Aldosterone Synthase *CYP11B2* Gene Promoter Polymorphism in a Turkish Population With Chronic Kidney Disease

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Introduction. It has been shown that gene polymorphisms influence the development and progression of chronic kidney disease (CKD). Many studies have indicated that aldosterone synthase *CYP11B2* gene polymorphism (-344C>T) influences the aldosterone level, urinary aldosterone excretion, blood pressure, and left ventricular size and mass. We aimed to investigate whether there is an effect of *CYP11B2* -344 C>T polymorphism on the development of CKD in a Turkish population.

Material and Methods. A total of 240 patients with stage 5 CKD and 240 age- and sex-matched healthy individuals were included in the study. Genotyping of *CYP11B2* gene -344 T>C promoter polymorphism was carried out using polymerase chain reaction and restriction fragment length polymorphism methods.

Results. No significant differences were found in the genotype distribution of *CYP11B2* -344 C>T polymorphism between the patients and controls; however, -344 C>T polymorphism was significantly more frequent among the CKD patients with diabetes mellitus as compared to those with it (P = .02). Diabetic CKD patients with TC genotype had a 2-fold increased risk for development of the disease than the CKD patients without diabetes mellitus (odds ratio, 2.21; 95% confidence interval, 1.04 to 4.67).

Conclusions. Our study suggests that the *CYP11B2* gene -344 C>T polymorphism may have an effect on the development of CKD in diabetic patients.

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INTRODUCTION

Chronic kidney disease (CKD) has become a major public health problem. According to data from the National Health Insurance Corporation, CKD ranked first in the public health expenditure.[1,2] The disease requires long and the costly treatment. Chronic kidney disease is classified into 5 stages. The stage 5 CKD is described as end-stage renal disease (ESRD) and is associated with significant morbidity and mortality. Glomerulus filtration rates of patients at this stage are reduced to less than 15 mL/min/1.75 m^2, so these patients need renal replacement therapies such as dialysis or transplantation.[3] Hypertension, diabetes mellitus, and obesity have been observed as considerable leading etiologic factors for the development of ESRD.[4] In addition, gene polymorphisms of the renin-angiotensin-aldosterone system (RAAS) have also been shown in various studies to influence the development and progression of kidney disease.[5,6]

The aldosterone synthase, which is encoded by aldosterone synthase gene (*CYP11B2*), is a

cytochrome P450 enzyme. The terminal steps of aldosterone hormone synthesis in human adrenal zona glomerulosa cells are catalyzed by this enzyme.[7] The activity of the mineralocorticoid hormone may be influenced by various polymorphisms on the aldosterone synthase gene.[8] *CYP11B2* promoter polymorphism which occurs at -344 position of the gene by cytosine-thymine substitution is located in the regulatory region of the gene, which is a putative binding site for the steroidogenic factor 1. When the T-allele's in vitro affinity for steroidogenic factor 1 is compared to the C allele's, T allele has 5 times lower affinity than that of the C allele. Although, the polymorphism has no apparent impact on the transcriptional regulation of *CYP11B2*,[9] several studies have informed that *CYP11B2* polymorphism (-344 C>T) has influenced the serum aldosterone level,[10] urinary aldosterone excretion,[11] blood pressure,[12] and left ventricular size and mass.[13] Although there were many studies on the association of the gene polymorphism and kidney disease, results were incoherent.[5,7,14,15]

There is no study to show the relationship of the *CYP11B2* promoter polymorphism in neither patients with CKD nor patients with ESRD in any Turkish population. In this study, we aimed to investigate whether there was an effect of *CYP11B2* gene polymorphism (-344 C>T) on the development of CKD in a Turkish population.

MATERIALS AND METHODS

Study Population

A total 240 patients with ESRD on maintenance hemodialysis for a mean duration of 4.6 ± 4.3 years were recruited from several dialysis centers located in the Sivas city. A same number of age- and sex-matched unrelated healthy individuals without any known chronic disease history (such as hypertension, type 2 diabetes mellitus, and renal and cardiac diseases) were selected as a control group. A written informed consent was obtained from both study and control groups. The study protocol was approved by the local university ethics committee (2012/04/13).

