findings highlight the importance of renal disease and its complications. Hypertension and CKD are closely associated diseases [2] which show several clinical complications like elevated blood pressure, poor renal outcome via pressure natriuresis mechanism [3,4]. Hypertension and diabetes are the major causes for CKD and it is common among different populations *viz*. African American, Hispanic, Asian American and Indian [5,6]. The CKD has also been observed very commonly among female population when compared to male population [7,8].

The Renin-Angiotensin System (RAS) is a key regulator of blood pressure and kidney function. The contribution of the RAS system for hypertension is well documented but the role of RAS in CKD still remains unclear. However, different types of RAS blockers i.e. both angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers have been suggested to CKD patients in order to prevent severe kidney damage [9]. Treatment with RAS inhibitors have been differed for major inter individual differences and still it is questionable to predict responders based on known pathophysiological characteristics [10]. Thus, different polymorphisms in RAS components are the major factors to contribute for its heterogeneous association among CKD patients [11].

In RAS pathway angiotensin converting enzyme-1 (ACE-1) is an important enzyme which determined the vasoactive peptide Angiotensin-II. The plasma and tissue ACE levels may also be determined by ACE insertion/deletion polymorphism. Insertion (I) or deletion (D) of a 287 bp fragment in the 16th intron of ACE gene is mainly related to kidney problems among the hypertensive patients [12]. Moreover, the D allele has played a vital role in the failure of the renoprotective action of ACE inhibitors [13,14].

The goal of the present study is to assess the role of ACE I/D polymorphism in CKD patients of Tamilnadu population. We explored the association between ACE I/D polymorphism and CKD in Tamilnadu population between the age group of 30 and 70 years.

2. Subjects and methods

2.1. Study population

A total of 147 CKD patients and 211 control subjects were included in this study. This work was started after the approval of Institutional Ethics Committee. Blood samples (5 ml) were collected from CKD patients and healthy volunteers between the age group of 30–70 years from the clinics after obtaining the informed consent form from the participants. All the cases included in this study are under CKD stages (II–V) as diagnosed and identified by the nephrologists. Individuals with normal kidney function with matched age, sex and location were selected as controls. All the necessary clinical data and family history were recorded in the questionnaire for all the participants. All the participants belonged to the Dravidian ancestry living in Tamilnadu, south Indian population. Control samples were also collected from the volunteers of same ethnicity. Genomic DNA was extracted using modified Miller's protocol [15] and it was quantified spectrophotometrically by OD_{260}/OD_{280} ratio. Genotyping was carried out with the allele specific primers method. Primers 5′GCCCTGCAGGTG TCTGCAGCATGT-3′ (sense primer) and 5′GGATGGCTCT CCCCGCCTTGTCTC-3′ (antisense primer) produced 319 and 597-bp amplicons for D and I alleles, respectively. The primers were 5′-TGGGACCACAGCGCCCGCCACTAC-3′ (sense primer) and 5′-TCGCCAGCCCTCCCATGCCCAT AA-3′ (antisense primer). The reaction yields a 335-bp amplicon only in the presence of an 'I' allele and no product in samples homozygous for DD [16].

2.2. Statistical analysis

Chi-square statistics was used to compare the differences in ACE genotype frequencies between case and control subjects. The strength of the association between genotype frequencies was calculated by the odds ratio (O) and 95% confidence interval (CI). Statistically significant accepted value was $p < 0.005$. Hardy–Weinberg equilibrium (HWE) was used to test the frequencies of the marker alleles by allele counting method. All the statistical calculations were carried out using PLINK 1.07 [17] and STATA 11.0.

3. Results

Association studies between genetic polymorphisms are hallmark for detecting the complex diseases. However, studying the relationship between allele and genotype frequencies of candidate genes in the affected and unaffected subjects to understand the genetic etiology of complex human traits is an efficient method for molecular dissection of the disease pathogenesis. Based on these, we determined the possible association between ACE I/D polymorphism and CKD patients among the south Indian subjects.