Genomic DNA Extraction

One milliliter of peripheral blood samples were collected into ethylenediaminetetraacetic acid-containing tubes from both patients and controls. Total genomic DNA extraction was performed from 100 µL of whole peripheral blood by using an Invitek kit extraction technique (Invisorb spin blood; Invitek, Berlin, Germany), according to manufacturer's instructions, and was stored at -20°C until analyzed.

Genotyping

Genotyping of *CYP11B2* (-344 T>C) gene promoter polymorphism was carried out using polymerase chain reaction and restriction fragment length polymorphism methods. Polymerase chain reactions were made in a reaction volume of 25 mL, containing 10 pmol of forward primer (5′-CAGGAGGAGACCCCATGTGAC-3′) and reverse primer (5′-CCTCCACCCTGTTCAGCCC-3′),[16] 5 nmol each of four deoxynucleotide triphosphates (Fermentas), 1 unit of Taq DNA polymerase (Fermentas), 10 mmol/L of Tris-hydrogen chloride (pH, 8.3 at 25°C), 50 mmol/L of potassium chloride, 1.5 mmol/L of magnesium chloride, and 50 ng of genomic DNA. Polymerase chain reaction conditions consisted of an initial denaturation at 94°C for 3 minutes followed by 32 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, followed by 1 cycle of 72°C for 5 minutes. Polymerase chain reaction amplification was performed on an Applied Biosystems Gene Amp polymerase chain reaction system 9700 (USA) thermal cycler. In the restriction fragment length polymorphism method, the 537 bp amplified polymerase chain reaction products were digested with restriction endonuclease HaeIII, according to the manufacturer's instructions (Fermentas) and then separated on 3.0% agarose gel and visualized under ultraviolet light using ethidium bromide staining. Alleles of the polymorphism were identified -344T and -344C according to the absence or presence of a HaeIII restriction site (GGCC), respectively. Individuals with fragments including to 273 bp, 138 bp, and 126 bp were detected as homozygous TT genotype and individuals with fragments including 202 bp, 138 bp, 126 bp, and 71 bp as homozygous CC genotype. In addition, individuals with the five fragments concerning 273 bp, 202 bp, 138 bp, 126 bp, and 71 bp appeared on the agarose gel were identified as TC heterozygous genotype (Figure).

Statistical Analysis

Statistical analyses were carried out using the

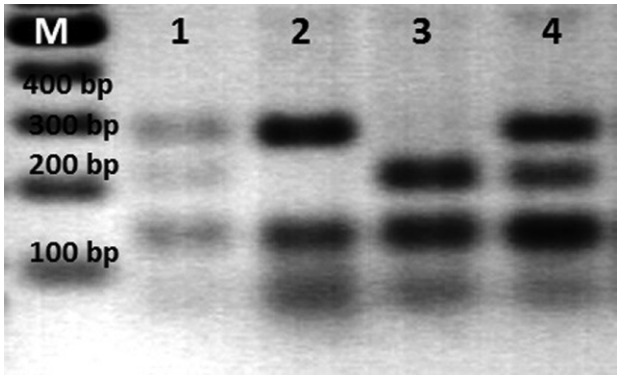

Illustration of *CYP11B2* -344 T>C polymorphism on agarose gel of 2%. M-GeneRuler 100-bp DNA leader, 1,4-heterozygous genotype (TC) including fragments of 273 bp, 202 bp, 138 bp, 126 bp, and 71 bp, 2-homozygous wild type genotype (TT) including fragments of 273 bp, 138 bp, and 126 bp, 3-homozygous polymorphic type genotype (CC) including fragments of 202 bp, 138 bp, 126 bp, and 71 bp.