Among the patients ($n = 147$), 100 (68.02%) are males and 47 (31.98%) are females, whereas in control subjects 130 (61.61%) are males and 81 (38.39%) are females. The mean age of patients is 53.8 ± 12.3 years for male patients and 54.40 ± 10.42 years for female patients. The mean age of control subjects ($n = 211$) is 43.71 ± 14.17 years and 43.90 ± 13.57 respectively for males and females (Table 1).

Genotype distributions of ACE I/D polymorphism were compatible with Hardy–Weinberg equilibrium (HWE) expectation in cases and control subjects. D allele is more prevalent in cases than controls among the allele/genotype frequency distributions which are shown in Tables 2 and 3. I is the minor allele and D is the major allele.

Genotype, allele frequencies and odds ratio were calculated for the variants to test the association of ACE I/D gene polymorphism with CKD. Results are reported in Table 3.

Table 1 Basic characteristics (mean, standard deviation) of study sample.

	Cases ($n = 147$)	Controls ($n = 211$)
Male	100	130
Female	47	81
Age (years), male	53.9 ± 12.3	43.7 ± 14.1
Age (years), female	54.4 ± 10.4	43.9 ± 13.5

N: sample size.

Table 2 Distribution of allele frequencies in cases and controls stratified gender wise.

SNP	Gender	Minor allele	Freq. allele case	Freq. allele control	χ^2	OR	95% CI	*P*-value
ACE I/D	All	I	0.52	0.46	2.09	1.25	0.72–2.22	0.15
	Male		0.47	0.49	0.78	0.85	0.58–1.73	0.38
	Female		0.49	0.42	1.17	1.33	0.76–2.32	0.28

χ^2: Chi-square with 1 degree of freedom; OR: odds ratio.

Table 3 Distribution of genotype frequencies of RAS gene polymorphisms in patients and control subjects.

SNP	Gender	Genotype	Case% (n = 147)	Control% (n = 211)	OR	95% CI	*P*-value
ACE I/D	All	II	30.61 (45)	24.17 (51)	Ref		
		ID	42.17 (62)	44.07 (93)	1.32	0.79–2.21	0.285
		DD	27.21 (40)	31.75 (67)	1.48	0.84–2.59	0.172
	Male	II	28 (28)	25.38 (33)	Ref		
		ID	38 (38)	51.53 (67)	1.50	0.79–2.84	0.219
		DD	34 (34)	23.07 (30)	0.7487	0.37–1.51	0.420
	Female	II	23.40 (11)	25.93 (21)	Ref		
		ID	51.06 (24)	32.10 (26)	0.75	0.37–1.51	0.420
		DD	25.53 (12)	41.98 (34)	2.40	1.05–5.51	0.038*

N: sample size; OR: odds ratio; CI: confidence interval; Ref: reference.
* $P < 0.05$: statistically significant.

Table 4 Distribution of ACE I/D polymorphisms (dominant and recessive model) in patients and control subjects.

SNP	Gender	Model	TEST	Case (N = 147)	Control (N = 211)	OR	95% CI	*P*-value
ACE I/D	All	II + ID Vs DD	DOM	107/40	144/67	1.24	0.78–1.98	0.356
		II Vs ID + DD	REC	45/102	51/160	1.38	0.86–2.21	0.176
	Male	II + ID Vs DD	DOM	66/34	100/30	0.58	0.32–1.04	0.070*
		II Vs ID + DD	REC	28/72	33/97	1.14	0.63–2.06	0.656
	Female	II + ID Vs DD	DOM	35/12	47/34	2.11	0.96–4.65	0.062*
		II Vs ID + DD	REC	11/36	21/60	0.87	0.38–2.02	0.751

DOM: dominant model; REC: recessive model; N: sample size; OR: odds ratio; CI: confidence Interval; DOM model: when DD is present only, it will cause diseases, REC model: when D is in homozygous or heterozygous it will cause diseases.
* $P < 0.05$: statistically significant.