Table 1. Characteristics of Patients With Chronic Kidney Disease and Healthy Controls*

Characteristics	Patients (n = 240)	Controls (n = 240)
Age, y	55.9 ± 15.6	53.0 ± 15.6
Sex		
Male	128 (53.3)	142 (59.2)
Female	112 (46.7)	98 (40.8)
Haemodialysis duration, y	4.6 ± 4.3	…
Hypertension	127 (52.9)	…
Diabetes	65 (27.1)	…
Hypertension and diabetes	43 (17.9)	…
Causes of kidney disease		
Unknown	77 (32.1)	…
Hypertension	45 (18.8)	…
Diabetes	42 (17.5)	…
Nephrolithiasis	17 (7.1)	…
Polycystickidney	15 (6.3)	…
Glomerulonephritis	10 (4.2)	…
Others	34 (14)	…

*Values in parentheses are percentages.

SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). The Hardy-Weinberg equilibrium was performed by a chi-square test. The independent-samples *t* test was used to compare the mean age between the patients and controls. Genotypes frequencies were compared between the patients and control groups using the chi-square test. As an estimation of relative risk of the disease, odds ratios (OR) were calculated on the basis of 95% confidence intervals (CI). *P* values less than .05 were considered to be significant.

RESULTS

Demographic findings of the 240 patient and 240 control groups are shown in Table 1. The mean of age was similar between patients and controls. The patient and control groups consisted of 53.3% and 59.2% men, respectively. A family history of kidney disease was observed in 11.2% of the patients, especially in mothers and sisters of them. Furthermore, there was no other kidney disease in 82.6% of the patients before the onset of CKD. Hypertension and type 2 diabetes mellitus were found in 52.9% and 27.1% of the individuals with CKD, respectively. In addition, 17.9% of the patients were determined to have both hypertension and diabetes mellitus.

Genotype frequencies of *CYP11B2* promoter polymorphism was detected to deviate from Hardy-Weinberg equilibrium in the controls but not in the patient group. Distrubition of the *CYP11B2* (T344C) polymorphism including TT, TC, and CC genotypes were 24.2%, 55%, and 20.8% in the patients and 19.2%, 57.5%, and 23.3% in the controls, respectively (Table 2). There were no significant differences observed in the distributions of genotypes between the patients and the controls (chi-square, 1.85; $P = .39$). The genotype distributions of the *CYP11B2* gene polymorphism among patients with and without diabetes mellitus were found to be significantly

Table 2. *CYP11B2* (-344) T>C Genotypes of Patients With Chronic Kidney Disease and Healthy Controls and Risk Analysis Results*

Genetic Characteristic	Patients	Controls	*P*	Odds Ratio (95% Confidence Interval)
Genotypes				
TT	58 (24.1)	46 (19.2)	…	Reference
TC	132 (55.0)	138 (57.5)	.25	0.75 (0.48 to 1.19)
CC	50 (20.8)	56 (23.3)	.21	0.70 (0.41 to 1.21)
Alleles				
T	248 (51.7)	230 (47.9)	…	Reference
C	232 (48.3)	250 (52.1)	.94	1.01 (0.78 to 1.31)

*Values are frequencies (percentages).

Table 3. Genotypic Distribution Among Patients With and Without Diabetes Mellitus*

Genotypes	Diabetes (n = 65)	No Diabetes (n = 175)	*P*	Odds Ratio (95% Confidence Interval)
TT	11 (16.9)	47 (26.9)		Reference
TC	45 (69.2)	87 (49.7)	.03	2.21 (1.04 to 4.67)
CC	9 (13.8)	41 (23.4)	> .99	0.93 (0.35 to 2.48)

*Values are frequencies (percentages).

Table 4. Genotypic Distribution Among Patients With and Without Hypertension*

Genotypes	Hypertension (n = 127	No Hypertension (n = 113)	*P*	Odds Ratio (95% Confidence Interval)
TT	32 (25.2)	26 (23.1)		Reference
TC	75 (59.1)	57 (50.4)	.87	1.06 (0.57 to 1.99)
CC	20 (15.7)	30 (26.5)	.12	0.54 (0.25 to 1.16)

*Values are frequencies (percentages).