3.1. ACE I/D polymorphism

We could not find a significant association for the ungrouped data of ACE I/D polymorphism. However, when we segregated into male and female population, we found that DD homozygous has a significant effect among females. The distribution of DD genotype did not differ significantly between CKD patients and controls. Slight increase in the frequency of DD (31.75%) homozygous was observed among patients when compared to control subjects (27.21%) (Table 3). The presence of "I" – allele carriers (homozygous DD and heterozygous ID) in case and control subjects (Table 3) suggests its protective role in male population. In Table 4, the distribution of DD genotype is more dominant (both cases and controls) when compared to ID and II genotype based on trend estimation. This association was estimated as odds ratio 0.58 with 95% CI 0.32–1.04 for male population and as odds ratio 2.11 with 95% CI 0.96–4.65 for female population (Table 4).

We estimated the genetic power using the ACE I/D polymorphism as an example; an 80% power should have to detect the linkage between CKD and D allele at type I error of 0.05 when the sample includes 147 cases and 211 controls. We also performed post hoc exploratory analyses to examine the relationships of the polymorphisms with cases and control subjects. Genetic power estimation showed that 147 cases and 211 controls had >80% power to detect the linkage between ACE I/D variant and CKD in the south Indian population.

4. Discussion

CKD is associated with many major cardiac and osteoporosis complications [18]. It mainly affects older population (>50 years) [19]. Therefore the mean age for male CKD subjects is 53.90 ± 12.33 and for females is 54.40 ± 10.42 in our study population. We could not find significant changes among the studied population [OR (CI) = 1.25 (0.72–2.22), p = 0.15]. This result was consistent with previous investigations in different parts of the world *viz*. UK [20], Denmark [21], Germany [22] and Japan (χ^2 = 1.63, p = 0.44) [23]. "I" allele is the minor allele in our case with OR (CI) = 1.25

(0.72–2.22). This result was similar to the already reported in Asian individuals [OR (CI) = 1.40 (1.23–1.59)] and Caucasian individuals [OR (CI) = 1.12 (1.04–1.21)] [24].

We found that DD genotype is associated with female patients. Similar observations were also reported in different populations. In 2010, Mansoor et al., [25] have reported that the ACE I/D polymorphism is associated with female population [OR (CI) = 0.81 (0.52–1.26) and *p* value 0.35] rather than male population [OR (CI) = 1.95 (0.99–3.85) and *p* value 0.05]. In another study by Pinon et al. [26] found that the DD genotype is the risk factor for the development of renal disease. Further, a meta-analysis from Lin et al. [24] stated that the gender dependent effect of the ACE I/D polymorphism has been observed very commonly among different populations. Thus, we segregated to male and female populations and the DD genotype was found to be the major risk determinants of CKD among female (OR = 2.40) population. This result was also supported by previous results from France [27] and Mexican population [26]. Furthermore, recently our group [28] reported that female is more prone to hypertension after menopause in Tamilnadu population. Most of the females in our studied population are post-menopausal women. Based on these observations we hypothesized, the DD genotype is associated with increased activity of the ACE and subsequent CKD among female population.

Our study has few limitations: (i) The study was conducted with low number of sample size. (ii) We included one kidney center in our study; More CKD patients from different kidney centers can be included for better interpretation for the role of ACE I/D polymorphism or the progression of CKD. (iii) The CKD has been caused by different diseases such as hypertension, diabetes and diabetic nephropathy. The heterogeneity of the CKD is also one of the limitations in our study. Moreover there are no measurements available to calculate the ACE levels in order to correlate with ACE I/D polymorphisms in this study. However, the samples are from homogenous genetic background, therefore they may not be affected by unmeasured confounding factors of population stratification.

In conclusion, DD genotype is significantly more prevalent in the CKD females than in general Tamilnadu population, suggesting the deteriorative effect of the genotype on CKD progression in females only.

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