Table 5. Genotypic Distribution Among Patients With and Without Hypertension and Diabetes Mellitus*

Genotypes	Hypertension and Diabetes (n = 43)	No Hypertension and Diabetes (n = 197)	*P*	Odds Ratio (95% Confidence Interval)
TT	6 (14.0)	52 (26.4)		Reference
TC	29 (67.4)	103 (52.3)	.06	2.44 (0.95 to 6.24)
CC	8 (18.6)	42 (21.3)	.40	1.65 (0.53 to 5.13)

*Values are frequencies (percentages).

different (chi-square, 7.30; $P = .02$; Table 3). We did not define any significant association among the frequencies of genotype when hypertensive CKD patients were compared with nonhypertensive CKD patients (Tables 4 and 5).

DISCUSSION

Several factors are responsible for the development of CKD. Frequencies of these reasons may be changed among countries and ethnic groups and sex. Diabetes mellitus, hypertension, chronic glomerulonephritis, and urologic diseases are the most important etiologic factors for CKD in Turkey.[17,18] In this case-control study, we did not find significant differences neither in genotypic nor allelic frequencies of the promoter polymorphism of *CYP11B2* gene between the ESRD patients and healthy controls. The number of the individuals with heterozygous genotype among both patients and controls was detected approximately 2-fold higher than the numbers of individuals with other genotypes (CC and TT) in the current study. In addition, the frequencies of the C allele and T allele were found about 0.52 and 0.48, respectively, in the control group.

Although our results are concordant with results of a previous study which was investigated *CYP11B2* -344 T>C promoter polymorphism in Sivas (Turkey) population,[19] and different Caucasian populations,[10,20] they are not similar to the findings among Japanese, White African, and South Asian populations.[21-23] It may be suggested that distribution of allelic variant may change according to geographic areas and ethnic groups. Three studies in 2000s reported that the polymorphism did not predispose carriers to the development of ESRD,[5,7,24] similar our results; whereas, Fabris and coworkers determined a significant association between *CYP11B2* gene polymorphism and kidney failure in the hypertension population.[14] However, they have also suggested that renin-angiotensin-aldosterone system phenotype may be an independent risk factor for kidney failure in hypertensive patients because both controls and cases suffered from essential hypertension in their study.[14] In another study, Tamaki and colleagues suggested that the effect of *CYP11B2* gene on kidney damage might be mediated by genotype-dependent differences in aldosterone levels,[16] that higher aldosterone levels were reported in the -344C allele of the gene.[25] We did not detect any association with genotypes and alleles of *CYP11B2* polymorphism in hypertensive patients in our study. In addition this result has confirmed to a previous study which

was performed in Turkey.[19]

It has been demonstrated that there is a genetic factor underlying diabetic nephropathy development in an observation that only 20% to 30% of all diabetic patients afflict from diabetic nephropathy.[26] In different populations, the roles of the renin-angiotensin-aldosterone system gene polymorphisms in the pathogenesis of diabetic kidney disease have been investigated.[27-29] Aldosterone as an important component in the pathogenesis of advanced diabetic kidney disease independent of arterial blood pressure and plasma angiotensin II levels has been indicated by recent experimental evidence.[30] Although we found that there was a significant association of the aldosterone gene polymorphism in the diabetic CKD patients compared to those without diabetes, there was not any association in CKD patients with hypertension compared CKD patients without hypertension. In addition, it has been found that the diabetic CKD patients with CT genotype have 2-fold higher risk than other patients for kidney disease (OR, 2.21; 95% CI, 1.04 to 4.67). Parasad and colleagues also found a significantly association between *CYP11B2* -344 T>C polymorphism and chronic renal insufficiency in diabetic patients, similar our results. Besides, they reported that T allele and TT genotype polymorphism seemed to be predisposing factors to renal insufficiency in their study group (OR, 1.57; 95% CI, 1.16-2.14 and OR, 1.81; 95% CI, 1.21-2.7, respectively).[31]

CONCLUSIONS

CYP11B2 (-344 T>C) promoter polymorphism may be a risk factor for development of CKD in patients with diabetes mellitus, while there was not any correlation between the polymorphism and hypertension on development of CKD in our study group. There are some limitations in our research as expression of aldosterone synthase gene that we did not analyze at levels of protein and mRNA. In addition, our study sample size was small. We think that further research in various populations with large sample size is needed to support these results.

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CONFLICT OF INTEREST

None declared.

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